1-1-2019

Tree Peony Species Are a Novel Resource for Production of α-Linolenic Acid

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Citation Information

Kilaru, Aruna; Xie, Lihang H.; Zhang, Qingyu; and Zhang, Yanlong. 2019. Tree Peony Species Are a Novel Resource for Production of α-Linolenic Acid. Poster Presentation. *American Society of Plant Biologists, Annual Meeting*,, San Jose, CA. https://plantbiology.aspb.org/wp-content/uploads/2020/01/Plant-Biology-2019-Posters.pdf

Tree Peony Species Are a Novel Resource for Production of α-Linolenic Acid

Plant Biology 2019 Posters

All Poster Presentations will take place in Hall 2 of the San Jose McEnery Convention Center.

1:30 PM - 3:00 PM	**Boechera and the Evolution of (No) Sex** Through sexual reproduction, the allelic combination of an offspring that is better suited to its particular environment is the basis of agricultural selection. This so called hybrid vigour, however, can not be transmitted to a following generation, as further reproduction leads to dispersal of the desired traits in the progeny. Apomixis, the asexual reproduction through clonal seeds, occurs only in a percentage of Angiosperms, but would have tremendous impact if applied to crops. It would permit the fixation of hybrid vigour by clonal generations, eliminating the costly effort of yearly producing the hybrids anew. A great leap forward has been taken recently by the introduction of the Pennisetum apomeiotic PsASGR-BABY BOOM-like locus in monocot crops such as maize and rice, successfully producing unreduced progeny. However, attempts to replicate the phenotype in dicots have failed, and the mechanism is not well understood. We have been exploring candidate genes for apomeiosis that have been described in Arabidopsis thaliana, but, by themselves, do not lead to the formation of a clonal embryo. We use the apomixis model genus Boechera, which contains acessions that can reproduce sexually or apomictically, in obligatory and facultative modes, to look into these genes. We looked for potential key regulators of reproductive mode by comparing them in sexual and apomictic lineages, and if the evolution of the gene could be linked to the rise of these lineages. We have found that some not only have marked differences in sexual and apomictic accessions, but also that some are represented in multiple copies in apomictic accessions, while others have mutations that lead to apo-specific changes in the aminoacid sequences. These findings suggest that the mis-regulated apomeiosis pathways that arose in Boechera are quite different that the ones in grasses, and this might help us understand how to bring apomixis to dicot crops. Co-author(s): Ueli Grossniklaus, Dmitry Smetanin Primary Poster Presenter: Sofia Nobre
1:30 PM - 3:00 PM	**CLAVATA3-mediated post-translational regulation of WUSCHEL provides stem cell maintenance robustnes** The spatial and temporal control of gene expression and growth patterns is required for the proper development of organisms. In Arabidopsis, the stem cell promoting transcription factor WUSCHEL (WUS) forms a concentration gradient across the cells of the shoot apical meristem (SAM). Proper regulation of the WUS concentration gradient is necessary for the maintenance of the stem cell population

and coordinated differentiation of stem cell progeny. At the cell level, the WUS gradient is in part managed by regulation of nuclear and cytoplasmic partitioning, as WUS accumulation in the SAM was dramatically altered when nuclear retention or nuclear export function was mutated. The diffusion of WUS protein across adjacently connected cells through plasmodesmata is regulated in the cytoplasm, while nuclear partitioning regulates WUS stability and also sequesters WUS from diffusing across cells. At the tissue level, cytokinin, an asymmetrically localized signal of the rib meristem overlapping the WUS domain, enriches the nuclear localization of WUS by promoting stability and nuclear retention. CLAVATA3 (CLV3) is the central zone signal which restricts WUS transcription. WUS, in a concentration-dependent manner, regulates CLV3 expression: low or high WUS levels activating or repressing CLV3 expression, respectively. Monitoring the fluorescently tagged WUS protein, we observed promotion of nuclear enrichment and stability of WUS protein upon exogenous CLV3 peptide treatments suggesting the CLV3 promoter can sense and respond to WUS levels accordingly for SAM homeostasis. As earlier studies have shown that the SAM can withstand large fluctuations in CLV3, the transcriptional and post-translational regulation acting in tandem provide robustness to the maintenance of the stem cell maintenance through precise control of the WUS protein gradient. Using mathematical modeling, we alter WUS synthesis rates and CLV3 levels to study quantitatively the robust maintenance of SAMs.

Co-author(s): Kevin Rodriguez,
Weitao Chen,
Venugopala Reddy

Primary Poster Presenter: Alexander Plong

1:30 PM - 3:00 PM

Developmental genetics of corolla tube formation: role of the tasiRNA-ARF pathway

More than 80,000 angiosperm species produce flowers with petals fused into a corolla tube. As an important element of the tremendous diversity of flower morphology, the corolla tube plays a critical role in many specialized interactions between plants and animal pollinators (e.g., beeflies, hawkmoths, hummingbirds, nectar bats), which in turn drives rapid plant speciation. Despite its clear significance in plant reproduction and evolution, the corolla tube remains one of the least understood plant structures from a developmental genetics perspective. Through mutant analyses and transgenic experiments, here we show that the tasiRNA-ARF pathway is required for corolla tube formation in the monkeyflower species Mimulus lewisii. Loss-of-function mutations in the M. lewisii orthologs of ARGONAUTE7 and SUPPRESSOR OF GENE SILENCING 3 cause a dramatic decrease in abundance of TAS3-derived small RNAs and a moderate up-regulation of AUXIN RESPONSE FACTOR 3 (ARF3) and ARF4, which lead to inhibition of lateral expansion of the bases of petal primordia and complete arrest of the upward growth of the inter-primordial regions, resulting in unfused corollas. By using an auxin reporter construct, we discovered that auxin distribution is continuous along the petal

primordium base and the inter-primordial region during the critical stage of corolla tube formation in the wild-type, and that this auxin distribution is much weaker and more restricted in the mutant. Together, these results suggest a new conceptual model highlighting the central role of auxin directed synchronized growth of the petal primordium base and the inter-primordial region in corolla tube formation.

Co-author(s): Blake Meyers,
Janelle Sagawa,
Yao-Wu Yuan,
Matthew Strobel,
Vandana Gurung,
Qiaoshan Lin,
Pamela Diggle,
Lauren Stanley,
Rui Xia

Primary E-Poster Presenter: Baoqing Ding

1:30 PM - 3:00 PM

Genetic Diversity of tomato germplasm developed by Texas A&M breeding programs

Genetic variation developed in plant breeding programs is fundamental to creating new combinations that result in cultivars with enhanced characteristics. Over the years, tomato (Solanum lycopersicum) breeding programs associated with the Texas A&M University system have developed morphologically diverse lines of tomatoes selected for heat tolerance, fruit quality, and disease resistance to adapt them to Texas growing conditions. Here we explored the intraspecific genetic variations of 322 cultivated tomato genotypes, including 300 breeding lines developed by three Texas A&M breeding programs, as an initial step toward implementing molecular breeding approaches. Genotyping by sequencing using low coverage whole-genome sequencing (SkimGBS) identified 10,236 high-quality single-nucleotide polymorphisms (SNPs) that were used to assess genetic diversity, population structure, and phylogenetic relationship between genotypes and breeding programs. Model-based population structure analysis, phylogenetic tree construction, and principal component analysis indicated that the genotypes were grouped into two main clusters. Genetic distance analysis revealed greater genetic diversity within than among the products of the three breeding programs. The germplasm developed at Texas A&M programs at Weslaco, College Station, and by Dr. Paul Leeper exhibited genetic diversity ranges of 0.175–0.434, 0.099–0.392, and 0.183–0.347, respectively, suggesting that there is enough variation within and between the lines from the three programs to perform selection for cultivar development. The SNPs identified here could be used to develop molecular tools for selecting various traits of interest and to select parents for future tomato breeding.

Co-author(s): Renesh Bedre,
Kranthi Mandadi,
Kevin Crosby,

Devi Kandel

Primary Poster Presenter: Carlos Avila

Group VII ERF orchestration of the hypoxia-responsive network in Arabidopsis thaliana, rice, & maize

Cereals--namely maize, rice, and wheat--are the leading agricultural crops of the world. However, their lucrative harvest is in continual jeopardy, as major cereal-producing countries are heavily prone to weather hazards, flooding being most prominent. Maize (Zea mays) is the largest contributor to global course grain trade and highly susceptible to the low-oxygen (hypoxic) conditions posed by flooding events. Discoveries in Arabidopsis thaliana and flood-tolerant rice have increased understanding of hypoxia response such that translating effective survival mechanisms to flooding-intolerant crops is feasible. While manipulation of the ETHYLENE RESPONSE FACTOR Group VII (ERF-VII) transcription factors, which orchestrate the hypoxia-responsive gene regulatory network, increased low-oxygen survival in these species, little is known about the maize orthologs (ZmERF-VIIs). Here we present a survey of the evolutionary conservation of ERF-VIIs, in both sequence and function, as well as the cis-regulatory elements with which the ZmERF-VIIs preferentially interact. We determined that the hypoxia-responsive promoter representative, ALCOHOL DEHYDROGENASE 1 (ZmADH1), was most highly transactivated in transfected protoplasts by the constitutive ZmERF 13 and 8, as well as the hypoxia-inducible ZmERF 12 and 1. The ADH1 promoter contains both an Anaerobic Response Element (ARE) and Hypoxia Responsive Promoter Element (HRPE) cis-element, both of which can be transactivated by the highly-studied Arabidopsis ERF-VII, RELATED TO APETALA2 12 (AtRAP2.12). ZmERF 13, the most AtRAP2.12-like in sequence, is the only candidate maize ERF-VII to mimic this quality, for ZmERF 12, 8, and 1 only transactivated through the HRPE. Flooding poses an escalating threat to global crop productivity, presenting the agriculturally crucial maize as an ideal candidate for submergence-resistance modification via ERF-VII-targeting manipulation.

Primary Poster Presenter: Sonja Winte

Identification of tissue-specific miRNAs from Cannabis sativa

MicroRNAs (miRNAs) are single-stranded, endogenous non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. Usually, miRNAs are 20-24bp in length. Cannabis sativa is one of the oldest cultivated plants and is unique for naturally producing cannabinoids (e.g., THCA). Recently, these phytocannabinoids recognized for their clinical application, especially for the treatment of epilepsy. However, the regulatory mechanism of cannabinoids is still enigmatic. Even though thousands of miRNAs from various plant species have been identified either by experimentally approach using direct cloning or by computational methods. Also, the knowledge of miRNAs mediated regulation of these cannabinoids from C. sativa is still lacking. Therefore, we decided to identify

tissue-specific miRNAs from C. sativa using next-generation sequencing approach sRNA-seq. Fifteen samples from five tissues and three chemotypes' plants ware used for small RNA-sequencing (sRNA-seq). From the sequencing experiment, a total of 241 million raw reads generated, of them, 135 million retained after passing through filtering steps and used for further downstream processing. From the complete miRNA prediction analysis, 249 miRNAs (Leaf=70, Root=22, Calyx=53, Young trichome=65, mature trichome=39) predicted. From these, 38 unique miRNAs identified, belonging to 20 miRNA families. Five candidates ubiquitously expressed in all the studied tissues, 14 have a high level of expression in the vegetative tissue, 11 miRNA families abundantly expressed in the reproductive phase organ. Two miRNAs were exclusively highly expressed in young trichomes. To functionally validate these two miRNA and their role in cannabinoid regulation, VIGS and CRISPR knockouts experiments are ongoing. Identification and validation of this miRNA mediated regulation of cannabinoids would be useful to exploit the potential cannabinoid-based pharmaceutical industry.

Chair and Concurrent Symposium Speaker: Oliver Kayser

Primary Poster Presenter: Tajammul Hussain

Identifying and Characterizing Cis-regulatory Elements in Maize and Sorghum

Cis-regulatory elements are DNA sequences that interact with nearby genes and affect their transcriptional activity. In eukaryotes, cis-regulatory elements have been shown to convey important tissue- and cell-type specific effects. Cis-regulatory elements affect gene expression by interacting with proteins, such as transcription factors. The interaction of proteins with cis-regulatory elements causes these regions of DNA to be less tightly wound around histones and become more accessible. We are using this accessibility, as determined by efficiency of transposase activity, to identify regions in maize and sorghum that interact with proteins and likely serve a regulatory function. Sets of regions identified through Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) are currently being tested for minimal promoter and enhancer activity. These regions are array-synthesized and cloned into barcoded plasmids which are transiently expressed in either tobacco, or maize and sorghum leaf-mesophyll protoplasts. Sequencing of barcoded reporter gene mRNA allows for the quantification of the transcriptional effects of each synthesized region. Preliminary data from transient tobacco expression has identified minimal promoter sequences that interact with the known 35S tobacco mosaic virus enhancer. Additionally, the 35S enhancer was identified among other non-enhancer sequences in a pilot test aimed specifically at finding enhancers. The next step will be to test how these accessible regions affect gene expression natively in both maize and sorghum using transient protoplast expression.

Primary Poster Presenter: Jackson Tonnies

Identifying Chemical Genetic Players in Repression of ABA Signal Transduction and Pathogen Defense

Being sessile organisms, plants have evolutionarily developed a variety of mechanisms to protect themselves from biotic and abiotic stressors such as pathogen exposure, drought conditions, and heavy metal contamination. The phytohormone, abscisic acid (ABA), is a major stress hormone in Arabidopsis thaliana essential to resistance of abiotic stressors, such as drought conditions. In addition to mediating drought tolerance, however, ABA has been found to interfere with pathogen resistance signaling as well by increasing susceptibility to pathogens. Recent discoveries demonstrated a novel interference in plant stress signaling--ABA signal repression by pathogen defense pathway activation. In a chemical genetics approach it was found that exposure to an isolated newly identified small molecule, [5-(3,4-dichlorophenyl)furan-2-yl]- piperidine-1-ylmethanethione (DFPM), down-regulates ABA-signaling via pathogen resistance signaling pathways in plants. This exposure-induced phenotype is consistently evident in A. thaliana. However, the molecular and cellular mechanisms of this pathogen signaling repression of ABA signal transduction are poorly understood. On one end, we have discovered a resistant to dfpm inhibition of aba (rda1) mutant via a forward chemical genetics screen. On the other, we have identified (amiRNA mutant) lines insensitive to DFPM induced root growth arrest. Further functional and genetic characterization of these two mutants will elucidate this bi-directionality of cross-interference in plant responses under simultaneous stress exposure and will help predict the effects of changing environmental conditions and contribute to future efforts in improving food production and safety.

Chair and Concurrent Symposium Speaker: Jiyoung Park, Tae-Houn Kim

Primary Poster Presenter: Chia-Yu Tsai

Increasing soybean oil yield through targeted gene silencing and overexpression

Soybean oil content is lower than most oilseed crops at 16-22% of dry weight. We are developing soybean germplasm with improved oil content and seed yield through rational metabolic engineering. To do this, we are primarily targeting the enzyme acetyl-CoA carboxylase (ACCase), which catalyzes the entry point into fatty acid biosynthesis, carboxylating acetyl-CoA to malonyl-CoA. In most non-gramineous oil-storing plant tissues, malonyl-CoA for FA biosynthesis is supplied by a tightly-regulated heteromeric form of ACCase, localized to the chloroplast. Increasing ACCase activity can provide a "push" of fatty acids into FA biosynthesis, resulting in increased oil production. We have developed and are testing soybean lines 1) that incorporate a gene silencing cassette that reduces expression of a family of negative regulators of fatty acid biosynthesis called biotin/lipoyl attachment domain containing-proteins (BADCs) or 2) that overexpress a modified form of the limiting subunit of heteromeric ACCase, α-carboxyltransferase (α-CT). These strategies have demonstrably increased oil content in the model organisms

Arabidopsis thaliana and Camelina sativa. We are characterizing these engineered putative high-oil soybean lines and are propagating them to the point of stable inheritance, at which point these traits will be stacked and populations evaluated in the field. We are also pursuing further strategies to improve soybean seed yield and oil content, including by repressing α-CT interactor protein (CTI), a new ACCase interactor (and putative inhibitor) that our lab recently discovered (Ye et al., unpublished), and by boosting seed sink strength through overexpression of an engineered form of sucrose synthase (SUS). Additional novel regulators of FA biosynthesis are being identified through traditional biochemical techniques and via co-expression meta-analyses of massive RNA-seq data sets with a software tool that we have developed (RNA-see).

Chair and Concurrent Symposium Speaker: Jay Thelen,
Ethan Myers,
Yajin Ye

Primary Poster Presenter: Eric Fedosejevs

Integration of Literature about Gene Functions in the PhyloGenes database using an ETL pipeline

We are developing a web application called PhyloGenes (PhyloGenes.org) that displays pre-computed phylogenetic gene trees along with experimental gene function data to facilitate inference of unknown gene functions in plants. For some genes, the known functions have been captured in the form of Gene Ontology (GO) annotations. However a large body of functional information from the literature has not been captured as GO annotations. In order to make this literature readily accessible to researchers within PhyloGenes, we developed an Extract, Transform, and Load (ETL) pipeline. The pipeline fetches literature data from UniProt database in XML format, converts the data into lists of strings, and stores the data into a Solr database. After this, we used an ExpressJS backend API to pull data from Solr, and VueJS framework to retrieve and display the literature linked to genes in PhyloGenes.

Chair and Concurrent Symposium Speaker: Peifen Zhang,
Qian Li,
Tanya Berardi,
Paul Thomas,
Leonore Reiser,
Anushya Muruganujan,
Huaiyu Mi,
Trilok Privithi,
Swapnil Swapnil,
Eva Huala

Primary Poster Presenter: Efrain Cuellar

Learning molecular biology by studying genes potentially affecting biofuel production in Arabidopsis

We taught our 300-level molecular biology class of 7 students by picking a research question and seeing where it led us rather than following a fixed curriculum. We used a workshop format where we met twice a week, mixing lectures with lab procedures, and we tailored lectures to the current status of the project and learned procedures as they were needed. The project we chose was studying genes that might affect biofuel production in Arabidopsis. Each student first identified a target gene and justified why it might affect biofuel production and then designed primers to measure its expression. They were then tested on DNA, and on RNA extracted from seedlings and from rosette leaves, cauline leaves, and flowers of Col-0 subjected to various treatments. No obvious differences were detected in the expression of any of these genes in any of these tissues or treatments. In the process we covered most of what we would have covered in a traditional course, but the students enjoyed the format better and said they learned more.

Chair and Concurrent Symposium Speaker: Alivia Womelsdorf, Kelvin Mejia, Kedene Clarke

Primary Poster Presenter: William Terzaghi

NAPPA-Nano-ESI-MS as a tool for biochemical characterization of plant cell wall glycosyltransferases

Glycosyltransferases (GTs) are responsible for the synthesis of all carbohydrates on earth including plant cell wall polysaccharides. Advances in bioinformatics have allowed the identification of numerous GT genes. However, biochemical characterization of cell wall GTs is far from complete, primarily because: 1-many GTs are membrane proteins and hard to purify, 2-GT protein levels are generally low, and 3-high-throughput tools for GT activity screening are lacking. Our goal is to adapt a combination of analytical tools to advance functional characterization of GTs in terms of biochemical function, protein-protein interactions (PPIs), and reaction product analysis. Here, we combine Nucleic Acid Programmable Protein Array (NAPPA) technology with Nano-Electrospray Ionization Mass Spectrometry (Nano-ESI-MS). NAPPA uses cell-free expression systems to produce either N or C-terminal tagged fusion proteins directly from microarrayed DNA templates. Subsequent enzyme and PPI assays are then performed in 96-well plates coated with anti-tag antibodies. Nano-ESI-MS is used for product detection and characterization. Nano-ESI-MS a soft ionization method that has a high salt tolerance and can be used to directly analyze products from GT reactions on 96-well plates. Here, we've tested four non-processive GTs (xyloglucan and arabinogalactan-protein fucosyltransferases, xyloglucan xylosyltransferase, and xylan glucuronosyltransferase) and three processive GTs (mixed-linkage glucan, xyloglucan, and mannan synthases). Optimization included testing anti-tag antibody dilutions as well as tag position, assay buffer preferences, and cell-free expression mix dilutions. Preliminary data show that 1-tag location affects the

enzyme activity of some GTs, 2-cell free expression systems produce active processive and non-processive GTs, and 3-Nano-ESI-MS detects reaction products for several GTs. Our results present a framework for a high-throughput pipeline for plant GT functional genomics.

Chair and Concurrent Symposium Speaker: Qi Wang,
Vel Murugan,
Ahmed Faik,
Hao Chen,
Matrika Bhattarai

Primary Poster Presenter: Michael Held

NAPPN: the North American Plant Phenotyping Network

The North American Plant Phenotyping Network (NAPPN) is an association of scientists and researchers in the rapidly evolving area of plant phenomics, formed as a regional partner of the International Plant Phenotyping Network (IPPN). Vision: The field of phenomics will become so advanced that the tools that would enable researchers to analyze and quantify phenotypic data become turn-key, thus enabling plant biologists to treat phenotypic prediction as a routine component of plant biological investigation and development. Mission: NAPPN seeks to achieve this vision by Accelerating the visibility and impact of advanced plant phenotyping research Maximizing existing synergies, identify and reduce potential bottlenecks, and facilitate collaboration spanning disciplines, locations and facilities across the region and beyond Incentivizing mutually beneficial research between public and private sectors Promoting a framework for data standards that facilitate data access and sharing Increasing the visibility and impact of plant phenotyping as a tool to enable plant sciences research beyond its own current research community Facilitating the interdisciplinary training needed for effective basic and translational plant phenotyping research Values: The NAPPN involves diverse stakeholders including, but not limited to, researchers, developers, and consumers of phenotyping technologies across all organizational dimensions. To do so, NAPPN relies on and encourages open communication and welcomes participation by individuals from diverse backgrounds, areas of study, and organization types. Members share involvement and interest in plant phenomics and are welcome from all ranks and levels of training, stature, and expertise. Join us to learn more about how NAPPN can help you connect with others working across the broadest spectrum of Plant Biology, Engineering, and Data Science disciplines to advance predictive plant phenomics.

Chair and Concurrent Symposium Speaker: Argelia Lorence,
Sindhuja Sankaran,
David LeBauer

Primary Poster Presenter: Carolyn Lawrence-Dill

1:30 PM - 3:00 PM

NLP2 is Required for Expression of Leghemoglobins

Leguminous plants and nitrogen-fixing bacteria called rhizobia form a symbiotic relationship in which rhizobia are taken up by root nodule cells into organelle-like structures called symbiosomes. In the symbiosomes the bacteria differentiate into large bacteroids which fix atmospheric nitrogen into ammonia through nitrogenase, a highly energy-intensive process. The energy for nitrogen fixation therefore requires a steady supply of O2 to maintain bacterial respiration. However, at the same time, O2 levels must be kept low since rhizobial nitrogenase is highly O2-sensitive. In nodule N-fixing cells the oxygen balance is achieved by the action of O2-binding leghemoglobin proteins. Medicago truncatula forms indeterminate nodules which are comprised of several zones, the meristem, infection zone, interzone and the N-fixation zone. We investigated NLP2, a gene belonging to the NIN-like Protein (NLP) transcription factor family, which has high expression in the N-fixation zone. A Tnt1-insertion mutant in the NLP2 gene had strongly reduced nitrogenase activity as shown using the acetylene reduction assay. Transcript profiling of nlp2 showed that all 12 leghemoglobin genes present in Medicago have strongly reduced expression in nlp2 nodules. To further understand the relationship between leghemoglobin expression and NLP2, we analysed the promoters of the 4 most highly expressed leghemoglobins. The result showed that these promoters contain NREs (Nitrate Response Elements), which are known direct targets of NLP transcription factors. Our results findings indicate that NLP2 is required for the expression of LgHbs and is essential for N-fixation in nodules.

Co-author(s): Suyu Jiang,
Jeremy Murray,
Jian Feng,
Giles Oldroyd

Primary Poster Presenter: Suyu Jiang

Photosynthesis in Tomato Genotypes with Differing Levels of Fatty Acid Desaturation

Loss of function of Fatty Acid Desaturase 7 (FAD7) in tomato alters the fatty acid profiles of chloroplast membranes and enhances resistance to aphids. We hypothesize that FAD7 may also influence photosynthesis because of its effects on the chloroplast membrane. We compared chlorophyll content, chlorophyll fluorescence, and stomatal density in isogenic tomato lines with normal (WT) and impaired FAD7 function (the spr2 mutant). Chlorophyll content was lower in the mutant than in wild-type (WT) plants, although the maximum quantum efficiency of photosystem II (Fv/Fm) was significantly higher in mutants than in WT plants. The density of stomata was also found to be lower in spr2 compared to WT plants. CO2 response curves suggested higher in vivo Rubisco activity in mutants under saturating light intensity (1200 µmol/m2/sec). These data indicate that the aphid-resistant spr2 mutant has enhanced photosynthetic activity due to enhancements in both the light-dependent and light-independent reactions of photosynthesis.

Potentially these alterations in primary metabolism may contribute to defense in spr2.

Chair and Concurrent Symposium Speaker: Fiona Goggin

Primary Poster Presenter: Janithri Wickramanayake

Plant hnRNPs participate in light-regulated alternative splicing

Plant photoreceptors tightly regulate gene expression to control photomorphogenic responses. Although gene expression is modulated by photoreceptors at various levels, the regulatory mechanism at the pre-mRNA splicing step remains unclear. Alternative splicing (AS), a widespread mechanism in eukaryotes that generates two or more mRNAs from the same pre-mRNA, is largely controlled by splicing regulators that recruit spliceosomal components to initiate pre-mRNA splicing. The red/far-red light photoreceptor phytochrome participates in light-mediated splicing regulation, but the detailed mechanism remains unclear. By taking protein-protein interaction approaches, we demonstrate that Physcomitrella patens phytochrome 4 (PpPHY4) physically interacts with the heterogeneous nuclear ribonucleoprotein H1 (PphnRNP-H1) splicing regulator in the nucleus, a process dependent on red light. We show that PphnRNP-H1 is involved in red-light-mediated phototropic responses in P. patens, and binds with higher affinity to the pre-mRNA-processing factor 39-1 (PpPRP39-1) splicing factor in the presence of red-light-activated phytochromes. Furthermore, PpPRP39-1 associates with the core component of U1 small nuclear RNP (PpU1C) in P. patens. Genome-wide analyses demonstrated the involvement of both PphnRNP-H1 and PpPRP39-1 in light-mediated splicing regulation. Our results suggest that phytochromes target the early step of spliceosome assembly via a cascade of protein–protein interactions to control pre-mRNA splicing and photomorphogenic responses.

Chair and Concurrent Symposium Speaker: Chueh-Ju Shih, Hsiang-Wen Chen, Hsin-Yu Hsieh

Primary Poster Presenter: Shih-Long Tu

Plant sphingolipid glycosylation, and its role in plant-microbe interactions and cell wall biosynthe

Glycosylinositol phosphorylceramides (GIPCs) are a class of glycosylated sphingolipids found in plants, fungi and protozoa. They are extremely abundant in the plant plasma membrane, estimated to form ~25-40 % of total lipids, but almost nothing is known about their function. GIPCs consist of a ceramide attached to a glycan headgroup via a phosphate group. Recently we have identified the first three Arabidopsis proteins involved in the headgroup biosynthesis - IPUT1 (a UDP-glucuronic acid glycosyltransferase; Rennie et al. 2014), GONST1 (a GDP-mannose transporter, Mortimer et al. 2013) and GMT1 (a GDP-mannose glycosyltransferase, Fang et al. 2016). Plants lacking functional copies of these proteins are either pollen

lethal (iput1) or have extreme developmental defects (gonst, gmt1), despite the lipid portion of the GIPC being unaffected. This implies a critical function for the GIPC glycan headgroup in membrane function. Here, we identify a new Golgi-localized protein involved in GIPC headgroup biosynthesis in both Arabidopsis, rice and Medicago - GINT1 (GLUCOSAMINE INOSITOLPHOSPHORYLCERAMIDE TRANSFERASE1. The products of GINT1 (glucosamine decorated GIPCs) are specific to seeds in Arabidopsis, but are found ubiquitously in rice. Atgint1 shows a mild germination phenotype but Osgint1 is seedling lethal. Medicago GINT1 RNAi plants have an impaired ability to form symbioses with arbuscular mycorrhizal fungi or nitrogen-fixing bacteria. We are now using our collection of GIPC GTs as a toolbox with which to explore GIPC function, and in particular the role of the glycan headgroup. This has been spurred by our recently published discovery that the length of the GIPC glycan headgroup can influence plant-pathogen interactions (Lenarcic et al. 2017).

Primary Poster Presenter: Jenny Mortimer

PRC2-Mediated H3K27me3 Contributes to Transcriptional Regulation of FIT-Dependent Iron Deficiency Re

Iron is an essential micronutrient for nearly all organisms, but excessive iron can lead to the formation of cytotoxic reactive oxygen species. Therefore, iron acquisition and homeostasis must be tightly regulated. Plants have evolved complex mechanisms to optimize their use of iron, which is one of the most limiting nutrients in the soil. In particular, transcriptional regulation is vital for regulating iron in plants, and much work has revealed the role of transcription factors on this front. Our study adds novel insights to the transcriptional regulation of iron homeostasis in plants by showing that chromatin remodeling via histone 3 lysine 27 trimethylation (H3K27me3) modulates the expression of FIT-dependent genes under iron deficiency. We provide evidence that FIT-dependent iron acquisition genes, IRT1 and FRO2, as well as FIT itself are direct targets of PRC2-mediated H3K27me3. In the clf mutant, which lacks the predominant H3K27 tri-methyltransferase, induction of FIT, FRO2, IRT1, and other FIT-regulated genes in roots is significantly higher under iron deficient conditions than in wild type. Furthermore, we observe that clf mutants are more tolerant to iron deficiency than wild type, indicating that gene expression levels appear to be limiting the plants ability to access iron. We propose that H3K27me3 attenuates the induction of FIT-target genes under iron deficiency and hypothesize that this may serve as a mechanism to restrict the maximum level of induction of iron acquisition genes to prevent iron overload.

Primary Poster Presenter: Kaitlyn Tsuyuki

Probing the photoprotective effects of heat under high light conditions in cowpea leaves

High light (HL) and heat stress often co-occur under field conditions. HL stress can cause detrimental effects on PSII efficiency via photoinhibition. Interestingly this effect is minimized under high temperature (HT) conditions. Using two different cowpea lines that have differing sensitivity to HL, we observed that prolonged exposure to HL caused decline in PSII efficiency (ΦII) under normal temperature conditions. However, exposure to HL combined with either dynamic or square wave temperature increases improved PSII efficiency in both the HL sensitive and tolerant cowpea lines. To determine whether the increase in PSII efficiency under HL was due to increased rate of D1 repair, we treated cowpea plants with lincomycin and observed that under normal growth conditions, both genotypes had declines in PSII efficiency under prolonged exposure to HL. When temperature was increased during the HL treatment, there was an increase in ΦII inconsistent with the reduced D1 repair associated with lincomycin treatment. Based on this we hypothesize that reduced photoinhibition under combined HT and HL is independent of D1 repair but likely due to decreased rate of damage. Whilst, ΦII can be decreased via the photoprotective mechanism of NPQ, we found that HT dissipated NPQ through an increased ATP synthase activity. Contrarily, carbon assimilation declined at HT of 45℃ but analysis of A/Ci curves under ambient CO2 concentrations revealed that maximum carboxylation rate (Vcmax) was increased at 45℃, implying that the decline in A was not due to decreased rubisco activity but rather an increase in its oxygenation activity (photorespiration). Upon exposure to combined HL and HT under non-photorespiratory conditions (1% O2) no significant increases in PSII efficiency were observed, suggesting that photorespiration acted as an electron sink, thereby playing a photoprotective role under combined HT and HL stresses.

Chair and Concurrent Symposium Speaker: David Kramer, Donghee Hoh, Jeffrey Cruz

Primary Poster Presenter: Isaac Osei-Bonsu

Structure-function studies on Grapevine Red Blotch Virus to elucidate disease etiology

Grapevine Red Blotch Virus (GRBV), a member of family Geminiviridae, has been reported as the causative agent of grapevine red blotch disease (GRBD), which has become a major threat for the grapevine production in the United States. GRBV is an inducer of, as well as a target of host posttranscriptional gene-silencing (PTGS) and has evolved anti-silencing processes as a counterdefense mechanism. Viral silencing suppressor proteins inhibit host antiviral responses upon infection, allowing viruses to infect host plants effectively. However, the resistance or susceptibility of the host to an infection depends on the arms race between the host defense against the pathogen and silencing suppression by the pathogen. In order to understand the molecular mechanisms of GRBD and its pleiotropic symptoms, we propose a working model where GRBV silencing suppressor proteins negatively regulate the documented autoregulatory loop miR828/TAS4, up-regulating the

anthocyanin positive regulators MYBA5/6/7 and thereby anthocyanin levels in GRBV-infected grapevines. Furthermore, we hypothesize that high levels of anthocyanin produced in vegetative tissues of infected plants might be a visual or olfactory cue for vectors to transmit the disease relatively faster to the other hosts. Toward this, we aim at characterizing all GRBV-encoded ORFs in both virion (V1, V2 & V3) and complementary strands (C1, C2 & C3) for their potential to act as host PTGS silencing suppressors. We will also characterize interactions between the candidate viral suppressors and target host proteins by yeast-two-hybrid screens to broaden our understanding of GRBD etiology and further to develop disease resistance strategies that could benefit wine and table grape industries. Keywords: Anthocyanin, Posttranscriptional gene-silencing, Red blotch, Viral suppressors Funding: supported by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, Agreement Number 18-0296-000-SA to C.R. and S.S.

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Primary E-Poster Presenter: Heshani De Silva Weligodage

Time-course of resveratrol and piceid synthesis by Japanese Knotweed seedlings

Japanese knotweed is an invasive plant in various parts of the United States that reproduces asexually, via the propagation of the rhizome, and sexually via dioecious flowering. It has the highest level of resveratrol and its glycosylated derivative, piceid, of all studied plants. Resveratrol is a stilbene produced in response to environmental stress by several plant species with antineoplastic, anti-inflammatory, and antiaging properties in animals. Prior studies showed that the rhizomes of Japanese knotweed have the highest levels of resveratrol and piceid; however, an inventory recording the levels of these chemicals across the plant was never reported. In this study, HPLC-MS data showed that resveratrol and piceid levels were highest in the dermis and pith, respectively. Additionally, resveratrol and piceid levels were higher in females for all studied plant tissues. We also show that Japanese knotweed seeds have no detectable levels of resveratrol or piceid, and a germination time course revealed that resveratrol and piceid production began within a day after germination, peaked between 3 and 8 days after germination then settled to a lower steady rate. This coincided with the expression of the gene for stilbene synthase, the key enzyme in resveratrol synthesis.

Chair and Concurrent Symposium Speaker: Alivia Womelsdorf,
Kelvin Mejia,
Kedene Clarke,
Donald Mencer,
Kristen Petrasko,
Lauren Johnson,
Junior Alvarado-Rosario,

Kenneth Klemow

Primary Poster Presenter: William Terzaghi

TRANSPORTER OF IBA1 Links Auxin and Cytokinin to Influence Root Architecture.

Developmental processes that control root system architecture are critical for soil exploration by plants, allowing for uptake of water and nutrients. Conversion of the auxin precursor indole-3-butyric acid (IBA) to active auxin (indole-3-acetic acid; IAA) modulates lateral root formation. However, mechanisms governing IBA-to-IAA conversion have yet to be elucidated. We identified TRANSPORTER OF IBA1 (TOB1) as a vacuolar IBA transporter that limits lateral root formation. Moreover, TOB1, which is transcriptionally regulated by the phytohormone cytokinin, is necessary for the ability of cytokinin to exert inhibitory effects on lateral root production. The increased production of lateral roots in tob1 mutants, TOB1 transport of IBA into the vacuole, and cytokinin-regulated TOB1 expression provide a mechanism linking cytokinin signaling and IBA contribution to the auxin pool to tune root system architecture.

Chair and Concurrent Symposium Speaker: Lucia Strader

Primary Poster Presenter: Suresh Damodaran

Understanding the Role of Type IVb Pili in the Rhizobium-Legume Symbiosis

Legumes, such as soybeans, peas, and alfalfa engage in a symbiotic relationship with rhizobial soil bacteria, which leads to the conversion of atmospheric nitrogen to biologically available forms of nitrogen. Bacterial attachment to the root is a critical step in infection and symbiosis. Sinorhizobium meliloti, possess type IV pili (Tfp), which are extracellular filaments used to mediate adhesion, cell-cell interaction, motility, and DNA uptake. In pathogens, Tfp are also important for virulence. Tfp are classified into two types, type a (Tfpa) and type b (Tfpb), based on the features of their pilin subunits, which serve as building blocks for pili. Tfpa are best known for their function in twitching motility, whereas Tfpb, which are commonly found in pathogens, function as host-colonizing factors by mediating tight adherence to host cells. The role of Tfpb in the interaction between plant symbionts or pathogens and their host plants is not well understood, mainly because they have only recently been recognized in genome mining and the pili structures have not been visualized. S. meliloti possesses Tfpb of the Flp (fimbrial low-molecular-weight protein) subfamily, which are organized in polar and uni-lateral bundles. Thus far, two gene clusters have been analyzed: one on the chromosome (pilA1) and one on the pSymA megaplasmid (pilA2). Both pili systems are expressed in S. meliloti cells attached to root hairs and promote the establishment of an effective symbiosis. Deletion of pilA1 and pilA2 results in reduced infection. Furthermore, the induction of abortive infections by the Flp-defective mutants sometimes result in nodules with unusual morphology. More recently, genome mining has identified a third gene

cluster: pilA3. To date, the role of pilA3 remains completely uncharacterized. The goal of this project is to assess the role of pilA3 in adhesion to soil and roots, in infection, in establishing and maintaining a successful symbiosis with the legume host.

Chair and Concurrent Symposium Speaker: Alex Napior

Primary Poster Presenter: Nancy Fujishige

Unmasking the mysteries of floral initiation in Carya illinoinensis through RNA-Seq analysis.

Carya illinoinensis (pecan) is native to North America and belongs to the Juglandaceae family. Pecan nuts contain high and healthful levels of mono-unsaturated fats and antioxidants and are currently being produced in several areas throughout the world. Carya illinoinensis is heterodichogamous with genotypes that are either protandrous or protogynous. A major constraint in pecan breeding programs is the extensive juvenile phase which typically lasts 8-15 years. To date, the transition from vegetative to reproductive phase and the genetic mechanism of flower initiation in pecan is unknown. Year to year fluctuations in pecan nut numbers per tree (alternate bearing) is a constraint for commercial pecan production and is thought to occur as a function of variation in floral initiation. RNA-Seq studies followed by qRT-PCR was used to examine the gene expression involved in the floral initiation of protandrous and protogynous genotypes. In this study, 52 RNA libraries were constructed from bud tissues at different time points through the growing season of a protandrous ('Western') and protogynous ('Wichita') genotype. The data obtained from this analysis suggests that floral initiation may occur in a two-step pattern. The first step occurs in the buds the summer prior to flower bloom with a second decision step that occurs in the beginning of Spring prior to bloom. This study evaluated the expression levels of the different copies of the known flowering genes to determine the active form of the genes involved in the initiation of flowering. DEGs between protandrous ('Western') and protogynous ('Wichita') cultivars was also evaluated to differentiate the genes involved in formation of catkin and pistillate flowers. These data show significant differences in expression of known flowering genes between protandrous and protogynous genotypes. Gene expression analysis of these transcriptomes allow us to increase our understanding of the floral initiation mechanism in pecan.

Chair and Concurrent Symposium Speaker: Jeremy Schmutz, Jerry Jenkins, Jane Grimwood, Nolan Bently, Patricia Klein, Jennifer Randall, Richard Heerema, LJ Grauke

Primary Poster Presenter: Hormat Rhein

Using CRISPR-Cas9 to Unravel the Mystery of Monolignol Translocation

Lignin is a complex polymer deposited in the plant secondary cell wall. The aromatic polymer is key to forming structural materials that support the growth and development of vascular plants. Genetic approaches to perturb lignin in Arabidopsis have led to the modification of lignin composition, which are comprised of mainly three monolignol subunits: p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol. While perturbations have provided insight into the biosynthetic pathway of lignin and strategies for redesigning it in plants to improve biofuel technologies, a critical step during its synthesis is not well understood. An understanding of how the building blocks of lignin are moved out of the cell from the cytoplasm to the apoplastic space where polymerization takes place has potential to advance biofuel technologies. I have data that suggest a transporter protein plays a role in this step. Findings from my research could provide insights to regulate the amount of lignin in biofuel crops. I will discuss efforts using gene expression data and T-DNA knockout lines to identify this protein and other genetic approaches, including bulk-segregant analysis and CRISPR-Cas9 experiments, that support its role in lignin translocation.

Chair and Concurrent Symposium Speaker: Curtis Wilkerson

Primary Poster Presenter: John Tran

Abiotic: General

Do AAT1, DUR3, and PTR3 Nitrogen Transporters Contribute to N Mobilization During Leaf Senescence? (1100-010)
Hall 2

The final stage of leaf development is leaf senescence, a recycling event in which nitrogen and other nutrients are reallocated to developing tissues to promote growth. In Arabidopsis thaliana, transporters such PTR3, AAT1, and DUR3 which respectively function in transporting dipeptides, amino acids, and urea, have shown a parallel increase in mRNA abundance and H3K4me3 histone marks during leaf senescence. The aim of this study is to determine whether PTR3, AAT1, and DUR3 transporters function in the mobilization of nitrogen during leaf senescence in Arabidopsis. To address this aim, T-DNA insertion mutants of the three transporters will be studied. Single mutants have been used to construct double and triple mutant plant lines, and these higher order mutants will be tested to determine if there is a decrease in nitrogen mobilization to the seeds and an increase in Rubisco retention in the leaves during senescence. Rubisco is a highly-abundant leaf protein that is the major source of mobilized nitrogen. Western blot analysis will be conducted to quantify Rubisco levels during senescence in double and triple

mutants. Nitrogen elemental analysis will be conducted to determine the nitrogen content WT and triple mutant seeds. Rubisco decrease will be evaluated in three senescence systems that function in different time frames. From slowest to fastest, these are age-related, dark-induced-attached leaves and dark-induced-detached leaves. Upon completion, I expect higher order mutant lines with non-functional transporters to experience lower levels of nitrogen mobilization and higher levels of rubisco retention, thus supporting the role of AAT1, PTR3, and DUR3 transporters during leaf senescence. Currently, dark-induced senescence protocols using attached and detached leaves are being conducted on double mutants. Western blot and chlorophyll analysis results for double mutant lines will be presented.

Co-author(s): Judy Brusslan

Primary Poster Presenter: Daniel Martinez

5:00 PM - 5:30 PM

ATP Binding Cassette Proteins ABCG37 and ABCG33 function as potassium independent cesium transporte (1100-115 (Screen 4))
Hall 2

Radiocesium, accumulated in the soil by nuclear accidents is a major environmental concern. The transport process of cesium (Cs+) is tightly linked to the indispensable plant nutrient potassium (K+) as they both belong to the group I alkali metal with similar chemical properties. Most of the transporters that had been characterized to date as Cs+ transporters are directly or indirectly linked to K+. Using a combinatorial approach of physiology, genetics, cell biology and direct transport assay, here we identified two ATP-Binding Cassette (ABC) proteins, ABCG37 and ABCG33 as new Cs+ transporters. The gain-of-function mutant of ABCG37 (abcg37-1) showed hypersensitive response to Cs+-induced root growth inhibition, while the double knock out mutant of ABCG33 and ABCG37 (abcg33-1abcg37-2) showed resistance. Single loss-of-function mutant of ABCG33 and ABCG37 did not show any alteration in Cs+ response. Short term uptake experiment with radioactive Cs+ revealed reduced Cs+ uptake in abcg33-1abgc37-2 compared with wild type in presence or absence of K+. Potassium response and content were unaffected in the double mutant background confirming that Cs+ transport by ABCG33 and ABCG37 is independent of K+. Collectively, this work identified two ABC proteins as new Cs+ influx transporters, which act redundantly and independent of K+ transport pathway.

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Primary E-Poster Presenter: Abidur Rahman

5:00 PM - 5:30 PM

Towards identifying new components of CO2-regulation of stomatal movements in grasses using Brachypo (1100-116 (Screen 14))

Hall 2

Plants need to take up CO2 for photosynthesis while avoiding excessive water loss through transpiration. This vital process is regulated by specialized pores on the surface of leaves, the stomata. High CO2 concentrations in leaves induce stomatal closure while low concentrations trigger stomatal opening. The atmospheric CO2 concentration has been continuously rising since the industrial revolution, impacting stomatal gas exchange. Investigation of stomatal physiology and movements is crucial for a better understanding of plant-environment interactions. In grasses, stomata are surrounded by two groups of cells: the dumbbell-shaped guard cells and the stomatal subsidiary cells. In combination, these cells respond to both external and internal stimuli, thus enabling rapid stomatal opening and closing. However, the molecular mechanisms mediating stomatal movements in grasses remain to a large degree unknown. Using the reference grass species Brachypodium distachyon, a forward genetic screen was performed. Over 1,000 mutagenized lines were screened for changes in their canopy leaf temperature using real-time infra-red imaging. Differences in leaf temperature may indicate defective stomatal development or responsiveness. Using this approach, 21 mutant lines with consistent canopy leaf temperature changes compared to wild-type (WT) were selected and are currently being characterized. Interestingly, two of these mutant lines named "chill1" and "chill7" have impaired responses to elevated CO2 concentration but retain an intact stomatal closing response to the hormone abscisic acid in intact leaf gas exchange experiments, suggesting specificity. Moreover, stomatal indices in chill1 and chill7 lines are similar to WT stomatal indices. Whole Genome Sequencing (WGS) data suggest that new genes in CO2-mediated stomatal movement in grasses will be identified in these mutants.

Co-author(s): Morgana Sidhom,
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Primary E-Poster Presenter: Paulo Henrique De Oliveira Ceciliato

A role for calcium-dependent protein kinases (CPKs) in CO2-regulated stomatal movements in Arabidops (1100-118 (Screen 6))
Hall 2

Plants rely on uptake of CO2 to conduct photosynthesis while avoiding excessive water loss through transpiration at the same time. Regulation of gas exchange is therefore vital and mediated by specialized guard cells on the surface of leaves, that build stomatal pores. Low concentrations of CO2 cause stomatal opening, whereas elevated CO2 concentrations trigger stomatal closure. The atmospheric CO2 concentration has been steadily increasing since the industrial revolution.

Plants have to cope with this ongoing impact on gas exchange, making the investigation of stomatal physiology and movements crucial for our understanding of plant-environment-interactions. Classical studies have suggested a role for Ca2+ and protein phosphorylation in CO2 regulation of stomatal movements. Among the kinases involved in guard cell signaling, calcium-dependent protein kinases (CPKs) can sense and translate cytosolic elevation of the second messenger Ca2+ into specific phosphorylation events. The role of several of these CPKs in abscisic acid-mediated stomatal movements has been described previously. However, whether CPKs function in CO2-mediated stomatal movements remains unknown so far.We have generated homozygous higher order cpk mutants and analyzed their CO2-mediated stomatal response. Here, we present data showing that one of the high order mutant is impaired in the high CO2-mediated stomatal closure as well as stomatal opening in response to shifts to low CO2 concentrations in intact leaf gas exchange experiments. These findings identify Ca2+ sensory proteins that function in CO2 control of stomatal movements.

Primary E-Poster Presenter: Sebastian Schulze

Potential of Enhancing Crop Plant Abiotic Stress Tolerance and Biomass Utilizing Crassulacean Acid M (1100-119 (Screen 9))
Hall 2

Crassulacean acid metabolism (CAM) is a specialized type of photosynthetic CO2 fixation pathway that results in enhanced water-use efficiency (WUE) compared to C3 and C4 photosynthetic plants. Increasing frequencies and intensity of drought and other abiotic stresses including high salinity, extreme temperatures, and high light intensities are major constraints for global crop production. Notable progress has been made towards genetic engineering crop plants to improve tolerances to different abiotic stresses. One widely uses bioengineering approach is the overexpression of transcription factors (TFs) to modify complex traits including tolerance to abiotic stresses in crop plants. Here, we have used abiotic stress-responsive TFs from CAM plants to improve abiotic stress tolerance in Arabidopsis presumably by activating regulatory mechanisms that mediate stress tolerance adaptations as CAM plants are naturally adapted to drought and other abiotic stresses. Previously, we identified several candidate TFs as key regulators of either CAM or water-deficit response or both in the obligate CAM plant, Kalanchoe fedtschenkoi. Of these TFs, functions of the K. fedtschenkoi NAC83 (KfNAC83) and KfbZip TFs are not known in CAM or C3 photosynthesis plants, but their A. thaliana orthologues display potential roles in abiotic stress responses and development. We have functionally characterized these TFs via overexpression in A. thaliana to determine their roles in abiotic stress responses and development. Overexpression of KfNAC83 TF in A. thaliana enhanced the drought and salt tolerance of transgenic lines, as well as enhanced plant growth and development. Remarkably, KfNAC83 overexpression lines showed a significant increase in integrated WUE with increased biomass productivity up to 42% compared to wild-type plants. Results of the

phenotyping and potential improvement of abiotic stress tolerance, WUE, and productivity of utilizing CAM TFs will be discussed.

Co-author(s): John Cushman,
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Primary E-Poster Presenter: Kumudu Rathnayake

Why is the explosive and environmental pollutant 2,4,6-trinitrotoluene (TNT) toxic and how do plants (1100-120 (Screen 10))
Hall 2

It is estimated that in the U.S. alone, 10 million hectares of military land are contaminated with munitions of which 2,4,6-trinitrotoluene (TNT) is a major component. TNT is highly toxic and recalcitrant to biodegradation and its progressive accumulation in soil, plants and groundwater is a significant concern at military sites. The U.S. DoD estimated that the clean-up of unexploded ordnance, discarded military munitions and munition constituents on its active ranges would cost between $16 billion and $165 billion. Explosives pollution is, however, a global problem with large amounts of land and ground water contaminated, including polluted sites dating back to the First and Second World Wars. A fundamental understanding of the phytotoxicity of TNT, and the enzyme systems plants use to detoxify it, will allow the development of robust plant systems to contain, re-vegetate and remediate explosives pollution effectively in situ. Towards this, we have established that, in Arabidopsis thaliana, monodehydroascorbate reductase 6 (MDHAR6) is responsible for the majority of TNT phytotoxicity. Present in the mitochondria and plastids, MDHAR6 catalyzes the one-electron reduction of TNT to produce a nitro radical, with its spontaneous regeneration back into TNT releasing superoxide. Thus in the presence of only catalytic amounts of TNT, this futile cycle depletes cellular NADH and causes oxidative damage within sensitive organellar environments. To remove TNT from the cellular environment, and the damaging activity of MDHAR6, distinct members of xenobiotic detoxification gene families are expressed, including oxophytodienoate reductases (OPRs), uridine diphosphate (UDP) glycosyltransferases (UGTs), glutathione transferases (GSTs) and cytochrome P450s. Ways in which the knowledge of TNT toxicity and its detoxification can be used to remediate explosives-contaminated environments will be presented.

Co-author(s): Emily Johnston,
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Neil Bruce,
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Primary E-Poster Presenter: Elizabeth Rylott

Abscisic acid-independent stomatal CO2 signal transduction pathway and

convergence of CO2 and ABA si (1100-117 (Screen 11))
Hall 2

Stomatal apertures regulate gas exchange and water loss in response to environmental cues in plants. Both elevated [CO2] and the plant hormone abscisic acid (ABA) rapidly induce stomatal closure. However it is unclear and has remained a matter of debate whether ABA signaling is involved in elevated [CO2]-triggered stomatal closure. We have combined genetics, in vivo ABA-reporter imaging, time-resolved gas exchange, guard cell patch clamp, and biochemical analyses to study the convergence mechanisms between ABA and CO2 signal transduction pathways in regulating stomatal closure. We found that guard cells of strong ABA synthesis mutants, nced3/5 and aba2-1, retain the rapid response to [CO2] elevation. While in AABA receptor sextuple mutant pyr1/pyl1/2/4/5/8, rapid elevated [CO2]-induced stomatal closure is not disrupted but delayed. Patch-clamp analyses indicated that slow-type anion channels in the guard cells of nced3/5 double mutant and pyr1/pyl1/2/4/5/8 ABA receptor sextuple mutant leaves can be robustly activated by [CO2] elevation. Using a real-time ABA FRET nano-reporter, ABAleon2.15, and an ABA-responsive promoter reporter, pRAB18-GFP, we found that ABA concentration and ABA signaling in guard cells are not obviously altered by [CO2] elevation. Unexpectedly, in gel kinase assays showed that elevated CO2 does not activate OST1 protein kinase activity in guard cells, even though ost1 mutants show an impaired CO2 response. Together our analyses show that primary CO2 signal transduction mechanisms do not signal via the early ABA signal transduction pathway. Furthermore, our findings indicate that basal ABA signal transduction can modulate and amplify CO2-induced stomatal closure. In addition, our study leads to a model that CO2 signal transduction triggers stomatal closure via an ABA-independent pathway downstream of OST1/SnRK2.6. New transcriptomic and additional data will be present that correlate with this model.

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Primary E-Poster Presenter: Po-Kai Hsu

Reactive Oxygen Species mediated membrane remodeling under nitrogen limitation in Chlorella sorokini (1100-121 (Screen 9))
Hall 2

Chlorella sorokiniana, a microalgae, is known to accumulate Triacylglycerol (TAG) under nutrient limitation conditions such as nitrogen starvation. TAG accumulation under nitrogen starvation is coupled with extensive chlorosis. Transmission electron micrographs on nitrogen-limited cells show extensive reduction in chloroplast

membranes. By 14C labeling studies, we show that pre-existing membrane lipids, especially the chloroplast lipid monogalactosyldiacylglycerol (MGDG), serve as a significant source of fatty acid for TAG synthesis. To understand the molecular mechanism of TAG synthesis and accumulation under nitrogen limitation conditions we carried out a transcriptomic analysis. RNAseq analysis showed that the expression of catalase and superoxide dismutase genes are downregulated. These enzymes are involved in quenching Reactive Oxygen Species (ROS). ROS levels are significantly higher in nitrogen starved cells, prompting us to hypothesize that ROS bursts are involved in activating membrane remodeling and channeling fatty acid from membrane lipids to TAG. Ectopic induction of ROS by inhibiting superoxide dismutase activity promoted membrane remodeling. This validated our hypothesis that ROS burst under nitrogen limitation condition is an inducer of membrane remodeling. Upon further analysis we found that expression level of NADPH oxidase, a key enzyme involved in oxidative burst, was increased. Chemical inhibition of this enzyme was able to partially save MGDG from degradation, indicating that, ROS generated under nitrogen limitation comes from the activity of NADPH oxidase. Taken together, we show that, under nitrogen limitation Chlorella sorokiniana cells produce ROS from the activity of NADPH oxidase, leading to the degradation of chloroplast membranes causing chlorosis.

Co-author(s): Wayne Riekhof,
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Primary E-Poster Presenter: Jithesh Vijayan

Roles of AGB1 and AGG3 in ABA and drought response at proteome level in Arabidopsis thaliana (1100-122 (Screen 7))
Hall 2

Abscisic acid (ABA) caused massive plant protein abundance changes plays regulatory roles in various physiological processes throughout plant growth and development and abiotic stress response. However, how plant balance plant growth and abiotic stress response via ABA signaling remain unclear. In this study, by using knockout mutants of the Arabidopsis thaliana Gβ and GɣIII subunit, we provide precise evidences that plants differentially requires Gβ and GɣIII to modulate photosynthesis pathway in response to ABA and water stress response. Both mutants showed higher photosynthesis indicated by lower leaf temperature and higher Fv/Fm under normal growth condition than WT. However, only agb1 showed in higher leaf temperature than WT during dehydration condition. Besides, both mutants exhibited greater ROS accumulation than WT, suggesting Gβ and GɣIII function in redox metabolism. To investigate roles of Gβ and GɣIII proteins in ABA and drought stress response, we conducted the whole and redox proteome analysis. Data showed that ABA and/or Gβ or GɣIII control abundance and/or redox status changing proteins as well as biological processes. Interestingly, Gβ and GɣIII differentially and negatively regulate redox protein changes but positively redox

protein changes in response to ABA. In addition, comparison of the whole and redox proteome demonstrates the correlation between oxidation state and abundance of proteins, including photosynthesis- and redox-related proteins. Reduced photosynthesis caused by ABA and/or Gβ or GγIII may attribute to downregulated abundance of light reaction- and electron transport-related proteins, which result from their increased oxidation state. Finally, our results provided basic important information of protein changes caused by ABA and G-proteins and their combination could be potentially used for further functional analysis.

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Primary E-Poster Presenter: Chien Ha

1RNA binding protein GRP7 and 8 are capable for cytoplasmic-destined FeSOD activation in Arabidopsis (1100-033)
Hall 2

Iron (Fe), a transition metal ion, is essential for multiple cellular functions, while excess Fe accumulation causes cytotoxic through the production of reactive oxygen species. Metallochaperones deliver ion cofactors to the specific physiological partners through direct protein-protein interactions. However, only three Fe chaperones are known. We identified two potential glycine-rich proteins (GRPs): AtGRP7 and AtGRP8, to be putative Fe chaperone for the cytosolic-destined Fe-containing superoxide dismutase FeSOD (ΔTP-FSD1) activation in Arabidopsis. By using yeast two-hybrid, bimolecular fluorescence complementation (BiFC), co-immunoprecipitation (Co-IP), and pull-down assay confirmed the interaction between GRPs and ΔTP-FSD1. These data revealed that GRPs interacts with ΔTP-FSD1 and this interaction enhance FeSOD activity. Thus, GRP7 and GRP8 are candidates to be Fe chaperone and involved in the intracellular distribution of Fe in Arabidopsis.

Co-author(s): Yi-Hui Wang

Primary Poster Presenter: Tsung-Luo Jinn

A mechanistic framework coordinates root suberization during development and stress response in Arab (1100-013)
Hall 2

Suberin lamella forms protective barrier in plant tissues against biotic and abiotic stresses. This hydrophobic structure assembles widely in various cell types during plant development and in response to stress-induced hormones. However, it remains unclear how developmental programs interplay with stress responses to direct the spatiotemporal precision of suberin deposition. Here we provide evidence that SHORT-ROOT (SHR) mediates the regulatory network through a group of MYB

transcription factors to direct the specific suberization in root endodermis. Both SHR and MYBs promote the ABA level in Arabidopsis, but SHR mediated regulation appears to be independent of ABA induction despite these two pathways could share overlapping downstream modules. Compared to the fast and transient induction by ABA, SHR mediates a slow readout of developmental program that promotes suberization. In addition, defective Casparian strip triggers a complementary enhanced suberization possibly via a SCHENGEN3 (SGN3) mediated sub-network in SHR pathway. Developmental regulation and environmental stimulus act in parallel but form an interacting framework to provide plasticity of plant suberization in response to endogenous and exogenous cues.

Co-author(s): Chunhua Wang,
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Primary Poster Presenter: Shuang Wu

A New Ratiometric ROS Bio-reporter as A Potential Toolkit for Studying the Function of Reactive Oxyg (1100-014)
Hall 2

Reactive oxygen species (ROS) are spontaneously generated during plant growth and when plants experience various stresses. Although accumulation of ROS can be toxic to plants, it also acts as signaling molecules, which are closely associated with adapting to stresses. Tight regulation of ROS homeostasis is required to adapt to stress and survive. However, in vivo spatiotemporal information of ROS dynamics is still largely undefined, in part due to the limited range of available ROS bioreporters. In order to understand the dynamics of ROS changes and their biological function in adapting to stresses, a quantitative ROS transcription-based bioreporter was developed using a promoter fusion stratagem. This bioreporter uses a ROS-responsive promoter from ZAT12 to drive Green Fluorescent Protein (GFP) expression and to compare the resulting expression to a constitutively expressed Red Fluorescent Protein (mCherry). The ROS-bioreporter, ZAT12p-ROS, was used to assess ROS response to oxidative stress (H2O2), salt stress (100 mM NaCl), and pathogen related elicitor flg22. The ZAT12p-ROS bioreporter showed increases in the ratio values of GFP to mCherry signals within 10 to 30 min post stress treatments, consistent with stress-induced ROS accumulation in the hypocotyl and root. Such stress-associated ROS signals correlated with the induction of abiotic/biotic stress responsive markers such as RbohD, ZAT12, SOS2, and PR5 suggesting the ZAT12p-ROS provides a robust indicator of increased ROS, which is related to stress responses. Based upon the temporal response patterns and magnitude of signal increases, the ZAT12p-ROS bioreporter appears to be suitable for cellular mapping of ROS changes in response to abiotic and biotic stresses.

Primary Poster Presenter: Won-Gyu Choi

A novel approach to provide insight on the regulation of Postharvest Chilling Injury in tomato (Sola (1100-003)
Hall 2

Most tropical and subtropical produce are so cold-sensitive that refrigeration reduces shelf-life and quality. Tomato fruit experiences Postharvest Chilling Injury (PCI) when stored at 0-12.5°C. Symptoms include surface pitting, and uneven ripening and decay, due to metabolic and physiological dysfunction. Unlike tomato, Arabidopsis thaliana can cold-acclimate partly due to the CBF family of transcription factors (AtCBF1-3). The ectopic and constitutive overexpression of AtCBFs led to higher chilling tolerance but had negative developmental effects in tomato plants, and fruit response to cold stress was not tested. Constitutive overexpression of CBF1 from the cold-tolerant wild tomato relative Solanum habrochaites (ShCBF1) resulted in higher cold-tolerance in Arabidopsis plants. This suggests that increasing the control of transgenic CBF1 expression could be useful to study PCI in tomato fruit without detrimental effects on plant development. We hypothesize that CBF1 overexpression will increase chilling tolerance and ameliorate PCI symptoms during refrigeration. In this study, Micro-Tom tomato plants were independently transformed with three constructs: a dexamethasone system to chemically trigger AtCBF1 expression, and a stress-inducible promoter (RD29A) to induce ShCBF1 or SlCBF1 specifically when fruit are refrigerated. Fruit were stored at 2.5 (PCI-inducing) or 12.5°C (control, non-PCI inducing) for 1- 3 weeks, and transferred to 20°C to promote PCI symptoms. To assess changes in whole-plant cold tolerance, the photosynthetic performance of transgenic lines was measured under cold stress. Results showed that high expression of transgenic CBF1 in fruit as determined by qRT-PCR, was linked to accelerated senescence and an aggravation of PCI symptoms, both quantified by objective color and surface pitting scores. This suggest that PCI may offer an evolutionary advantage in tomato by accelerating fruit breakdown for seed dispersal under extreme stress conditions.

Co-author(s): Diane Beckles

Primary Poster Presenter: Karin Albornoz

A tetratricopeptide repeat (TPR) domain containing protein is involved in the regulation of Fe homeo (1100-040)
Hall 2

Iron (Fe), an indispensable micronutrient for plant growth and development. Its deficiency affects the crop productivity and nutritional values worldwide. To increase the Fe uptake and storage in plants, identification and characterization of novel regulator(s), behind this Fe-deficiency response are needed. Even though the identification of several positive regulators in Fe-deficiency response provided many hints to understand the Fe homeostasis mechanism, the Fe sensing and signal transduction machineries are not understood clearly. In order to identify new regulators involved in Fe homeostasis, forward genetic mediated Ethyl Methane Sulfonate (EMS) mutagenesis approach was used to identify new components involved in Fe homeostasis. EMS generated mutant, which has a phenotype of non-

responsive to Fe deficiency (nrf2) was screened using the reporter line with an IRON REGULATED TRANSPORTER 1 (IRT1) promoter driven luciferase (PIRT1:LUC) construct in Arabidopsis Col-0 background. This reporter system gets activated within 2-3 days under Fe deficient conditions and repressed under Fe sufficient conditions. Several Fe-deficiency induced genes including IRT1, FRO2, bHLH38, bHLH39, bHLH100, bHLH101, FIT and GRF11 were down regulated in nrf2. In addition, nrf2 also showed several developmental defects such as less seed yield, early flowering phenotype, low Fe content in shoot compared to the reporter line (Col-0). Besides, the signaling molecule nitric oxide (NO) also increased in nrf2 suggesting the involvement of NRF2 in FIT mediated Fe deficiency response. Further, NRF2 was identified as a transcriptional activator which encodes for a tetratricopeptide repeat (TPR) domain containing protein using next generation sequencing (NGS) analysis. Genetic cross between nrf2 and the T-DNA mutant showed similar phenotype and considered to be allelic. Taken together, our data suggest that NRF2 is involved in the regulation of Fe homeostasis.

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Primary Poster Presenter: Surjit Singh

Abiotic Stress Response Functions of the Obligate CAM Plant NF-Y Transcription Factor (1100-007)
Hall 2

Abiotic Stress Response Functions of the Obligate CAM Plant NF-Y Transcription Factor

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An algorithm to measure root hair response to abiotic stresses in microscopy images (1100-031)
Hall 2

Improving nutrient and water uptake in crops is one of the major challenges to sustain a fast-growing population that faces increasingly nutrient limited soils. Root hairs, which are specialized epidermal cells, compromise up to 70% of the total root surface area. Therefore, root hairs are important drivers of nutrient and water uptake from the soil. Microscopy provides a mean to record root hairs as digital

images. However, quantifying root hairs in microscopy images remains a bottleneck because of their geometry and their complex spatial arrangement. Describing root hairs manually is based on a limited selection of representative root hairs and is only possible in cases, where length and density are sufficiently low to trace individual root hairs. We present a method to automatically quantify phenotypic traits of root hairs in digital microscopy images. Our method uses a machine learning approach that classifies root hair, parent root and the image background. We define local metrics to quantify relatedness between root hair segments that are separated by crossing root hairs or blobs of two or more root hairs. Based on our local metric we can detect individual root hairs by resolving these complexities in a globally optimal way. As a result, we measure the root hair traits, length, number and density. We demonstrate our method on examples of rice, maize and common bean under phosphor, nitrogen and potassium stress. Preliminary results suggest that our measurements of root hair traits strongly correlate with manual measurements (Pearson-correlation up to 0.9 in length). We expect that our method distinguishes subtle differences between genotypes and treatments on the basis of the extracted traits. We believe our study paves a way towards identifying the genetic control of root hair traits and increased agricultural production in future.

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Primary Poster Presenter: Peter Pietrzyk

An Australian wild rice relative contributes to improved heat tolerance in carbon assimilation (1100-019)
Hall 2

Rice production worldwide is subject to many abiotic stresses, with the interaction between water supply and temperature being primary among them. There is growing interest in heat stress and its impact on both vegetative and reproductive growth. Wild relatives within the genus Oryza occur throughout the tropics, including two species largely endemic to the savannah of northern Australia (O. meridionalis and O. australiensis) where day temperatures regularly exceed 40 degrees during seedling establishment. This presentation will report on mechanisms of heat tolerance in the assimilation of carbon by O. australiensis, concentrating on the role of Rubisco activase, a heat-sensitive chaperone that activates Rubisco. The discovery of a heat-tolerant ortholog of Rubisco activase will be described, as well as details of its interaction with Rubisco under temperatures up to 45 degrees. The complexity of this interaction and the possibility of other players in the evolution of heat-tolerant photosynthetic systems is freely acknowledged but the case is also made for stress-tolerant alleles of key genes from wild crop relatives being a major plank of improved heat tolerance under rapidly warming regimes. This role is illustrated by experiments in which the O. australiensis Rubisco activase was expressed in O. sativa under day temperatures of 45 degrees, where grain yield was improved by more than two-fold by the wild rice transgene.

Primary Poster Presenter: Brian Atwell

An eukaryotic elongation factor 2 from Medicago falcata (MfEF2) confers cold tolerance (1100-004)
Hall 2

An eukaryotic translation elongation factor-2 (eEF-2) plays an important role in protein synthesis, however, investigation on its role in abiotic stress responses is limited. A cold responsive eEF2 named as MfEF2 was isolated from yellow-flowered alfalfa (Medicago sativa subsp. falcata), a forage legume with great cold tolerance, and transgenic tobacco plants overexpressing MfEF2 were analyzed in cold tolerance and proteomic profiling was conducted under low temperature in this study. MfEF2 transcript was induced during cold treatment. Overexpression of MfEF2 in trasngenic tobacco plants resulted in enhanced cold tolerance. Compared to the wild type, transgenic plants showed higher survival rate after freezing treatment, higher levels of net photosynthetic rate (A), maximum photochemical efciency of photosystem (PS) II (Fv/Fm) and lower levels of ion leakage and reactive oxygen species (ROS) production after chilling treatment. iTRAQ-based quantitative proteomic analysis identified 336 differentially expressed proteins (DEPs) from leaves of one transgenic line versus the wild type after chilling treatment for 48 h. GO and KEGG enrichment were conducted for analysis of the major biological process, cellular component, molecular function, and pathways of the DEPs involving in. It is interesting that many down-regulated DEPs were grouped into “photosynthesis” and “photosynthesis-antenna”, as subunits of PSI and PSII as well as light harvesting chlorophyll protein complex (LHC), while many up-regulated DEPs were grouped into “spliceosome”. Our results suggest that MfEF2 confers cold tolerance through regulating hundreds of proteins synthesis under low temperature conditions. The elevated cold tolerance in MfEF2 transgenic plants was associated with down-regulation of the subunits of PSI and PSII as well as LHC with reduced ROS production, and upregulation of proteins involving in splicesome that promotes alternative splicing of pre-mRNA under low temperature.

Primary Poster Presenter: Haifan Shi

Arabidopsis F-BOX STRESS INDUCED 1 (FBS1) destabilizes two nuclear-localized WD40 repeat-like superf (1100-041)
Hall 2

The ubiquitin 26S proteasome system selectively degrades cellular proteins, where E3 ubiquitin ligases select targets for removal. One type of E3 ligase is an SCF complex that uses F-box (FBX) proteins substrate adaptors. Plant genomes encode hundreds of FBX proteins, suggesting a broad range of targets, but the vast majority of FBX functions are unknown. Our research group uses publicly available gene expression data to guide hypotheses regarding biological functions of previously uncharacterized Arabidopsis FBX genes. We have identified F-BOX STRESS INDUCED 1 (FBS1) as a gene rapidly induced by a range of abiotic stresses and significantly co-expressed with other established stress regulators. FBS1 gene

knockout plants have no immediately obvious phenotype under both normal and stress conditions. However, over-expressing FBS1 causes stress-related phenotypes, including severely stunted growth and excessive anthocyanin production, suggesting that removal of an FBS1 ubiquitylation target might be part of a stress response pathway. We have identified an FBS1 interacting protein by yeast two-hybrid screening, FBS Interacting Protein 1 (FBIP1), belonging to the WD40 repeat-like superfamily of proteins, a family that includes important co-repressors of gene expression. A second FBIP protein, FBIP2, is also encoded in Arabidopsis and it also interacts with FBS1. Bimolecular fluorescence complementation (BiFC) assays show that FBS1 and FBIP1/FBIP2 interact in the nuclei of plant cells and that the 26S proteasome inhibitor, MG132, is required to visualize their interaction. Increasing abundance of FBS1 in Agrobacterium co-infiltration assays correspondingly decreases FBIP abundance. Collectively, our work shows that previously unrecognized nuclear proteins, FBIP1/2, are likely targets of SCF(FBS1) and are possibly degraded to enact FBS1-dependent stress responses. Our current work further tests this hypothesis and investigates genetic interactions between FBS1 and FBIPs.

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Primary Poster Presenter: Bryan Thines

At3g24570 encodes a homolog of MPV17 enhances abiotic stress tolerance in plants (1100-020)
Hall 2

Drought is one of the severe environmental stresses limiting the growth and yield of plants. We identified a desiccation stress response gene, PyMPV17 from Pyropia yezoensis that is extremely desiccation-tolerant marine red algae, shares sequence homology with MPV17 associated with mitochondrial DNA depletion syndromes in humans. Ten MPV17 homologs, MPV17/PMP22 are found in Arabidopsis genome. Among them, At3g24570 encodes a polypeptide sharing the highest sequence homology with MPV17 as well as PyMPV17. The expression of At3g24570 in sym1, yeast MPV17 ortholog, cells complements the ethanol growth defect at 37 °C. Fluorescence of the At3g24570-GFP fusion protein was detected in mitochondria such as MPV17, suggesting that At3g24470 is a functional ortholog of MPV, AtMPV17. The knock out mutants of the AtMPV17 are showing normal grow and seed development. However, the KO mutants of the AtMPV17 are more sensitive to ABA and mannitol during germination. Sensitivity of the KO mutant to ABA and mannitol are complemented by overexpression of the AtMPV17 gene. Total malondialdehyde content in the transgenic plant overexpressing the AtMPV17 gene were lower than those of the control plants under stress conditions. These results suggest that the AtMPV17 reduces oxidative damage and contributes to the mechanism of tolerance for abiotic stress tolerance in plants.

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Primary Poster Presenter: Jiwoong Wi

Characterization of a CAM halophyte Homeodomain Type Transcription Factor in C3 Arabidopsis thaliana (1100-009)
Hall 2

Abiotic stresses negatively affect plant growth and development globally, limiting the production of food, feed, fuel, and fiber to meet the demand of an ever-growing human population. Thus, there is need for developing novel strategies to increase abiotic stress tolerance in crop plants. One of these strategies is exploring transcription factors (TFs) in extremophytes, such as crassulacean acid metabolism (CAM) plants to improve crop plants ability to withstand increasing abiotic stresses due to climate change. CAM plants are naturally adapted to abiotic stresses and contain genetic components (i.e., TFs) that might increase abiotic stress tolerance when introduced into C3 plants. However, functions of CAM TFs for their stress-adaptive characteristics are unknown. Here, we determine the functions of the McHB-12 gene, a TF found in the facultative CAM halophyte, Mesembryanthemum crystallinum. This gene orthologue in Arabidopsis thaliana (AtHB-12) is responsible for controlling a network of genes in salt and drought stress responses. The McHB-12 gene was overexpressed in the C3 plant, A. thaliana to determine its regulatory role in abiotic stress responses. Preliminary results show that T1 transgenic plants have increased salt tolerance (200 mM NaCl) and biomass compared to wild type. Further analyses on water-deficit stress performed on soilless potting mix and salt stress will be discussed in context of its potential translational applications to C3 crop plants to increase their abiotic stress response. In addition, findings could be useful in understanding the regulatory basis of this transgene in abiotic stress response of the facultative CAM halophyte.Keywords: Abiotic stress, McHB-12, CAM

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Comparative Physiological Analysis Reavels Role of NR-derived Nitric Oxide in Cold Tolerance of Fora (1100-125 (Screen 11))
Hall 2

The role of nitric oxide (NO) signaling in cold acclimation of forage legumes was investigated in this study. Medicago sativa subsp. falcata (L.) Arcang. (hereafter M. falcata) is a forage legume with higher cold tolerance than M. truncatula, a model legume. Cold acclimation treatment resulted in increased cold tolerance in both M. falcata and M. truncatula, which was suppressed by pretreatment with tungstate, inhibitor of nitrate reductase (NR), and 2-phenyl-4,4,5,5-tetramethylimidazoline-1-

oxyl 3-oxide (PTIO), scavenger of NO. Likely, NITRATE REDUCTASE 1 (NIA1) but not NIA2 transcript, NR activity and NO production were increased after cold treatment. Treatments with exogenous NO donors resulted in increased cold tolerance in both species. Superoxide dismutase (SOD), catalase (CAT), and ascorbate-peroxidase (APX) activities and Cu,Zn-SOD2, Cu,Zn-SOD3, cytosolic APX1 (cAPX1), cAPX3 and chloroplastic APX1 (cpAPX1) transcript levels were induced in both species after cold treatment, which was suppressed by tungstate and PTIO. Treatment with exogenous NO resulted in enhanced activities of SOD, CAT and APX. Moreover, higher levels of NIA1 transcript, NR activity, NO production, and antioxidant enzyme activities and transcripts were observed in M. falcata as compared with M. truncatula after cold treatment. The results suggest that NR-derived NO production and upregulated antioxidant defense are involved in cold acclimation in both species, while the higher levels of NO production and its derived antioxidant enzymes are associated with the higher cold tolerance in Medicago falcata as compared with M. truncatula.

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Primary E-Poster Presenter: Zhenfei Guo

1:30 PM - 3:00 PM

DETERMINING THE ROLE OF CDT1 UNDER METAL STRESS IN ARABIDOPSIS

Cadmium is naturally available in the surface level of soil around the world and is also increased by human activity. Cadmium is toxic, so plants have mechanisms to deal with this stressor. Cadmium tolerance 1 (CDT1) is a gene that is found in many plants including the model plant Arabidopsis. Does this gene help in cadmium tolerance in Arabidopsis? A homolog of CDT1 in rice has been found to bind Al in the cell wall; mutant plants lacking CDT1 are hypersensitive to aluminum presence (Xia et al., 2013). However, the function of CDT1 in Arabidopsis has not yet been studied. Two insertional mutants in the CDT1 promoter were isolated; to find the function of this gene, wildtype and mutant Arabidopsis seedlings were placed under abiotic stress to study their response. cdt1 mutants responded similarly to the wildtype seedlings under varying pH and under aluminum stress. One of the cdt1 mutants (cdt1-2) responded like wildtype to 25μM $CdCl_2$ while the other (cdt1-3) was hypersensitive. Gene expression analysis of cdt1-2 revealed over-expression of CDT1 under cadmium stress. Future gene analysis will help scientists better understand the role of the CDT1 gene in environmental response.

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Primary Poster Presenter: Chloe Michalopoulos

Developing functional relationships between sesame (Sesamum indicum

L.) growth development and nitr (1100-016)
Hall 2

Plants, being sessile, are subjected to multiple environmental stresses of varying intensity over the course of their life cycle. Among the stresses, low fertility, drought, salinity, and non-optimal temperatures limit crop productivity all over the world. Functional relationships between leaf nitrogen (N) and crop growth are prerequisites to developing management tools to optimize productivity in the field. An outdoor pot-culture experiment was conducted to determine N deficiency effects on sesame, cv. "Seasaco S40" growth and development. Plants were grown in pots filled with fine sand and irrigated with full-strength Hoagland's nutrient solution from emergence to ten days after sowing (DAS). After that, the five nitrogen treatments were imposed: one treatment of full-strength Hoagland's nutrient solution (control, 100% N), and four reformulations of Hoagland's nutrient solution containing reduced N at 60, 20, 10 and 0% of the control. Treatments were maintained until plants were harvested at 31 DAS. Growth, including several root traits, photosynthesis, and leaf N were measured at the end of the experiment. Maximum growth rates were achieved at 5.3 g N kg-1. Even though all growth rates declined with lower leaf N, leaf area expansion was more sensitive to leaf N among the shoot growth and developmental parameters. Among the root traits, root volume was more sensitive to leaf N than other parameters. Among the plant-dry components, leaf dry weight had the greatest decrease while the root/shoot ratio increased under N deficiency. The functional algorithms and critical leaf N levels for various growth processes will be useful for modeling and for managing sesame crop in the field.

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Primary Poster Presenter: K. Reddy

Distortion of the N/C ratio homeostasis in small Chlamydomonas cells suggests a lower limit on N/C s (1100-035)
Hall 2

Maintenance of a constant nuclear to cell volume ratio (N/C) is a conserved trait in eukaryotic cells, though the absolute N/C ratio varies by species and by cell type. The green alga Chlamydomonas reinhardtii (Chlamydomonas) uses a multiple fission mitotic cell cycle where cells have a prolonged G1 phase, during which they can grow more than ten-fold in size. G1 is followed by rapid alternating rounds of DNA syntheses and mitosis (S/M) to produce uniform- sized daughters. We took advantage of the large cell-size range observed in Chlamydomonas cultures to test the limits of N/C across different sizes. N/C was tracked in live cells using a nuclear-localized ble-GFP fusion protein as an in vivo marker for nuclei. We found that N/C remained nearly constant at around 5% across the cell cycle in wild-type haploid and diploid cells, and in cell-size mutants. By comparison, the shoot meristem cells

and epidermal cells of Arabidopsis have an N/C ratio of 20%. The genome of Arabidopsis (135Mb) is only slightly larger than that of Chlamydomonas (120 Mb), indicating that genome size is not the major parameter which sets N/C. We also observed a distortion of N/C in the smallest Chlamydomonas cells suggesting that there may be a lower limit of the N/C ratio which is constrained by the physical packing of DNA. To test this idea we examined the lower limit of the N/C ratio in diploid cells and found it to be higher than in haploid cells, a result that is also consistent with physical constraints on N/C set by nuclear DNA content. We examined N/C in cell size mutants from the retinoblastoma tumor suppressor pathway and found the N/C value to be identical to wild-type, but observed more cells with distorted ratios among the smallest cells from the mat3/rb mutant. Interestingly, nuclear shape was also distorted in mat3/rb mutants, a result that is under investigation using mat3/rb cells from different cell cycle stages.

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Primary Poster Presenter: Dianyi Liu

Divergence in Thermostability of Mitochondrial DnaK/HSP70 Nucleotide Exchange Factors in Arabidopsis (1100-045)
Hall 2

The divergence of duplicate genes may contribute to organismic adaptation to heat stress, but few examples are available in this regard. In the Arabidopsis thaliana genome, two nuclear genes encode mitochondrial GrpEs, MGE1 and MGE2, the nucleotide exchange factors of DnaK/HSP70 chaperone. MGE1 and MGE2 are derived from a recent whole genome duplication event and respond differentially to high temperature. MGE2 is heat-inducible, while MGE1 is constitutively expressed. Disruption of MGE2 increases sensitivity to prolonged moderate high temperature at the seedling stage. Heterologous expression of MGE2 but not MGE1 restored the growth of E. coli grpE mutant cells at elevated temperatures, suggesting that MGE2 is more thermostable than MGE1. In this study, we directly compared the thermostability of the purified recombinant MGE1 and MGE2 by circular dichroism spectroscopy. The temperature midpoints of the unfolding transition (Tm) of MGE1 and MGE2 were about 38 and 46°C, respectively, indicating that MGE2 is more stable than MGE1 at a higher temperature. Domain swapping between the two homologous proteins showed that the N-terminal region, including an unstructured sequence and a long α-helix domain, is the primary determinant of the thermostability. Although MGE2 contains a conserved sequence derived from an exonized intron within the N-terminus unstructured region, deletion of this sequence did not substantially affect protein thermostability in vitro and complementation of E. coli and Arabidopsis heat sensitive mutants. Together, our results suggest that Arabidopsis MGE1 and MGE2 had diverged not only in transcriptional response but also in the thermostability of the encoded proteins, which may contribute to the adaptation of the plant to different temperature ranges.

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Do AAT1, DUR3, and PTR3 Nitrogen Transporters Contribute to N Mobilization During Leaf Senescence? (1100-010)
Hall 2

Co-author(s): Judy Brusslan

Primary Poster Presenter: Daniel Martinez

Effects of CO2 enrichment and elevated temperature on potato plants grown in SPAR chambers (1100-052)
Hall 2

Potato is the most important non-grain crop in the world. Therefore, understanding of the potential impact of climate change on potato production is critical for future global security. In order to investigate the effect of increasing CO2 concentration and temperature on growth, yield and photosynthesis of potatoes, the physiological performance of potato plants grown under elevated temperature, increased CO2 concentration, separately and in combination, was examined. Potato plants were grown in SPAR (soil plant atmosphere research) chambers and exposed to four different conditions, current climate, elevated temperature (+4℃), increased CO2 concentration (800 μmol mol-1) and combined treatment conditions. Elevated temperature reduced rapidly stomatal conductance and canopy net photosynthetic rate during late growth stage and led to a decrease in the dry weight and tuber yield. In the increased CO2 condition, stomatal conductance and chlorophyll content were decreased, but biomass and tuber number were increased compared to the current climatic conditions. However, there was no significant difference in the yield of tuber. In the combination of elevated temperature and CO2 enrichment, growth, canopy net photosynthetic rate and dry weight of potato plants were significantly higher than that of other conditions. As a result, yield was also increased due to the larger size than the number of tubers. Interestingly, the contents of chlorophyll, magnesium and phosphorus were lowered in the two conditions with increased CO2 concentration, and the results showed that the C/N ratio was increased due to decreased nitrogen content. In conclusion, this study suggests that temperature elevation may be a negative factor in the growth and yield of potatoes during the late growing season, but the combined increase of temperature and CO2 concentration can have a positive effect on potato growth and productivity.

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Primary Poster Presenter: Jung-Il Cho

Effects of High Ambient Temperature on Reproductive Stage in EMS-mutant Heat Tolerant Tomato (1100-038)
Hall 2

Harsh temperature is an extreme consequence of climate change and global warming directly reduces quality and yield of tomato. National BioResource Project Japan (NBRP) has been created an EMS mutant tomato collection based on Micro-Tom (WT) with over 16,000 lines. The preliminary population screening indicates heat tolerant mutant line HT7 showed higher pollen viability and higher yield under heat stress (HS). To evaluate the effects of high temperature on reproductive stage, we characterized the phenotype of WT and HT7 in 35oC/25oC (16h light/ 8h dark, 44% humidity) for two months. Interestingly, WT leaf stomata density was 11.3% higher than HT7. HT7 leaves were darker green with higher concentration of chlorophyll at 10 days after heat stress (DAHS) and HT7 produces fewer lateral shoots than WT at 60 DAHS, respectively. HT7 produced 25% less flower number than WT, but HT7 flowers had two times higher in total grain pollens, viable pollens and non-viable pollens. HT7 pollens germinated up to 5% although WT reached approximately 1%. WT pollens were damaged before releasing by observation flower at anthesis. Taken together, HT7 had two times higher in fruit setting rate, resulting that HT7 fruit yield was two times higher than WT. HT7 green fruits remained full jelly, while WT significantly reduced it causing the abnormal and dehydrate fruits in WT. Typically, HT7 produced several normal fruits containing seeds, but WT could not produce any seeds. To identify the responsible gene(s) which regulate(s) the unique phenotype in HT7, we performed the NGS mapping and detected a single strong QTL. This QTL peak was also found in bulked F2 plants with HT7 phenotype, but not in bulked F2 sample with WT phenotype. It is suggested the QTL is associated with HT7 phenotype.

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Primary Poster Presenter: DUNG PHAM

Effects of Ionizing Radiation in Maize Lines from Mexico and Central America (1100-049)
Hall 2

Maize is one of the most important food crops around the world. It is also a species that with high diversity. Recent works suggest that global warming may affect cloud formation and increase exposure to damaging UV-B ionizing radiation. This type of radiation creates double strand breaks on DNA that might lead to cell death, delayed growth and reduced pollen fertility. In this study we have used homozygous lines from the CIMMYT center in Mexico, local Costa Rican purple grain landrace "Pujagua", local Costa Rican lines CR5 and CR7, Thai line SUWAN1 and the American line B73. We exposed seeds to gamma radiation at doses of 5 and 15 Gy, and to no radiation as a control. Root morphology traits were analyzed with a scanner and WinRHIZO® software one week after germination. Statistical analyses were performed with InfoStat software. Tests included ANOVA and the DGC test. Results indicate that regarding root area, landrace Pujagua, CML349, CML455, CML456, and CML457 exhibited high values compared to the control. Meanwhile the SUWAN1 and CML242 lines showed a reduction compared to the control. Regarding surface area CML349, CML456, CML457 had high values compared to the control while the opposite occurred with SUWAN1. Root length increased in lines CML456 and CML457 while in line CML 242 a reduction was observed. In all these three variables lines CML456 and CML457 showed the highest values. Our results suggest that in maize there is natural genetic variation regarding responses to UV-B radiation that may be harnessed for plant breeding purposes.

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Primary Poster Presenter: Pablo Bolaños-Villegas

Examining Maize Nitrogen Stress Responses using Carbon-11 (1100-011)
Hall 2

Agriculture in the 21st century faces formidable challenges as the economically empowered global population grows and placing unprecedented and taxing demands on the planet's natural resources. Aside from water resource availability, nitrogen management is perhaps the next most critical component for most agronomic systems. Maize especially requires large inputs of nitrogen fertilizer for healthy growth and crop development. Globally, staple grains account for roughly 43% of caloric intake by the human population and corn reflects 36% of caloric intake totaling 1016 million metric tons consumed annually. Success to increasing corn yields hinges on developing strategies that will enhance crop nitrogen utilization. Here, we examined key physiological and metabolic features of plant abiotic stress that contribute to increased root growth under nitrogen limiting growth. Using radioactive C-11 (t1/2 = 20.4 m), we administered 11CO2 to intact leaves of 3-week old maize plants that were subjected to a 10-fold reduction in their nitrate supply, and measured changes in the partitioning of 'new' carbon into mobile substrate pools that supported increased root allocation of those resources in response to nitrogen stress. This research is supported by Agriculture and Food Research Initiative Award No. 2017-67013-26216 from the USDA National Institute of Food and Agriculture.

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Primary Poster Presenter: Alexandra Bauer

First insights into the structure-function relationship of the DNA damage regulator SOG1 (1100-030)
Hall 2

Due to their sessile lifestyle, plants are sensitive to very distinct types of cues compared to animals. When it comes to stress response this implies that they had to evolve unique ways to cope with environmental treats. A central transcription factor involved in regulating DNA damage response pathway (DDR) in plants is SUPRESSOR OF GAMMA RESPONSE 1 (SOG1), that belongs to the plant specific NAM (no apical meristem), ATAF1,2 and CUC2 (cup-shaped cotyledon) (NAC) family. As such SOG1 takes on the role of the mammalian p53, which is absent in plants. By combining plant in vivo studies with molecular biology and biophysics we investigate SOG1 structure and function. In this study we present the first structural data on SOG1, showing that it consists of a folded DNA binding domain (DBD) (NAC domain) and a large intrinsically disordered C-terminal domain (CTD). SOG1 forms stable dimers in solution through its NAC domain forming the functional unit. This is corroborated by mutagenesis of key residues in the DBD (R139 and G155) that shows that the NAC domain is essential for DNA binding. Interestingly, SOG1 contacts DNA in a highly non-specific manner. Alongside sliding experiments, this suggests that the CTD allows SOG1 to scan the DNA in search of its cognate site. To investigate the role of post-translational modification (PTM) on DNA binding specificity we assayed the binding of SOG1 phosphomimetics on Ataxia telangiectasia and Rad3 related (ATR) and Casein kinase 2 (CK2) phosphorylation sites. Moreover an additional level of regulation coordinates SOG1 function, as NAC proteins are known to form heterodimers with other NAC proteins. We found that SOG1 heterodimerizes with ANAC044, a closely related NAC protein. This study emphasizes that SOG1 function is probably regulated on multiple levels in vivo both due to its modular nature consisting of a folded and unfolded domain that can be prone to different PTMs and the formation of heterodimers with other NAC proteins.

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Primary Poster Presenter: Manon Demulder

Functional Analysis of Mitogen-Activated Protein Kinase 9 in Arabidopsis thaliana (1100-023)

Hall 2

Mitogen-Activated Protein Kinase 9 (MPK9), a Group D MAP kinase from the model plant Arabidopsis thaliana, is a positive regulator of the reactive oxygen species-mediated abscisic acid (ABA) signaling pathway which leads to stomata closure. Its role in the regulation of stomatal movement has been established, yet questions remain regarding additional physiological roles and its direct regulators and substrates. In transgenic lines expressing the fusion protein GFP::GUS under the control of a 2kb-long MPK9 promoter, MPK9 expression was limited to guard cells and pollen grains. By promoter deletion, we observed that a 1kb-long promoter region was sufficient to preserve this specific tissular expression. Subcellular localization studies of the protein fused to GFP in Arabidopsis transgenic lines showed that MPK9 is mostly cytosolic, although dot-like signals could also be observed. By yeast-two-hybrid assay, we identified several potential protein partners for MPK9, which are currently under validation. In addition, in germination assays, MPK9 T-DNA mutants were less sensitive to ABA, suggesting a new physiological role for the protein and warranting further gene expression studies during embryo development. Taken together, these results contribute to a better understanding of the role of MPK9 in Arabidopsis growth and development, and opens new avenues of investigation.

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Primary Poster Presenter: Fabien Jammes

Genome-wide identification and characterization of sHSP in Pyropia yezoensis, marine red algae (1100-005)
Hall 2

Small heat-shock proteins (sHSPs) play important role in life cycle as well as stress tolerance in plants. But, a little is known about sHSP gene family and their function in red algae. Pyropia yezoensis is one of the most valuable and cultivated marine red alga (Rhodophyta). Based on the draft genome sequence of P. yezoensis, we identified sHSP gene family including 6 members. Pyropia sHSPs are classified into two different subfamilies according to amino acid sequence similarity, expression pattern and predicted cellular localization. PysHSP19.2, PysHSP19.3, PysHSP19.6 and PysHSP20.3 of the subfamily I are response strongly to heat and detected as granules in the cytoplasm. PysHSP25.8 and PysHSP28.4 of the subfamily II are response more strongly to H2O2 than heat and are predicted localization in subcellular organelle such as plastid or mitochondria. Phylogenic analysis also show that the PysHSP25.8 and PysHSP28.4 of the subfamily II are branched into sHSP subfamily CVI of Arabidopsis. These results demonstrate that sHSPs of Pyropia, marine red algae are consist of two subfamily. Molecular and physiological function of the PysHSP25.8 in subfamily II will be posted.

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Grapes could suffocate under drought and high temperatures (1100-022)
Hall 2

Cell death in the mesocarp of berries occurs late in ripening, and may influence berry sensory attributes and water retention. Cell death in Shiraz is associated with berry shrivel, higher berry sugar concentration, higher potential alcohol in wine, and yield losses of up to 30%. The objective of this study was to investigate the association between berry internal [O2] and berry cell death. Using an oxygen micro-sensor, steep [O2] gradients were observed across the skin. [O2] decreased toward the middle of the mesocarp in Chardonnay, Shiraz and Ruby Seedless grapes. As ripening progressed, the minimum [O2] approached zero in the seeded cultivars and correlated to cell death pattern across the mesocarp. Seed respiration declined during ripening. [O2] increased towards the central axis corresponding to the locule space visualised using x-ray micro-CT. Locule spaces connect to pedicel lenticels, which are critical for berry O2 uptake as a function of temperature. Blocked lenticels caused hypoxia in Chardonnay berries. A factorial field experiment, comprising two thermal and two irrigation regimes, indicated water deficit increased the rate of cell death and was associated with lower mesocarp [O2] relative to well-watered vines. Berry respiration and total berry porosity decreased during berry ripening. Further research into the role of berry gas exchange on berry quality and cultivar selection for adapting viticulture to a warming climate is warranted.

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GWAS identifies loci associated with cold-deacclimation processes in canola (1100-026)
Hall 2

Deacclimation of winter annual crops such as winter canola can result in crop damage following unusual warm temperatures in the late fall or early winter- a phenomenon predicted to become more common with increased global warming. Identifying loci and the underlying genes controlling deacclimation processes could allow for the development of winter canola that is less responsive to such unseasonal events. Using a diversity panel of over 400 winter canola varieties, we developed testing parameters and phenotyped the population for damage following freezing conditions (4 hrs at -10C) after fully acclimated plants (4 weeks at 5C) were deacclimated for 3 days (20 C day and 10C night with 12 hr photoperiod).

Experiments were repeated three times, each with three technical replicates for the entire population (9 total replicates per variety). Various statistical models were used to identify several loci associated with survival following freezing on chromosomes CNN random, ANN random, and C06 random. Potential candidate genes included METHIONINE OVER-ACCUMULATOR 2, VIP3, AGO2, flower-specific, phytochrome-associated protein phosphatase 3, OSBP(oxysterol binding protein)-related protein, phytochrome kinase substrate 4, synaptotagmin 3, UUAT1 sugar transporter, Transducin/WD40, and a Nucleic acid-binding, OB-fold-like protein. Several of these were shown to be differentially expressed following deacclimation in a well replicated RNAseq study. In an initial experiment, mutants of these genes in arabidopsis generally acclimated as well as wild type (easily surviving -14C for 4 hrs following 3 weeks at 5 C), but several showed different deacclimated patterns relative to wildtype (had reduced or greater freezing survival follow 3 days deacclimation at 20 C relative to wild type).

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How freezing tolerance is regulated at the chloroplast envelope membrane
(1100-036)
Hall 2

Internal cellular membranes must have their lipid composition remodeled for plants to survive low temperatures. One mechanism necessary for freezing tolerance of the chloroplast envelope membranes is well defined. An enzyme named "Sensitive to Freezing 2" (SFR2) changes monogalactolipid into oligogalactolipids at temperatures below freezing. Interestingly, SFR2 activity does not respond to initial cool temperatures, it only responds to barely tolerable freezing temperatures. Here, we show that SFR2 is post-translationally regulated by modifications and changes to cytosolic acidification. We show that freezing increases cytosolic acidification and that proton pumps at both the plasma and vacuolar membranes participate in maintaining the acidification during low temperatures. Finally, quantitative measurements of SFR2 activation in a large number of plant species with diverse phylogenetic backgrounds shows that SFR2 is likely responding to membrane damage in some, if not all species. We conclude that plant low temperature sensing and response is likely a continuum rather than a switch, and that internal cellular membranes have systems set up to respond to damage in a diverse set of abiotic stresses.

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Identification and transcription profiling of the NHL (NDR1/HIN1-like) gene family in rice (1100-039)
Hall 2

The functions of NHL (NDR1/HIN1-like) genes in plant responses to biotic stress and abiotic stresses have been reported. Although the NHL family have been well-studied in Arabidopsis, little is known about NHLs in rice. In this study, we have identified 103 OsNHL genes and determined conserved domains in the predicted OsNHL proteins. These OsNHL members were classified into six groups, based on the presence or absence of the three key protein motifs. To understand the relationship of OsNHLs to each other, the conserved domains, chromosomal distribution, phylogenetic relationships, and gene structure of these protein-coding genes were analyzed in detail. The transcription profile of OsNHL genes in various tissues, organs, and developmental stages were further analyzed to obtain information of the functions of these genes. In addition, five OsNHL genes displayed differential expression in aleurone cells treated with abscisic acid (ABA) and gibberellin (GA). Together, this work has provided foundation to the characterization and further functional studies of OsNHL family in rice.

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Involvement of a heat-inducible ERF in the control of the sugar/acid balance in grape berries (1100-051)
Hall 2

One of the main consequences of global warming in grapevine consists in the rise of berry K+ and sugar concentrations at harvest. This trend already observed since several decades in Europe (and further) results in wines with increasing alcohol contents, more flat, and with decreased ageing potential. The control of the sugar/acid balance of the berries would allow maintaining the typicity, the quality and the market value of French wines. This requires a better understanding of the molecular basis of K+ and sugar accumulations along grape berry development and in response to climate change. To this aim, the characterization of the molecular repertoire of genes and regulatory networks involved in the fine-tuning of the fruit sugar/acid balance at harvest was initiated using Cabernet Sauvignon fruiting cuttings. Differentially expressed genes from flesh cells upon drought and/or high temperature stress were identified through RNA-SEQ analysis. Here, we describe the functional characterization of a heat-inducible transcription factor belonging to the large ERF family (Ethylene Response Factor). VvERFhs is ubiquitously expressed in vine and highly inducible upon heat stress (HS). A RNA-SEQ analysis of transgenic grape cells overexpressing dominant and dominant-negative versions of VvERFhs was conducted. Several putative VvERFhs target genes linked to K+ or

sugar accumulation were identified. Complementary approaches (Electrophoretic mobility shift assays, yeast-one-hybrid, and dual-luciferase assays) are on the way to evaluate the ability of VvERFhs to bind and transactivate the promoters of these different genes. In conclusion, the present work should contribute to open new research lines through the identification and characterization of key players involved in the control of berry quality and might provide an improved basis for the selection of vines better adapted to the ongoing climate change.

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Low temperatures induce photoinhibition and limit altitudinal distribution of the dioecious plant Pi (1100-050)
Hall 2

Mediterranean plants are well adapted to resist hot and dry summers and mild cold winters characteristic from this climate region. However, some of these plants are highly sensitive to extreme cold temperatures which also limits its altitudinal distribution. Here we aimed at unravelling adaptation mechanisms of the Mediterranean dioecious plant Pistacia lentiscus to winter photoinhibition. Male and female plants of this genera were selected from a natural population and followed for 12 months to evaluate photoinhibition degree and peroxidation damage, as well as chemical photoprotective mechanisms to restore redox balance. Moreover, in order to understand how cold limits their altitudinal distribution, we also selected three natural populations of Pistacia lentiscus at three altitudinal levels (360, 530, 730 m a.s.l) to determine if the degree of photoinhibition could be a crucial factor for its distribution. Our results show higher photoinhibition when temperatures decrease at winter both in males and females with values of maximum efficiency of photosystem II under 0.6 in February, which was the coldest month. This photoinhibition period is coupled to an increase of anthocyanin biosynthesis which counteracts photoinhibitiory damage and allows shoot recovery when temperatures warm up. Besides, the population at the highest altitude where this mastic tree could be found at 730m shows a higher photoinhibition degree than the other two populations. These results indicate that low temperatures are fundamental to determine Pistacia lentiscus distribution and the control of photoinhibitory damage through anthocyanin biosynthesis is essential to overcome cold periods.

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Primary Poster Presenter: Paula Muñoz

Members of Plasma Membrane Intrinsic Proteins Involve in Arsenic and

Boron Transport in Oryza Sativa (1100-025)
Hall 2

Plasma membrane intinsic proteins (PIPs) belong to the subfamily of Aquaporins (AQPs) which are involved in transporting water and small molecules including metalloids such as arsenic (As) and boron (B) in plants. Arsenic is a highly toxic element for humans while boron is required by plants but even a slight change in its concentration causes toxicity or deficiency to the plant. We have studied four rice PIP genes (OsPIP1;3, OsPIP2;4; OsPIP2;6 and OsPIP2;7) for their role in As and B transport in plants. Heterologous expression of these PIPs in Xenopus oocytes and yeast showed As and B transport. Transgenic Arabidopsis overexpressing these four OsPIPs showed strong tolerance to AsIII and B, without any significant accumulation in the plant as compared to wildtype controls. Further, to understand their in-planta functions, we knocked down the expression OsPIP1;3 and OsPIP2;6 genes using RNAi. The RNAi lines showed enhanced tolerance to AsIII and a significant reduction in As accumulation in root and shoot tissues. Collectively, these preliminary data indicated a prominent role of the selected PIP genes in As transport and tolerance/sensitivity in rice. Further experiments for As and B influx and efflux assays as well as total As accumulation in mature seeds are in progress. Additionally, we are also exploring the soil amendment assays for reducing the As uptake and accumulation in rice using various sulfur compounds. Rice grown in soil amended with sulfur compounds showed strong tolerance to As and reduced total As accumulation in root and shoot tissues. In order to understand the role of sulfur compounds for ameliorating As toxicity at molecular levels, we plan to use the RNA-Seq approach to identify the differentially regulated genes and gene networks in rice, which will be helpful to develop strategies for further reducing As uptake and accumulation in rice grains.

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Multiple temperature inputs are combined in vernalization (1100-124 (Screen 8))
Hall 2

Plants use environmental signals such as temperature and light to align their developmental transitions with the seasons. One example of such a transition is flowering, the timing of which is very important for ensuring survival and reproductive success. Understanding how temperature is sensed by plants is a complicated but necessary task, particularly in view of warming future climates.In winter annual Arabidopsis thaliana, the gene FLOWERING LOCUS C (FLC) is a key floral repressor that stores the memory of winter, allowing flowering in spring. This is achieved by the epigenetic silencing of FLC by Polycomb Repressive Complex 2 in combination with the cold-induced VERNALIZATION INSENITIVE3 (VIN3). FLC silencing has previously been studied extensively in controlled-temperature

conditions. In this work we have studied FLC and VIN3 in natural field conditions using a combination of mathematical modelling and experimental methods. We set up field experiments in three sites in Northern Europe and assayed expression throughout autumn and winter. We developed a predictive mathematical model which we first fitted to FLC and VIN3 RNA data from the lab and the field, and then validated in a second field experiment. We found that plants respond to the daily extreme temperatures rather than just the average and utilise multiple temperature inputs, each of which is sensing at a different temperature range and timescale. Finally, we used the model to predict the response of vernalization to higher or more variable temperatures and found that the FLC shutdown will be altered, especially in regions with currently mild winters.

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Negative regulation of age-related developmental leaf senescence by the IAOx pathway, PEN1 and PEN3 (1100-029)
Hall 2

Exosomes are extracellular vesicles formed in response to pathogen infection and herbivory. Exosomes contain RNA and protein, and two abundant components of exosomes are the PEN1 syntaxin and the PEN3 ABC transporter. PEN3 transports multiple substrates, including indole glucosinolates (IG). A role for exosomes in the safe transport of bioactive IG catabolites to the apoplast has been proposed. We show early age-related leaf senescence in pen1/pen3 double mutants. In addition, double cyp79b2/cyp793 mutants, defective in the conversion of tryptophan to IAOx, the first step in IG biosynthesis, also display early age-related leaf senescence. pen1/pen3 lines have increased IG amounts while decreased amounts (1%) are found in cyp79b2/cyp79b3 lines, yet both show early senescence. We propose that exosomes can form in cyp79b2/cyp79b3 mutants, but the IG levels in these apoplastic vesicles are minimal. The pen1/pen3 mutants cannot form exosomes, and thus the high internal IG levels cannot incorporate into exosomes. The cyp79b2/cyp79b3 mutants also block camalexin synthesis, and thus camalexin could be important in senescence. However, pad3 and cyp71A12/cyp71A13 mutants, both defective in camalexin synthesis, do not senesce early. PEN2 encodes an IG myrosinase important for pathogen defense, but pen2 mutants do not show early senescence, indicating that different IG myrosinases are important for leaf senescence. The addition of the salicylic acid (SA) biosynthesis sid2 mutation to the pen1/pen3 mutant partially restores normal leaf senescence demonstrating that early leaf senescence in pen1/pen3 is partly dependent on SA. Mutations in other steps of the IG biosynthetic pathway do not show early senescence, likely a result of genetic redundancy of tandemly-duplicated gene family members. Overall, our genetic data support the hypothesis that exosomes

harboring IG-related molecules play an attenuating role, slowing down the rate of age-related developmental leaf senescence.

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Primary Poster Presenter: Judy Brusslan

Novel patterns of small heat shock protein transcript and protein levels in Arabidopsis (1100-037)
Hall 2

Plants respond to heat stress by synthesizing a diverse set of heat shock proteins which facilitate survival of high temperatures. We are most interested in the small HSPs (sHSPs), which are important ATP-independent molecular chaperones. Here we describe unusual patterns of transcript and protein levels of AtHSP17.6 (cytosolic class I, AT1G5340) and AtHSP22.0 (ER-localized, AT4G10250) in liquid grown Arabidopsis seedlings following severe, nearly-lethal heat shock treatments. When subjected to 39C heat stress treatments, where the temperature is raised gradually, and then held at 39C for 2 hr, both HSP transcript and protein levels accumulated to a maximum level by the end of the heat treatment period. While both HSP22.0 and HSP17.6 proteins persisted many hours, with half lives of approximately 24 hrs, their respective transcripts were nearly absent within 4 hrs of hs cessation. The response of seedlings exposed to sudden, 2 hr, 39C heat shocks was markedly different. First, as has been observed previously, both HSP transcript and protein levels immediately following heat shock were markedly lower compared to heat-stressed seedlings. Unexpectedly, even though sHSP mRNAs had decreased substantially, both sHSPs accumulated to high levels between 12-24 hr following the cessation of heat stress. In the case of HSP22.0, which is endomembrane-localized, the transcript decayed at a lower rate and the protein accumulated to higher relative levels than those observed for HSP17.6. We are studying the expression of HSP22.0 under these 39C heat shock conditions in greater detail. We are investigating the mechanisms by which shocked seedlings might preferentially stabilize HSP22.0 mRNA, and by which HSP22.0 mRNA is preferentially translated long after the cessation of heat-treatment.

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Primary Poster Presenter: Kenneth Helm

Overexpression of MfSAMS1 Improves Chilling Tolerance in Transgenic

Stylo (Stylosanthes guianensis) (1100-126 (Screen 13))
Hall 2

Stylo [Stylosanthes guianensis (Aublet) Sw.] is an important forage legume and cover crop in tropical and southern subtropical regions. Low temperature in winter is the major factor limiting its growth and survival in subtropical regions. S-adenosylmethionine synthetase (SAMS) is the key enzyme catalyzing the formation of S-adenosylmethionine (SAM), a precursor of polyamines synthesis. To improve chilling tolerance in stylo, MfSAMS1 from Medicago falcata was overexpressed in transgenic stylo plants. DNA blot hybridization and quantitative reverse transcription PCR analysis showed that MfSAMS1 was inserted into the genomes of transgenic plants and expressed. MfSAMS1 expression resulted in enhanced levels of SAM and spermidine (Spd) with increased chilling tolerance in transgenic plants. Polyamine oxidase activity and H2O2 levels were increased in transgenic plants as compared with the wild type. In addition, higher activities of superoxide dismutase (SOD) and catalase (CAT) were observed in transgenic plants as compared with the wild type. The results suggest that overexpression of MfSAMS1 promoted polyamine synthesis and oxidation, which in turn improved H2O2-induced antioxidant protection and protected transgenic plants against chilling stress.

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Overexpression of Multiple Genes in Arabidopsis to Improve Heat and Drought Tolerance (1100-021)
Hall 2

Soil salinity, drought, and heat are the major environmental factors that limit crop production in many places of the world including West Texas. To meet the increasing global demand for food and fiber, it is important to come up with novel technologies that are capable of improving crop production. Genetic engineering is a major approach that can generate plants capable of withstanding severe abiotic stresses, while improving the quality of the product. Heat stress is one of the major abiotic stresses which restricts plant growth and productivity. The plant photosynthetic rate declines at moderately high temperatures especially in plants like Arabidopsis thaliana, which results in a considerable loss in productivity. As the reduced photosynthetic activity can be attributed to the low thermostability of Rubisco Activase (RCA), to overcome this problem we have obtained thermostable RCA from a naturally heat tolerant plant type. SIZ1 is one of the SUMO E3 ligases present in the Sumoylation pathway, which is a post-translational regulatory process largely involved in abiotic stress responses. It has already been confirmed that the single gene overexpressing plants for both of these genes significantly improve heat and drought tolerance. Therefore, we hypothesize that co-overexpression of these two genes would lead to a higher heat and drought

tolerance in transgenic plants than single gene overexpressing plants. Thus, we are now introducing the RCA-SIZ1 gene construct into Arabidopsis to make the plants significantly more tolerant to heat and drought. Most recent findings of this project will be presented at the ASPB Annual Meeting.

Co-author(s): Hong Zhang

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PROTEIN L-ISOASPARTYL METHYLTRANSFERASES (PIMTs) play an important role in abiotic stress tolerance (1100-027)
Hall 2

PROTEIN L-ISOASPARTYL METHYLTRANSFERASE (PIMT: EC 2.1.1.77) is a protein repairing enzyme which repairs isoAsp mediated protein damage in organisms. However, the precise role of PIMT in plant stress tolerance is still elusive. Herein we report that stress induced PIMT activity due to transcriptional induction of PIMT coding genes (PIMT1 and PIMT2) is essential to restrict stress induced isoAsp accumulation in plants. Utilizing overexpression and knockdown approaches in Arabidopsis thaliana, we found positive correlation of PIMT activity to restrict isoAsp accumulation and tolerance to heat and oxidative stress in both seedlings and mature plants. The damage caused by induced stresses was assayed in terms of malondialdehyde content, chlorophyll estimation and H2O2 accumulation. Subsequent analysis on these transgenic lines suggests that under stress conditions, PIMT plays an important role in repairing isoAsp in proteins for efficient functioning of antioxidative enzymes like catalase and superoxide dismutase. Further biochemical studies revealed that antioxidative enzymes are indeed susceptible for isoAsp modification which leads to disruption of their functions under stressful conditions. PIMT restricts such isoAsp mediated damage and maintain catalytic proficiency of these enzymes. Taken together, our study strongly suggests that PIMT plays an essential role in protecting stress induced isoAsp mediated protein damage for efficient functioning for various proteins required for stress tolerance.

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Primary Poster Presenter: Shraboni Ghosh

Regulation of growth and stress tolerance of valuable crops by termination of ERECTA signaling (1100-018)
Hall 2

Abiotic stresses are serious threats to the sustainability of crop yields accounting for more losses in crop productivity. Use of modern molecular biology tools for engineering plants with enhanced stress tolerance is a major challenge in Plant Biology. Our study focusses on creating stress tolerant crops without yield penalty by the termination of ERECTA signaling, which is a major plant signaling pathway.

ERECTA family genes encode leucine-rich repeat receptor-like kinases that control major aspects of plant development such as elongation of aboveground organs, leaf initiation, development of flowers, and epidermis differentiation. Manipulation of these ERECTA family genes by expressing AtΔKinase gene associated with truncated ERECTA protein from Arabidopsis thaliana reduced plant size with enhanced stress tolerance in tomato (Villagarcia et al., 2012). Previous studies documented that Arabidopsis thaliana ERECTA family genes have sequence homology with various crops including soybean and rice (Villagarcia et al.,2012). In order to elucidate the importance of ERECTA signaling for development and stress response of food crops, we have disrupted ERECTA signaling by expression of truncated ERECTA protein from Arabidopsis (AtΔKinase) in soybean. The transgenic soybean plants were smaller and produced more branches with a reduced number of leaves and total leaf area compared to soybean wild-type. The transgenic AtΔKinase plants also exhibited increased tolerance to water deficit stress due to the reduction of total leaf area and more modest transpiration compared to wild-type plants. Since these results demonstrated the significance of ERECTA family genes for development and stress responses in soybean, it can be suggested that genetic manipulations with ERECTA signaling can be used for regulation of size and abiotic stress response of other commercially important crops including rice.

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Primary Poster Presenter: Sudha Shanmugam

Role of a Selaginella lepidophylla bHLH Transcription Factor under Water-deficit Stress Condition (1100-008)
Hall 2

The desiccation tolerant plant, Selaginella lepidophylla, can endure water loss down to 4% relative water content (RWC). Their desiccation tolerance ability allows them to be "resurrected" from extremely dry conditions in their natural habitats. However, the underlying regulatory basis of the resurrection trait is essentially unknown. Several candidate transcription factors (TFs) have been identified as regulators of the desiccation tolerance response from transcriptomics analysis of S. lepidophylla tissues containing just 4% RWC. One such transcription factor is a basic helix-loop-helix (bHLH) type that was highly expressed at 4% RWC compared to 100% RWC samples. The function of this TF is unknown, but the bHLH transcription factor family is known for their involvement in a variety of physiological processes including the regulation of plant responses to various abiotic stresses. To begin to understand this TF's regulatory role, we characterized the growth responses of T1 transgenic Arabidopsis seedlings to water-deficit stress via agar-based polyethylene glycol infused nutrient plate drought assays. Preliminary results indicated that T1 seedlings overexpressing SlbHLH have enhanced water-deficit stress tolerance. The results suggest that the SlbHLH TF might function in

water-deficit stress response without negatively affecting growth in a C3 plant. Specifically, transgenic T1 seedlings show increased plant height, rosette size, number of branches, and number of siliques compared to wild type. We discuss how a transcription factor-based approach using novel regulatory genes from resurrection plants might enhance crop plant drought tolerance. Such an approach might provide a potential transformative and facile means to ensure world food security by addressing one major challenge that we are facing today, sustainable food production under increasing frequencies and intensities of drought.

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Root hair phenotypes of nutrient uptake efficiency in early root development (1100-012)
Hall 2

Nutrient depletion in soils adversely affects crop yield. With soil nutrient deficits beingestimated at an average rate of 18.7 N, 5.1 P, and 38.8 K (kg ha -1 yr -1) globally, it poses apotential threat to food security. Having no respite from an increasing population, the worldneeds high performing crops adapted to nutrient deficiencies. Root hairs are responsible for~40% of the nutrient uptake by a plant. Most studies report changes in length and density after2-4 weeks of growth under deficiencies. In contrast, we observed distinct phenotypes of roothairs for N, P stress and control already at 3-5 days after germination. We observed them underan inverted light microscope for seedlings germinated in hydroponics with a stress and non-stress nutrient solution respectively. Our lead hypothesis is that nutrient uptake efficiency isalready characterized by early root hair phenotypes. To study the functions linked to the newlyobserved phenotypes, we develop a computational pipeline that characterizes length, densityand shape from the microscopy images. Our goal is to enable breeders to select for betterstress adapted bean varieties within a few days of germination.

Primary Poster Presenter: Ankita Roy

SENSITIVE to FREEZING 2 activation among multiple species through freezing, proton pump inhibition a (1100-048)
Hall 2

In order to survive freezing, plants use the protein SENSITIVE TO FREEZING (SFR2) on the outside of the chloroplast and alter the membrane by creating ogliogalactoplipids. SFR2 is a protein that is present in nearly all plants, but it is not always activated. When SFR2 is activated it produces Trigalactosyldiacylglycerol (TGDG), which is how the activation of SFR2 is measured through Thin Layer Chromatography analysis. SFR2 can be activated by a myriad of stressors: heat

stress, drought, salt toxicity, and freezing. Not every plant activates SFR2 at the same strength; plants have a maximum activation temperature at which maximum TGDG is produced. Sorghum and pea were compared to the activation in arabidopsis and the amount of TGDG produced was much lower in those plants while the temperature is much lower. During freezing, when SFR2 is active, the cytosol of a cell is acidified but the mechanism behind the acidification is still unknown. In order to better understand the how and where the protons are moving from into the cytosol the proton pumps on the plasma membrane and vacuole are inhibited chemically in the cells of Arabidopsis.

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Primary Poster Presenter: Samantha Surber

Stress memory in herbaceous plants: influence of repeated freezing events on plant productivity (1100-017)
Hall 2

Although damage caused by recurring stress events can be cumulative, following the initial event, the severity of damage for subsequent events can be reduced due to the phenomenon of stress memory. Prior exposure to freezing, for instance, can increase the subsequent freezing tolerance of plants. However, investigations of plant freezing stress memory have been primarily limited to woody species. The existence of freezing memory may have important consequences for herbaceous species in northern temperate regions, because declining snow cover may expose them to an increased frequency and intensity of freezing events. We investigated the effects of repeated freezing events on plant productivity over multiple growing seasons in five herbaceous species: Bromus inermis, Lolium perenne, Festuca rubra, Plantago lanceolata and Poa pratensis. We exposed the plants to various combinations of freezing in the early and late spring (2017), the fall (2017) and the following spring (2018). Control plants were frozen only once during the spring of 2018. Bromus inermis that experienced every freeze and the plants frozen in both the early and late spring had higher biomass than the controls. Similarly, Poa pratensis frozen in both the early and late spring had higher biomass than control plants. There were no differences in aboveground biomass between the control and the various treatments in Festuca rubra, but the plants frozen in early spring and fall had lower root biomass. Lolium perenne that experienced every freeze had lower root biomass compared to controls, and fall freezing had no effects on subsequent freezing tolerance for Plantago lanceolata. Our results reveal that for some herbaceous species, freezing stress memory can be retained over winter, such that it benefits plants that are exposed to spring freezing.

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Primary Poster Presenter: Ricky Kong

Structure-function relationships of different epidermal cell type cuticles in the maize leaf (1100-032)
Hall 2

The cuticle is the outer physical barrier of plants, establishing an important interaction interface with the environment. This hydrophobic layer consists of the lipid polymer cutin embedded with and covered by different waxes, providing protection against environmental stresses like desiccation, UV radiation, and pathogen attack. Thickness, structure, and chemical composition of the cuticle vary widely among different plant species, and even within a species, depend on organ identity, developmental stage, and growth conditions. Our project aims to identify the relationship between cuticle ultrastructure, composition and function of different epidermal cell types of the adult maize leaf. Here we present bulliform cell cuticles as an example of a specialized cuticle likely contributing to the cells' specific function. Bulliform cells are organized in longitudinal rows only on the adaxial side of most grass leaves, and are implicated to play a role in the leaf rolling response upon drought and heat stress. We were able to show increased shrinkage of bulliform cells during dehydration of the leaf, independently confirmed by analysis of mutants with increased bulliform cell content. Ultrastructural data showed distinct alterations in cuticular organization dependent on the cell type, which we related to differences in cuticle composition, analyzed by GC-MS, to identify crucial components of cuticular function in these epidermal cell types.

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Primary Poster Presenter: Susanne Matschi

Testing the heat tolerance of maize unfolded protein response mutants in the Enviratron (1100-043)
Hall 2

bZIP60 is one of the major transcription factors associated with the unfolded protein response (UPR) in plants. We are studying its role in conferring tolerance to heat stress. We subjected maize plants to diurnal temperature cycles in controlled environment conditions and asked whether the UPR is activated under these conditions, and if so, whether the UPR protects plants from elevated temperatures. We conducted our experiments in the Enviratron, a facility with an array of environmentally controlled growth chambers to test plant performance in different environments. Plant performance was monitored by a robot, that operates like a self-driving car, armed with sensors and working 24/7, traveling from chamber to chamber to record images and measuring physiological parameters. In our experiment, we subjected plants to four different temperature regimes in which the plants were grown with daytime temperatures reaching a maximum of 31°C, 33°C, 35°C or 37°C. We compared the performance of a Mu transposon insertion line

called bzip60-2 to the parent line, W22 and found that the mutation confers sensitivity to elevated daytime temperature of 37°C. We also took leaf samples to assess gene expression patterns via RNAseq analysis. A substantial number of differentially expressed genes (DEGs) were identified in the comparison between temperatures and genotypes. One of the findings was that heat shock genes were turned on at the higher daytime temperatures (35°C and 37°C) even through the temperatures were ramped up slowly through the virtual morning hours. Several, but not all of canonical UPR genes were upregulated in the parent line, W22, by the elevated temperatures. Some of the genes downregulated in the bzip60-2 mutant at elevated temperature include those involved in stress signaling, vesicle transport and hormone response. They may explain the greater sensitivity of the mutant to elevated temperatures.

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Primary Poster Presenter: Stephen Howell

The diversification of the Hsf gene family in Brassicas (1100-044)
Hall 2

Plant development is affected by abiotic stresses such as heat, cold, drought, and salinity. Plants being sessile have evolved adaptation strategies to cope with stresses. Timely and efficient response to abiotic stress is a key to plant survival, crop productivity, and yield loss. Hsf genes constitute an important family of transcription factors which play critical roles and act as central regulators in plant responses to different stresses. In comparison to animals, plants have a very high number of Hsfs. The largest Hsf gene family known till date is in Brassica napus. B. napus is an allopolyploid formed as a result of the natural hybridisation of genomes of B. rapa and B. oleracea approximately 7500 years ago. Gene family expansion can occur as a result of small scale and global duplication events. In this study, a comparative analysis of Hsf gene family in B. oleracea, B. rapa and B. napus highlighted the role of hybridisation and allopolyploidy in the evolution of the largest known Hsf gene family in B. napus. The presence of orthologous gene clusters, found in Brassica species, but not in A. thaliana, suggested that polyploidisation has resulted in the formation of new Brassica-specific orthologous gene clusters. Gene duplication analysis indicated that the evolution of Hsf gene family was under strong purifying selection in these Brassica species. High-level synteny was observed within B. napus genome. Conservation of physical location, the similarity of structure and similar expression profiles between the B. napus Hsf genes and the corresponding genes from B. oleracea and B. rapa suggests a high functional similarity between these genes. This study paves the way for further investigation of Hsf genes which play roles in various stresses and plant development.

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The Role of Myosin XI in the Gravitropic Response of Maize (1100-034)
Hall 2

Research of gravitropism within plant systems has yielded insight into its significance relating to efficient nutrient uptake and photosynthesis, but little is known about the specific molecular mechanisms behind this phenomenon in shoots. Statoliths, the starch granules responsible for gravity sensing in statocytes, sediment in the direction of the gravity vector. Given that myosin XI is thought to facilitate the movement of organelles within cells, this motor protein may thus have a significant role in proper gravity sensing. We found that maize mutant opaque1 (o1), which lacks myosin XI, exhibited a slightly altered gravitropic response. The rate of curvature in the shoot of opaque1 when subject to gravistimulation was less than that of wild type. This trend was found to be correlated with length of coleoptiles since longer coleoptiles curved away from the gravity vector to a greater degree than did shorter coleoptiles. Morphologically, there was little difference between the statoliths of opaque1 and wildtype. Even though opaque1 was found to localize around vasculature containing the starch sheath in shoots, we were unable to determine whether the delayed curvature of opaque1 was due to differences in shoot length or a direct consequence of opaque1 on the gravitropic response.

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Primary Poster Presenter: Anastasiya Andriyash

The Unfolded Protein Response and translational regulation in plants: Life without PERKs (1100-047)
Hall 2

Plants actively respond to a variety of abiotic stresses and to the administration of chemical ER stress agents by eliciting the Unfolded Protein Response (UPR). These stresses upregulate the expression of the canonical UPR genes and induce autophagy. Plants have two arms of the UPR signaling pathway, one arm involving the membrane-associated transcription factors, bZIP17 and bZIP28, and the other arm involving IRE1 and splicing of the mRNA encoding bZIP60. However, no homolog of PERK or any component of the PERK downstream signaling pathway has been reported in plants. So, the question has been raised whether the UPR controls global translation as it does in mammalian cells. In the model plant Arabidopsis, it has been demonstrated that an isoform of eIF2a is phosphorylated in a GCN2-dependent way, but it has not been shown that the phosphorylation attenuates translation. We used polyribosome profiling and ribosome profiling (ribo-seq) in maize to address the question whether translation is slowed by the UPR, and we found though polyribosome profiling and Sunset assays that global translation is only modestly downregulated, if at all, by the UPR. Although global translation is

not slowed, we observed through ribosome profiling that there is a decline in the efficiency of translation on a per RNA basis during the upsurge in expression of the canonical UPR genes. We interpret this to mean that not all of the canonical UPR gene transcripts are loaded onto polyribosomes in response to ER stress. We will describe a likely fate and subcellular localization of these RNA transcripts.

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The XBAT family of RING-type ubiquitin ligases and plant response to environmental stress (1100-015)
Hall 2

The ubiquitination pathway involves the attachment of ubiquitin, a small, highly conserved protein to select substrates. The attachment of a chain of ubiquitin molecules targets the modified protein to the multi-proteolytic 26S proteasome complex for degradation. At the center of the pathway is a large and diverse family of substrate recruiting ubiquitin ligases (E3s). The Arabidopsis genome is encodes for ~500 RING-type E3s, many of which are known to regulate abiotic stress signalling. Of interest are the seven XBAT (XB3 ortholog in Arabidopsis thaliana) E3s, each of which has a distinct role including regulating ethylene biosynthesis, abscisic acid (ABA) signalling, cell death and pathogen defense. I will discuss our recent findings for two members, XBAT31 and XBAT35, both of which are alternatively spliced to produce two isoforms. XBAT31.1, but not XBAT31.2, is involved in regulating iron deficiency response, increasing root iron uptake when availability is low. Overexpression of XBAT35.2, but not XBAT35.1, is known to induce cell death and reduce susceptibility to bacterial pathogens. The regulatory role of XBAT35.2 is linked to its ability to promote the proteasome-dependent degradation of Accelerated Cell Death 11 (ACD11) in the presence of pathogen. Also, XBAT35.2 promotes its own turnover and pathogen infection leads to stabilization of the E3. Interestingly, we have recently uncovered a role for XBAT35.1 and XBAT35.2 in abiotic stress tolerance. Expression of both isoforms increase in response to ABA and high salinity stress. However, the xbat35 mutant is more tolerant of salt stress, suggesting that, in contrast to its role in pathogen defence, the E3 is a negative regulator of abiotic stress response. We are continuing to examine the dual, but conflicting, roles of XBAT35, and the function of XBAT31 in stress tolerance by identifying substrates and determining how these enzymes are regulated to affect growth under suboptimal conditions.

Primary Poster Presenter: Sophia Stone

Towards doubling crop yield for dryland agricultural production system

(1100-024)
Hall 2

Abiotic stresses such as drought and salt cause significant crop losses annually. Climate change will likely make agricultural production more challenging, especially for countries that are already in lack of fresh water for irrigation. To feed the growing population in the world, we must increase food production by at least 50% to 70% in the next 20 to 30 years. Unfortunately, no more arable lands are available for growing crops in most countries today, and therefore we must produce more food with less land, less water, and less fertilizers. It was previously shown that overexpression of the Arabidopsis vacuolar pyrophosphatase gene AVP1 and overexpression of the rice SUMO E3 ligase gene OsSIZ1 could significantly increase tolerance to drought/salt and drought/heat stresses in transgenic Arabidopsis and creeping bentgrass, respectively. We hypothesized that there might be synergistic effects between AVP1-overexpression and OsSIZ1-overexpression, which could lead to even higher seed yields if these two genes are co-overexpressed. Indeed, we found that AVP1/OsSIZ1 co-overexpressing Arabidopsis plants were significantly more tolerant to stresses that came alone or in any combinations, and AVP1/OsSIZ1 co-overexpressing Arabidopsis plants produced more than 100% more seeds than wild-type plants under multiple stress conditions. We then introduced these two genes into cotton, and we found that OsSIZ1/AVP1 co-overexpressing cotton plants performed significantly better than AVP1-overexpressing, OsSIZ1-overexpressing, and wild-type cotton plants under single stress and multiple stress conditions in laboratory, as well as in the dryland agricultural production system of West Texas. Our research suggests that co-overexpression of AVP1 and OsSIZ1 is a viable strategy for engineering abiotic stress tolerant crops and substantially improving crop yields in low input and marginal soil conditions, providing a solution for food security in arid and semiarid regions of the world.

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Two interacting ERFs act downstream of EIN3 to regulate heat stress response in Arabidopsis (1100-006)
Hall 2

APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) transcription factors are integral components of environmental stress signaling cascades that regulate the expression of a wide variety of downstream genes related to stress response and development of plants. However, the role and the regulatory mechanism of AP2/ERF genes in heat stress response are not well understood. Here, we uncovered the role

of ethylene signaling and ethylene response factors (ERFs) in plant basal thermotolerance. We found that ethylene signaling mutants ein2-5 and ein3eil1 had decreased basal thermotolerance, whereas plants with constitutive ethylene response e.g. ctr1-1 mutants and EIN3 overexpressing plants had enhanced basal thermotolerance. Overexpression of ERF95, a direct downstream target of EIN3, and ERF97 also increased the tolerance of plants to heat stress. We further revealed that ERF95 interacted with ERF97 and that EIN3 physically bound to the promoter of ERF97 as well. Overexpression of ERF95 or ERF97 in ein3eil1 could rescue the phenotype of the mutants, further demonstrating that ERF95 and ERF97 were genetically downstream of EIN3. On the other hand, an erf95erf96erf97erf98 quadruple mutant (erfq) had decreased basal thermotolerance. Transcriptome analysis indicated that ERF95 and ERF97 similarly regulated many downstream genes including heat-induced HSFs, HSPs, and plant defensin genes. Thus, our study reveals that ethylene positively regulates basal thermotolerance and that the EIN3-ERFs transcriptional complex is a crucial module in heat stress response, thereby establishing a connection between ethylene and its downstream regulation in heat stress response of plants.

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Primary Poster Presenter: Jianyan Huang

Understanding the Role of Polyamines in Rice under Drought and Salt Stress (1100-028)
Hall 2

Abiotic stresses are important constraints on crop yield. Paddy-grown rice is particularly susceptible to drought and salt stress, which have negative effects on carbon and nitrogen intake that limit plant growth and grain yield. Polyamines (PAs), mainly putrescine (Put), spermidine (Spd), and spermine (Spm), are important molecules in plant metabolism and have been implicated in abiotic stress responses, both as protectors of plants from stress and preparing the plant for tolerance of stress. This has led to genetic manipulation of PA metabolism aimed at improving drought and salt tolerance in rice and other crops. Prior to overexpressing PA biosynthetic genes, we have profiled the response of a commercial rice variety to drought and salt stress. We found that PAs may be involved in recovery from stress, but levels during stress appear to fluctuate widely. To minimize sampling errors, we also studied differences in PA contents among different parts of the long, morphologically heterogeneous rice leaf. The results show that under drought, Put is increased in the sheath and decreased in the lamina as compared to the control, suggesting that the plant prioritizes protection of the meristematic tissues by compatible solute accumulation. There were no differences in Spd or Spm under drought. Furthermore, PA levels were higher under moderate salt stress than severe stress. In all cases, the PA levels were

significantly higher in the leaf blade than the sheath. This research will ultimately increase scientific understanding of abiotic stress tolerance for plant improvement.

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Primary Poster Presenter: Anna Haber

Genetic study of cold tolerance during germination and early seedling growth in rice (1100-123 (Screen 2))
Hall 2

Rice germination and productivity is constrained by low temperatures. The optimum temperature for rice germination is 30C, and temperatures lower than 30C are detrimental for germination and uniform plant stand. To evaluate the genetic basis of cold tolerance in rice, we conducted a genome wide association study of 283 rice accessions using an Illumina 7K SNP array developed at Cornell University. The experiment was conducted in a growth chamber under controlled conditions with no light under normal (30C) and low (13C) temperatures. Low temperature germinability, germination index, coleoptile growth under cold temperature exposure, plumule length after 4 days recovery at 30C from 13C and plumule growth rate after cold germination were measured. We identified 34 significant SNP markers associated with low temperature tolerance in the full set of 283 accessions, and 38 significant SNP markers across the subset of japonica accessions. Out of 38 GWAS sites identified in the japonica accessions, 8 sites were found to be colocalized with previously reported known genes for cold tolerance at germination/seedling stage. A few highly significant novel GWAS loci were identified in this study. Additionally, RNA-Seq transcriptomic data were generated from two contrasting phenotypes that may aid in the identification of potential candidates genes contributing to the traits. From this study, highly tolerant accessions and SNP markers associated with cold tolerance were identified, which can help lead to a better understanding of the genetic and molecular mechanisms of cold tolerance during germination and early seedling stage in rice.

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Primary E-Poster Presenter: Endang Septiningsih

Salt-responsive gene regulatory network (GRN) conserved in land plants (1100-127 (Screen 5))
Hall 2

High salinity limits plant growth through early-occurring osmotic stress and slowly-occurring ion cytotoxicity. Higher plants have various molecular mechanisms to cope with high salinity. However, little is known about when and how these

mechanisms were acquired during the evolution of life. To address the question, we set out to compare the structures of GRN in primitive (Marchantia polymorpha, Mp) and higher plants (Arabidopsis thaliana, At). First, we collected transcriptome data at different time points during salt treatment in both At and Mp, and classified salt-responsive genes into 10 groups based on their expression patterns over the time course. We further investigated transcription factors (TFs) enriched in each group in At and Mp to identify regulators exhibiting similar dynamic changes in both species. These transcriptome data, processed and visualized with co-expression network and Bayesian network methods, revealed GRNs mediating the early and late salt responses and hub TFs in the GRNs. Our predictions were then validated by phenotyping the knockout mutants and overexpression lines in At and Mp. Interestingly, although At and Mp have similar GRN structures, our analyses uncovered that predicted cis-regulatory elements (pCREs) are more divergent in the two species. Using machine learning with those pCREs as predictors, we have identified several species-shared and species-specific regulatory elements. This study provides new insights into the evolution, similarity and divergence of salt responsive mechanism in plants.

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Machine Vision HT Phenotyping Reveals Modulation of Early Maize Seedling Performance under Cold (1100-128 (Screen 3))
Hall 2

Emergence under cold temperatures is an important trait and therefore developing new, reliable, high throughput phenotyping tools is necessary to assist plant breeders. Seedling emergence is an important factor for yield, particularly under challenging planting conditions. In the US corn belt, maize is planted in early spring, as soon as soil temperatures are permissive to germination. At that time, temperatures often drop below normal, which can delay or even kill the seedling. Seed pre-treatments have been shown to improve germination in cold conditions in crops such as rice and cabbage, but are largely unpublished in maize. To assess the effects of pre-treatments on early maize cold tolerance, twenty-seven inbred parents of maize Nested Association Mapping (NAM) population were primed using a synthetic solid matrix and then tested for cold tolerance using a soil-based emergence assay. Primed kernels were incubated at 10°C for 5 days, and then transferred to 24°C for emergence. DSLR cameras were used to capture images every 30 min to obtain emergence profiles of each seedling. Emergence time was determined from the time-lapsed images and multiple measures including final emergence percentage, time to 50% emergence, and emergence rate were extracted for each genotype. The cold treatment reduced total emergence of

several genotypes. However, priming pre-treatment protected the sensitive genotypes allowing nearly full emergence. We also used single-kernel near infrared reflectance spectroscopy to determine seed density, weight, oil, protein, and starch for the kernels prior to planting. By combining kernel characteristics and emergence time, we found small, but highly significant correlations between the kernel and early seedling performance. Results show that our machine vision based HT phenotyping can be used in plant research and breeding applications.

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Morpho-agronomic characterization and generation of GA20-oxidase mutant tef lines using CRISPR/Cas (1100-129 (Screen 12))
Hall 2

Tef [Eragrostis tef (Zucc.) Trotter] is the most important grain crop of Ethiopia, where it serves as major staple food for more than two-thirds of Africa's second most populous nation. Until recently, cultivation of tef as human food grain was highly restricted to Ethiopia, but the crop is now produced in other parts of the world including the United States. Currently, the crop is gaining wider popularity across the globe due to its desirable nutritional and health benefits. Lodging, a permanent collapse of the culm from an upright position, is the number one yield-limiting factor despite efforts to develop lodging-resistant varieties since the late 1950s. Grain yield loss due to lodging is estimated to range from 15-45% depending upon the variety and the agro-climate. Phenotypic and agronomic traits variability of 367 accessions of the USDA E. tef collection was assessed under greenhouse conditions at the Nevada Agricultural Experiment Station. Hierarchical agglomerative clustering analysis, based on 10 agronomic traits, identified five distinct clusters. Clustering of accessions into groups will facilitate selection of parental lines and generation of mapping and backcross populations for subsequent genome-wide association studies. Principal component analysis showed that the contribution of plant height to overall trait variability was minimal. Naturally existing variability of culm height among the accessions is very low indicating that direct genetic manipulation of the trait is essential. Genome-editing investigation was conducted to generate semi-dwarf E. tef lines with reduced susceptibility to lodging by reducing the amount of bioactive GAs synthesized in the cells. CRISPR/Cas9 construct targeting GA20-oxidase and a DELLA protein encoding gene were designed and cloned.

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Abiotic: Light

Brassinosteroids inhibit flavonoid biosynthesis to coordinate growth and UV-B stress tolerance in pl (1100-130 (Screen 9))
Hall 2

UV-B light (Ultraviolet-B) is known to act as a potential stress. How could plant coordinate growth and UV-B tolerance is not well understood. We previously showed that UVR8 physically interacts with BIM1 and the functional dephosphorylated BES1 to inhibit their DNA-binding activities and transcription of growth-related genes, thus to inhibit Brassinosteroids (BRs)-promoted growth. Whether BR signaling is involved in UV-B stress responses remains unclear. We report here that BRs signaling inhibits flavonoid biosynthesis to coordinate growth and UV-B stress tolerance in Arabidopsis and crops. BRs inhibit UV-B stress tolerance via controlling flavonol biosynthesis. BES1, the master transcription factor of BR signaling, represses the expression of three PFG MYBs genes MYB11, MYB12, and MYB111, which control the biosynthesis of flavonol. BES1 directly binds to the promoters of these three MYBs in response to BR to repress their expression and flavonol accumulation. On the other hand, broad band UV-B, mainly the low-wavelength high-energetic UV-B light, down-regulates the transcriptional expression of BES1 to reduce its protein level, to promote flavonol accumulation. These results demonstrate that BR-activated BES1 inhibits flavonoid biosynthesis to promote growth, while UV-B stress suppresses the expression of BES1 to turn on defense, so plants could timely and efficiently switch from growth to UV-B stress defense.

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Phytochrome A plays a role in the regulation of carbon flux in dark-grown tomato seedlings (1100-131 (Screen 5))
Hall 2

The phytochrome (phy) gene family consists of multiple genes encoding photoreceptors with which plants perceive environmental information used to guide developmental decisions, such as germination, photomorphogenesis and flowering. In tomato phytochromes are encoded by five genes: PHYA, B1, B2, E, and F. As a general rule, red light activates phytochromes, while far-red light deactivates them, however, phyA is responsive to both wavelengths. PhyA is involved in early developmental processes during the seedling’s transition from growing in the dark to growth in light. The role of phytochromes during the dark growth phase, also known as skotomorphogenesis, has been the subject of considerably fewer research studies than those investigating their role in the light. We performed transcriptomic profiling and co-expression network analysis of tomato seedlings during the transition from dark to light growth. Our data suggest that phyA plays a role in the

regulation of enzymes involved in carbon flux through glycolysis, beta-oxidation, and the Krebs cycle. Our analysis also showed that phyA is involved in the regulation of several sucrose transporter SWEET genes before the plant is exposed to light. This coincided with slightly longer hypocotyls of dark grown phyA mutants compared to WT seedlings grown in the dark, and increased root growth in the mutants soon after the seedling reached light. Intriguingly, these data suggest that phyA might play a role in carbon distribution in the dark, possibly in anticipation of light growth.

Primary E-Poster Presenter: Andreas Madlung

SUPRESSOR OF PHYTOCROME A-105 (SPA1) functions as a bona fide serine/threonine kinase in Arabidopsis (1100-132 (Screen 7))
Hall 2

COP1/SPAs (SPA1-4) is an ubiquitin E3 ligase complex that plays dominant role in plant light signaling cascades by targeting multiple factors such as HY5 for ubiquitin mediated degradation. SPA1 was first identified as a suppressor of a weak phytochrome A mutant, phyA-105. Further analyses have shown that SPAs (SPA1-4) form stable protein complexes with the RING finger E3 ligase COP1 in plants. Genetic and biochemical evidences suggested that SPAs are promoting COP1 function in Arabidopsis. Despite numerous studies on SPA1 and its family members, however, it remained unknown what is the molecular function of SPA proteins in plants. With extensive biochemical and genetic analysis, we show that SPA1 act as a bona fide serine/threonine protein kinase in Arabidopsis. We also show that the kinase activity is essential for SPA1 function in the dark as well as in the early seedling de-etiolation process.

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Primary E-Poster Presenter: Inyup Paik

Network analysis reveals new connections between shade avoidance and defense signaling (1100-133 (Screen 4))
Hall 2

Plants have sophisticated mechanisms for sensing neighbor shade and responding through enhanced elongation and physiological changes to maximize their ability to compete for light. The shade avoidance response affects many different organs and growth stages, yet the signaling pathways underlying this response have mostly been studied in seedlings. To understand the signaling pathways operating in older plants, we analyzed a gene expression time course of adult shade avoidance in wild-type and shade avoidance mutants. With this data we established a signaling

cascade of hormone action during wild-type response to shade and used mutants to determine how genetic perturbation affects the cascade. We found pervasive misregulation of salicylic acid genes in many mutants, suggesting salicylic acid signaling to be an important shade avoidance growth regulator. Supporting our hypothesis, several salicylic acid pathway mutants reduced shade-induced and basal growth. The effect of these mutants on shade avoidance was specific to petiole elongation; neither hypocotyl nor flowering time responses were altered, thereby defining important stage-specific differences in the downstream shade avoidance signaling pathway. Shade treatment did not change salicylic acid levels, indicating salicylic acid mediation of shade avoidance is not dependent on modulation of salicylic acid levels.

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Primary E-Poster Presenter: Julin Maloof

Tandem Cysteines convert Arabidopsis phytochromes into violet sensors and confer dominant negative e (1100-134 (Screen 4))
Hall 2

Plant phytochromes (phys) are a small group of red/far-red light photoreceptors that regulate virtually every developmental stage throughout the plant life cycle. Linear tetrapyrrole (bilin) covalently bound by a single Cysteine residue within the GAF domain of the N-terminal photosensory module of phys renders phys photoconvertibility between biologically active far-red absorbing Pfr and inactive red absorbing Pr conformations. Here we report that mutating the highly conserved Histidine residue flanking of the bilin-binding Cysteine into a secondary Cysteine abolishes phy red/far-red absorbing capability but retains violet absorbance. PhyB harboring such an HC mutation (HCB) cannot complement phyABCDE quintuple null mutant under light condition of various wavelengths. Instead, HCB confers significant dominant negative effects in the wild-type plants, regardless if it is overexpressed or expressed at a level comparable to that of endogenous phyB. In the light conditions, HCB is exclusively retained in the cytosol and resists to light-induced protein degradation. Similarly, two known phyB alleles defective in nuclear import were tested to also exert dominant negative effects over the wild-type phyB. PhyA harboring the same HC mutation (HCA) has a marginal dominant negative effect on the wild-type phyA. At the molecular level, However, HCA expressed in the phyA mutant is completely resistant to red and far-red light-induced protein turnover. We conclude that cytosolic non-Pfr phys, as exemplified by HC alleles, interfere with the function of photoactive phys by heterodimerizing with and blocking them from importing into the nucleus.

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Primary E-Poster Presenter: Wei Hu

A glutamyl-tRNA synthetase interacts with FIN219 to regulate light- and hormone-mediated responses (1100-062)
Hall 2

Integration of light and phytohormones is critical for plant growth and development. Especially, the interaction between light and jasmonate signaling in regulating the balance of plant growth and defense responses is attractive and remains to be elucidated. Here, we report that a FIN219/JAR1-Interacting Protein 2 (FIP2) gene, encoding glutamyl-tRNA synthetase (GluRS), was identified by yeast 2-hybrid screen. Its mutant fip2-1 with reduced levels of FIP2 showed a hyposensitive long-hypocotyl and overexpression line (FIP2OE) led to a hypersensitive short-hypocotyl phenotype under far-red light conditions, suggesting that FIP2 may act as a positive regulator in photomorphogenesis. Further studies revealed that fip2-1 and FIP2OE, respectively, showed longer and shorter hypocotyl phenotypes than Col-0 in MeJA- and ABA-mediated inhibition of hypocotyl elongation under high FR light. Co-immunoprecipitation assays confirmed that FIN219 interacted with FIP2 via its C-terminal domain. Intriguingly, FIP2OE resulted in great accumulation of anthocyanin under FR light with or without methyl JA treatment, but fip2 mutant showed less anthocyanin as compared to Col-0. Besides, fip2 mutant resulted in a reduced number and size of chloroplasts and an increased accumulation of starch granules in mesophyll tissues under white light conditions. Surprisingly, the stomatal aperture in the leaf epidermis of FIP2OE was widely open under white light and MeJA as well as ABA treatments as compared to Col-0 under the same conditions. Taken together, these data indicate that FIP2 interacting with FIN219/JAR1 may play vital roles in response to light, jasmonates, and ABA to regulate various aspects of seedling development.

Co-author(s): Chin-Wen Chang

Primary Poster Presenter: Hsu-Liang Hsieh

Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light (1100-053)
Hall 2

The plant canopy functions as an aerial array of light-harvesting antennas. To achieve maximal yield, each leaf within this array and the array as a whole need to rapidly adjust to naturally occurring fluctuations in light intensity and quality. Excessive light stress triggers the closing of pores in leaves called stomata to minimize moisture loss. We found that different leaves within the canopy of an Arabidopsis thaliana plant, including leaves not directly exposed to light, coordinated stomatal closure in response to light stress by sending and receiving

rapid systemic signals. This response required the plant hormones abscisic acid and jasmonic acid and was mediated by a rapid autopropagating wave of reactive oxygen species (ROS) production. Furthermore, this response depended on the function of genes encoding the ROS-generating NADPH oxidase RBOHD and various stomatal regulators, such as the anion channel SLAC1, GHR1 (guard cell hydrogen peroxide resistant 1), and lipoxygenase 1 (LOX1). Our findings reveal that plants function as highly dynamic and coordinated organisms, optimizing the overall response of their canopies to fluctuating light intensities.

Primary Poster Presenter: Amith R Devireddy

Genome-Wide Alternative Splicing Pattern in Rice from Dark to Light Transition (1100-059)
Hall 2

Light is essential for the autotroph plants and is one of the most important factors that regulates development, physiology and cellular processes like metabolism, gene expression, cellular transport. Light induces reprogramming of gene expression and impacts alternative splicing of mRNAs. To explore the transcriptome modulation and alternative splicing patterns of rice genes operating in dark and light conditions, we carried out transcriptome analyses of rice seedlings grown in the continuous dark for 8 days after germination followed by 48hrs of continuous exposure to light. Assembled transcripts of dark and light samples cover ~80% of O. sativa japonica genome. A total of 14,766 (38%) genes were differentially expressed with majority upregulated in the light. Genes from 2-C-methyl-d-erythritol-4-phosphate (MEP) pathway were turned on in light and turned off in dark condition, whereas genes from mevalonate (MVA) pathway were turned on in the dark and off in the light. Of the total expressed genes >21% of the genes undergo light-regulated splicing. These genes are associated to circadian rhythm, nitrogen assimilation, MVA and MEP pathways. Intron retention (84.9%) was the most predominant alternative splicing event. This study will provide insight about the portion of the genome regulated in response to light at the level of alternative splicing by the different pathways and regulatory transcriptional network as well as help improve the rice genome annotation.

Primary Poster Presenter: Parul Gupta

Increased seed yield of pea (Pisum sativum L.) in response to inoculation with photoactivated Rhizob (1100-055)
Hall 2

Light-activation of a LOV-histidine kinase in cells of Rhizobium leguminosarum increases the number of nodules and the number of intranodular bacteroids on pea (Pisum sativumL.) roots grown in hydroponics systems (Bonomi et al., Proc. Natl. Acad. Sci. 109: 12135, 2012). We have investigated whether a similar response might be demonstrated under normal greenhouse conditions with pea plants grown in soil to determine whether the finding might ultimately prove beneficial in

agriculture. We have also extended the experiments to measure the effects both of light treatment of the bacteria and timing of inoculation on final bean yield. Pre-irradiation of cells of Rhizobium leguminosarum with blue light induces an increase in the number of functional nodules (those containing leghemoglobin) and ultimately seed yield both when the inoculation takes place with the onset of imbibition and four days after the onset when primary roots have emerged. However, inoculation four days after the onset of imbibition and in the presence of primary roots greatly increases both the number of functional nodules and seed yield compared to inoculation at the start of imbibition with or without light treatment of the bacteria. We have measured several growth and developmental parameters, including numbers of flowers per plant per week, pod weight and numbers of peas per pod. Our findings show photoactivated bacterium suppresses floral abortion, and significantly increases pea yield. We are currently carrying out field tests to determine whether light treatment and the timing of inoculation might lead to increased yield in an agricultural context.

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Isolation and Functional Characterization of Phytochromes from Ceratopteris richardii Brongn. (1100-066)
Hall 2

Plants rely on a diverse set of photoreceptors that sense the ambient light conditions and regulate numerous photomorphogenic pathways. Among the most heavily studied of these pigments are the ubiquitous red/far-red-absorbing phytochromes, yet the molecular mechanisms of phytochrome signaling and action are poorly understood in groups other than the angiosperms. Members of the CrPHY family of genes, encoding at least six phytochrome apoproteins from the genetic model fern Ceratopteris richardii, have been isolated and sequenced. The coding regions of several of these genes have been cloned into Gateway entry vectors and recombined into a variety of compatible destination vectors for subsequent particle bombardment of sporophyte callus tissue. The status of stable C. richardii transformants exhibiting genomic integration of constructs will be discussed, and results of light-dependent nuclear translocation of phytochrome-reporter fusion proteins will be presented. Constitutive ectopic overexpressing lines and RNAi knockdowns will be examined for photomorphogenic phenotypes in vivo and those findings compared with results obtained for transient expression studies performed previously. The efficacy of this system as a platform for understanding the mechanisms of signal transduction, interactions among photoreceptors, and the evolutionary history of these receptors will also be considered.

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Nucleus-to-plastid light signaling mechanism for initiating chloroplast biogenesis (1100-056)
Hall 2

Chloroplast biogenesis is initiated by principally the red and far-red photoreceptors, the phytochromes through the light-dependent activation of photosynthesis-associated genes encoded by both the nuclear and plastidial genomes, but how photoreceptors control plastidial gene expression remains enigmatic. Here we show that the photoactivation of phytochromes triggers the expression of photosynthesis-associated plastid-encoded genes (PhAPGs) by stimulating the assembly of the bacterial-type plastidial RNA polymerase (PEP) into a 1000-kDa complex. Using forward genetic approaches, we identified RCB (Regulator of Chloroplast Biogenesis) as a dual-targeted nuclear/plastidial phytochrome signaling component required for PEP assembly. Surprisingly, RCB controls PhAPG expression primarily from the nucleus by interacting with phytochromes and promoting their localization to photobodies for the degradation of the transcriptional regulators PIF1 and PIF3. RCB-dependent PIF degradation in the nucleus triggers the plastids for initiating PEP assembly and PhAPG expression. Thus, our findings reveal the framework of a nucleus-to-plastid phytochrome signaling mechanism linking photobody biogenesis to the regulation of chloroplast transcription.

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PCH1 and PCHL regulate light responses by interacting with PIF1 and mediate its degradation (1100-064)
Hall 2

Phytochrome B (phyB) is the primary red light photoreceptor and regulates growth and development of plants in response to ambient light environment. The level of active form of phyB is determined by the light conditions, such as direct sunlight or shade, but is also affected by a light-independent relaxation process, called thermal reversion, where phyB reverts from active to inactive state. In our previous study, we have demonstrated that two phyB-interacting proteins PCH1 and PCHL suppress phyB thermal reversion, resulting in plants with dramatically enhanced light sensitivity. To further elucidate the mechanism underlying PCH1 and PCHL's

function, we examined their light-related phenotypes and found that PCH1 and PCHL positively regulate many light responses including seed germination, negative gravitropism, and chlorophyll biosynthesis. Our quantitative real-time PCR results also showed that PCH1 and PCHL positively regulate many light-responsive gene expression and also the expression of the genes involved in the early stages of chlorophyll biosynthesis. These results prompt us to examine whether PHYTOCHROME INTERACTING FACTOR1 (PIF1) level is regulated by both PCH1 and PCHL. Our results showed that PCH1 and PCHL promote the degradation of PIF1 and negatively regulate the expressions of PIF1 target genes in response to light. By performing a series of interaction assays, we demonstrate that PCH1 and PCHL interact with PIF1 both in the dark and in the light, and the interaction is phyB-independent. However, PCH1 and PCHL facilitate the interaction between phyB and PIF1. Our study revealed a novel mechanism of PCH1 and PCHL regulating light responses by interacting with PIF1 and mediating its degradation.

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Phytochrome B's Impact on Resource Allocation in Brassica rapa (1100-063)
Hall 2

Conservative estimates suggest that food production must increase by 60-70% by 2050 to keep pace with population growth. This challenge to global food security is compounded by a lack of knowledge of how crop species will respond to a changing climate. Phytochrome B (phyB) has been shown to direct resource allocation in response to environmental stimuli such as elevated CO2. This study has focused on this phenomenon in Brassica rapa, a member of the mustard family that includes a large variety of popular vegetables such as broccoli, cauliflower, Brussels sprouts, as well as varieties used for oil production. B. rapa plants with mutations in the phyB gene make fewer seeds, have lower chloroplast density, reduced photosynthetic rate and decreased total chlorophyll levels. We are investigating which genes are regulated by phyB in leaves experiencing induced senescence or recovery. We hope that this will ultimately lead to the identification of targets for engineering more resource-efficient and higher-yielding varieties.

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Relevance of short wavelength UV-B radiation in natural light environments (1100-067)
Hall 2

Laboratory studies have shown that etiolated seedlings and light grown plants exhibit specific responses to UVB radiation which includes significant energy at wavelengths shorter than 300 nm (SW-UVB). We measured responses of 3 greenhouse-grown Texas native grasses to supplementary UVB (1.6 -2 kJ m-2day-1). A UVB source with output below 300 nm was used for one treatment; a second treatment used the same source filtered by cellulose acetate to attenuate wavelengths shorter than 300 nm. Plants were irradiated for 7 days. We took methanol extracts of leaves and measured UV absorption spectra of extracted pigments. We also measured leaf reflectance at 300 nm. All 3 species responded differently to the 2 UVB treatments. We placed similar plants on field sites in South Texas for 7-14 days under cellulose acetate or UVB-transparent Aclar. Solar spectra determined that UV radiation at 295 nm or shorter was present at these field sites. We measured absorption from methanol extracted pigments and leaf reflectance as above. Responses were not the same between greenhouse and field studies, but SW-UVB had effects on both. For example, under greenhouse conditions, Sideoats grama (Bouteloua curtipendula) showed a 60% reduction in UV absorbance with both UVB treatments and a 25% increase in reflectance ONLY in response to SW-UVB. Under field conditions the 2 UVB treatments exhibited different UV absorption spectra, but leaves showed no difference in leaf reflectance. Other species showed different combinations of changes in UV absorbing pigments and leaf reflectance in response to UV radiation. Despite this variability all species exhibited at least one response specific to SW-UVB under natural light conditions.

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Splicing variants constitute novel regulators of photomorphogenic development in Arabidopsis (1100-057)
Hall 2

Light regulates gene expression regulation at all levels of central dogma during photomorphogenesis, including alternative splicing (AS). However, the accurate determination of full-length splicing variants was greatly hampered by the short-read nature of commonly used RNA-seq technologies. To combat this limitation, we adopted PacBio isoform sequencing (Iso-seq) that offers advantages in long-read sequencing for the identification of full-length AS variants. Normalized cDNA libraries prepared from 4-d-old etiolated seedling with or without 4-h white light treatment were used for Iso-seq to achieve comprehensive and effective

identification of full-length AS variants. Our analyses revealed greater than 30,000 splicing variant models from ~16,000 gene loci and identified ~700 previously unannotated transcripts. Among them, 14,644 transcripts represented new gene models, and one-third of the loci producing AS variants contain two or more splicing events. Intron retention (IR) is most frequently observed, and some IR-containing AS variants show evidence of engagement in translation. Through tackling the biological functions of the splicing isoforms, our study showed the formation of heterodimers of transcription factors in their annotated and IR-containing AS variants. Moreover, transgenic plants overexpressing the IR-forms of two BBX family members exhibited light hypersensitive phenotypes, suggesting the regulatory roles of these IR isoforms in modulating optimal light responses during photomorphogenic development. Our results provide a new approach for identifying de novo synthesized AS variants that impose regulatory functions in de-etiolating Arabidopsis.

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The N-terminal end of the LRBs is important for E3 ligase assembly and Phytochrome regulation. (1100-065)
Hall 2

Plant growth and development is delicately tuned to the quality and quantity of light it receives. The phytochrome family of photoreceptors detects light in the Red/Far-Red light spectrum, and controls many aspects of growth and development. The Light Regulating BTBs (LRBs) are Cullin 3 based E3 ligases that have been found to add ubiquitin to the active form of phyB and the related family members (phyC-E) in a red light-dependent fashion. This results in Phy degradation, and represents a key point of regulation of red light responses in plants. We investigated the LRB protein sequence and discovered that the amino terminal end is extremely conserved and shares amino acid sequence similarity to a region in Cullins. This region is in close proximity to the neddylation site on Cullins 1 and 2. In order to determine the functional significance of this region, we introduced an N-terminal truncated-LRB1 protein into the lrb1/lrb2 double mutant. We found that the truncated LRB1 did not complement the lrb1/lrb2 mutant in plant growth and development assays, even though the full Length LRB was able to. We also discovered that this N-terminal region aides in Cul3 binding, which impacts Phy degradation, and is therefore necessary for proper red-light signaling in plants. Our research suggests that the N-terminal amino acids in the LRB proteins play an important role in E3 ligase assembly and ultimately regulation of Phy signaling in Arabidopsis thaliana.

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The Role and Expression of Phytochrome F in Tomato Development (1100-060)
Hall 2

Phytochromes are a type of red and far-red light sensing protein. Tomato (Solanum lycopersicon) has five phytochromes called phyA, phyB1, phyB2, phyE and phyF. Of these, phyE and F are the least studied. This study surveys the role and expression of phyF through three projects. In the first project, phyF expression levels were surveyed in whole seedlings for four genotypes: wild-type, and three new phyF mutant lines. In the second, the same four genotypes were grown for five days, some in continuous darkness and others in continuous red light, and the lengths of their hypocotyls and roots were compared. In the third, the same four genotypes were grown for 9 weeks, and a variety of adult phenotypes were observed, including but not limited to: plant height, leaf length, chlorophyll content, and internode length.

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Utilizing Sunlight More Efficiently with Semi-transparent Organic Photovoltaics: Solar-Powered Inte (1100-054)
Hall 2

Greenhouses have high energy demands for heating, cooling and supplemental lighting but provide higher, year-round plant production capacity and the potential for a more sustainable agriculture. The energy demands of greenhouses could be reduced and met by using organic photovoltaic (OPV) devices to selectively harvest light not utilized by plants for production of electricity. Our OPV devices are semi-transparent filters that allow certain wavelengths of light (red and blue) to pass through the filter while absorbing other wavelengths (green, violet, UV) to generate electricity to power the greenhouse. We tested three different OPV filters that absorb varying ratios of the wavelengths in the photosynthetically active region of the spectrum. The three OPV filters were coated onto glass and used as roofs on model greenhouses designed so that only filtered light reached the plants inside. These model greenhouses were placed in a growth chamber illuminated with a mixture of ceramic metal halide and incandescent lights to approximate the spectrum of natural sunlight. Lactuca sativa var. Red Oakleaf lettuce was chosen as a first test greenhouse crop to grow inside these model greenhouses. The lettuce growth, development and a variety of physiological parameters were measured throughout the experiment to characterize plant performance. Our results suggest that at least one of our OPV filters can be implemented in a full scale solar-powered greenhouse to produce commercially viable crops in a potentially energy neutral system.

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UVR8 mediated spatial differences as a prerequisite for UV-B induced inflorescence phototropism (1100-061)
Hall 2

In Arabidopsis hypocotyls, phototropins are the dominant photoreceptors for the positive phototropism response towards unilateral ultraviolet-B (UV-B) radiation. We report a stark contrast of response mechanism with inflorescence stems with a central role for UV RESISTANCE LOCUS 8 (UVR8). The perception of UV-B occurs mainly in the epidermis and cortex with a lesser contribution of the endodermis. Unilateral UV-B exposure does not lead to a spatial difference in UVR8 protein levels but does cause differential UVR8 signal throughout the stem with at the irradiated side 1) increase of the transcription factor ELONGATED HYPOCOTYL 5 (HY5), 2) an associated strong activation of flavonoid biosynthesis genes and flavonoid accumulation, 3) increased GA2oxidase expression, diminished gibberellin1 levels and accumulation of DELLA protein REPRESSOR OF GA1 (RGA) and, 4) increased expression of the auxin transport regulator, PINOID, contributing to local diminished auxin signalling. Our molecular findings are in support of the Blaauw theory (1919), suggesting that differential growth occurs trough unilateral photomorphogenic growth inhibition. Together the data indicate phototropin independent inflorescence phototropism through multiple locally UVR8-regulated hormone pathways.

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Abiotic: Salt and Minerals

Rice OsDOF15 contributes to ethylene-inhibited primary root elongation under salt stress (1100-135 (Screen 13))
Hall 2

Roots are important plant ground organs, which absorb water and nutrients to control plant growth and development. Adverse soil conditions directly affect root development. Ethylene represents a key regulatory signal for root development and salt response, but the underlying molecular mechanism of how ethylene-inhibited root growth is modulated in environmental changes remains poorly understood. Here, we show that a novel DOF transcription factor OsDOF15 positively regulates primary root elongation by regulating cell proliferation in the root meristem, via restricting ethylene biosynthesis. Loss-of-function of OsDOF15 impaired primary root elongation and cell proliferation in the root meristem, whereas OsDOF15 overexpression enhanced these processes, indicating that OsDOF15 is a key regulator of primary root elongation. This regulation involves the direct interaction of OsDOF15 with the promoter of OsACS1, resulting in the repression of ethylene biosynthesis. The control of ethylene biosynthesis by OsDOF15 in turn regulates cell proliferation in the root meristem. OsDOF15 transcription is repressed by salt stress, and OsDOF15-mediated ethylene biosynthesis plays a role in inhibition of primary root elongation by salt stress. Thus, our data reveal how the ethylene-inhibited primary root elongation is finely controlled by OsDOF15 in response to environmental signal, a novel mechanism of plants perceiving salt stress and transmitting the information to ethylene biosynthesis to restrict root elongation.

Primary E-Poster Presenter: Hua Qin

Salinity tolerance in Australian wild Oryza species: from physiology towards mechanism (1100-136 (Screen 15))
Hall 2

While cultivated rice (Oryza sativa) provides the primary source of nutrition for more than one-third of the world’s population, relatively little use has been made of the vast genetic diversity found in the wild species of Oryza worldwide for resistance to abiotic stresses. Salinity limits rice growth and yield, modern rice cultivars are highly sensitive to salinity, especially during early vegetative and reproductive stages. In an effort to address this problem, we evaluated accessions of O. australiansis and O. meridionalis endemic to the savannah of northern Australia. Plants were assessed at the seedling stage for their growth at sodium chloride concentrations up to 80 mM. Multiple accessions were compared with O. sativa genotypes ranging from salt sensitive (IR29) to tolerant (Pokkali). An initial greenhouse-based screening revealed substantial salt tolerance in some but not all native accessions. To validate this, non-destructive image-based phenotyping was performed at the Plant Accelerator, an Australian national plant phenotyping facility. The combination of our two screening experiments uncovered striking levels of salt

tolerance diversity among the Australian wild rice accessions tested and enabled analysis of their growth responses to a range of salt levels. With the aim of understanding the mechanism underlying this tolerance, we further investigated the tolerant and sensitive accessions through protein mass spectrometry (MS). Extracted proteins were quantified by tandem mass tags and two-stage MS. Over 3000 proteins were quantified, proteins significantly differentially expressed as compared with the control treatment and between accessions. A few transporters were found to be over-expressed in the tolerant line when compared to the sensitive line. The expression of some proteins were validated using RT-qPCR. Our results highlight the potential of exotic germplasm to provide new genetic variation for rice salinity tolerance.

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Protective role of acetate in mitigating copper stress in lentil seedlings: insights into antioxidan (1100-137 (Screen 2))
Hall 2

Roles of organic acids in enhancing plants tolerance against heavy metal toxicity are well known. However, the roles of acetate in reducing heavy metal toxicity have not yet been investigated. In this study, we combined pharmacological, physiological and biochemical approaches to investigate the mechanisms underlying acetate–mediated copper (Cu) toxicity tolerance in lentil (Lens culinaris). Results demonstrated that high dose (3 mM) of Cu reduced seedlings growth and chlorophyll content, while augmenting Cu content, and increasing oxidative damage in lentil plants through disruption of the antioxidant defense pathway. Principle component analysis clearly indicated that Cu accumulation and increased oxidative damage were the key factors for Cu toxicity in lentil seedlings. However, acetate pretreatment reduced Cu accumulation in shoots, increased proline content and improved the responses of antioxidant defense pathway. As a result, excess Cu–induced oxidative damage was reduced, and consequently seedling growth was improved under Cu stress conditions, indicating the role of acetate in alleviating Cu toxicity in lentil seedlings. Pharmacological study revealed that inhibition of acetate–induced overaccumulation of glutathione by buthionine sulfoximine increased oxidative damage in seedlings, demonstrating that acetate enhanced oxidative stress tolerance through glutathione-associated pathway. Taken together, acetate–induced reduction in Cu accumulation in lentil shoots rather than in roots and mitigation of oxidative damage are the major determinants for mitigating Cu toxicity in lentil seedlings. Our findings provide mechanistic insights into the protective roles of acetate in mitigating Cu toxicity in lentil, suggesting that application of acetate could be a novel strategy for the management of heavy metal toxicity and accumulation in crops.

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Salt-induced proline accumulation in Arabidopsis is regulated by light and is under control of ELONG (1100-138 (Screen 14))
Hall 2

Plants are exposed to constantly changing environmental conditions, among which the availability of light and water is crucial in determining growth and development. Proline accumulation is one of the sensitive metabolic responses to such extreme conditions. Proline content is augmented during high salinity or drought in an ABA-dependent and independent way and is regulated by light. We found that red and blue but not far-red light is essential for salt-induced proline accumulation, upregulation of Δ1-PYRROLINE-5-CARBOXYLATE SYNTHASE 1 (P5CS1) and downregulation of PROLINE DEHYDROGENASE 1 (PDH1) genes, which control proline biosynthetic and catabolic pathways, respectively. ChIP and EMSA assays demonstrated that the transcription factor ELONGATED HYPOCOTYL 5 (HY5) binds to G-box and C-box elements of P5CS1 and a C-box motif of PDH1. Salt-induced proline accumulation and P5CS1 expression were reduced in the hy5hyh double mutant, suggesting that HY5 promotes proline biosynthesis through connecting light and stress signals. Our results improve our understanding on interactions between stress and light signals, confirming HY5 as a key regulator in proline metabolism. This work was supported by NKFI Grants K128728, NN118089 and GINOP Project no. 2.3.2-15-2016-00001.

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The Arabidopsis Heat Shock Factor A4A (HSFA4A) is substrate of MAP Kinase 4 and regulates responses (1100-139 (Screen 1))
Hall 2

Extreme environmental conditions often represent combinations of various abiotic stress conditions such as heat with drought or high soil salinity. Heat shock factors are principal regulators of responses to high temperatures and to other stresses such as salinity, water deprivation or heavy metals. Their function in stress

combinations is however not known. The Arabidopsis heat shock factor A4A (HSFA4A) is implicated in salt and oxidative stress tolerance and is a substrate of MAP kinases MPK3 and MPK6. Here we show, that the HSFA4A gene is induced by salt, elevated temperature and combination of these conditions. Fast translocation of HSFA4A-YFP protein from cytosol to nuclei takes place in salt-treated cells. HSFA4A can be phosphorylated not only by MAP kinases MPK3 and MPK6 but also by MPK4 and Ser309 is the dominant MAPK phosphorylation site. In vivo phosphorylation data suggest that HSFA4A can be substrate of other kinases as well. Changing Ser309 to Asp or Ala has altered intramolecular multimerisation. Chromatin immunoprecipitation assays could confirm binding of HSFA4A to promoters of various target genes encoding the small heat shock protein HSP17.6A and transcription factors WRKY30 and ZAT12, key regulators of responses to biotic and oxidative stresses. HSFA4A overexpression enhanced tolerance not only to individual stresses but also to simultaneously applied heat and salt stresses through reduction of oxidative damage. Our results suggest that this heat shock factor is a component of a complex stress regulatory pathway, connecting upstream signals mediated by MAP kinases MPK3/6 and MPK4 with transcription regulation of a set of stress-induced target genes. This research was supported by OTKA NN110962, NKFI NN118089 and GINOP 2.3.2-15-2016-00001 grants.References: Perez-Salamo, et al. (2014). Plant Physiol 165(1): 319-334.Farago, (2018). Front Plant Sci 9: 219.Andrási et al., (2019) J. Exp. Bot. (In press).

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Alteration of plant growth performance under phosphate-limited stress by repressing a phloem-mediate (1100-140 (Screen 13))
Hall 2

Abiotic: Salt and Minerals

Primary E-Poster Presenter: Byung-Kook Ham

Alleviation of Salt-Induced Oxidative Stress of Cucumber (Cucumis sativus L.) by Exogenous Applicati (1100-072)
Hall 2

The present study was conducted to investigate the role of tebuconazole (TEB) and trifloxystrobin (TRI) in modulating reactive oxygen species metabolism and enhancing growth and physiology of cucumber plants (Cucumis sativus L. cv. Tokiwa) under salt stress (60 mM NaCl). Cucumber seedlings were grown semi-hydroponically in the glasshouse and 3-week after transplanting; they were exposed to two different doses of TEB and TRI solely and in combination with NaCl for further 6 days. The application of salt phenotypically deteriorated the cucumber plant growth caused yellowing the whole plant and significantly destructed the contents of chlorophyll and carotenoid. The oxidative damage was created under salt stress by increasing contents of malondialdehyde (MDA), other aldehydes, H2O2, and electrolytic leakage (EL) resulting from the inefficiency of antioxidant defense system. Furthermore, Na+ content increased in the leaf, stem, and root of cucumber seedlings under salt stress, whereas the K+/Na+ ratio and contents of K+, Ca2+, and Mg2+ were decreased. Exogenous application of TEB and TRI decreased the contents of MDA, H2O2, and EL by regulating the activities of antioxidants related enzymes and the contents of ascorbate and glutathione. Therefore, this study indicates that the exogenous application of TEB and TRI improved salt tolerance in cucumber plants by enhancing antioxidant defense systems.

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Carrot DcALFIN4 transcription factors confer abiotic stress tolerance in Arabidopsis thaliana. (1100-070)
Hall 2

The ALFIN-like transcription factors family are involved in several physiological processes such as root development and abiotic stress responses in plants. Carrot (Daucus carota) is middle tolerant to salt stress and the expression of DcPSY2, involved in carotenoid synthesis, is induced under salt treatment. Interestingly, the DcPSY2 promoter has ALFIN response elements. Through in silico analysis we identified two putative genes that encode ALFIN-like transcription factors, DcALFIN4 and DcALFIN7 in the carrot transcriptome, that are induced in carrot under salt stress. These genes encode nuclear proteins that transactivate the transcription of reporter genes in yeast. They also bind to the promoter of the carrot DcPSY2 by means of yeast monohybrid assay. Transgenic T3 A.thaliana homozygous lines that express DcALFIN4 and DcALFIN7 show a higher survival rate respect to control plants after chronic salt and drought stress. Particularly, DcALFIN4 transgenic lines present a better performance in stress treatments which correlates with the expression level of DcALFIN4 and AtPSY and an increment in carotenoids and chlorophylls. These results let us to propose that DcALFIN4 and DcALFIN7 encode

for functional transcription factors and DcALFIN4 provides tolerance to salinity and drought in plants.

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Chrysanthemum CmHSFA4 gene positively regulates salt stress tolerance in transgenic chrysanthemum (1100-071)
Hall 2

Soil salinization is one of the major issues threatening crop productivity worldwide as it hampers plant growth by inducing Na+ toxicity and oxidative stress. HSF transcription factors have involved in varieties of plants stress resistance however little information about HSFs in chrysanthemum are available. Here, we showed that expression of the chrysanthemum CmHSFA4, a homologue of the heat shock factor AtHSFA4a, is inducible by salt, and localizes to the nucleus. It is a transcription activator binding with HSE. Chrysanthemum overexpressing CmHSFA4 displayed enhanced salinity tolerance by limiting Na+ accumulation while maintaining K+ concentration, which is consistent with the up-regulation of ion-transporters CmSOS1 and CmHKT2. Additionally, the transgenic plants reduced H2O2 and O2·- accumulation under salinity, which could be due to up-regulation of ROS-scavenger activities such as SOD, APX and CAT as well as CmHSP70, CmHSP90. Together, these results suggest that CmHSFA4 conferred salinity tolerance in chrysanthemum as a consequence of Na+/K+ ion and ROS homeostasis.

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Epigenetic down-regulation of As(III) importer NIP3;1 by VIM1 in Arabidopsis (1100-076)
Hall 2

To understand a molecular mechanism for enhanced arsenite (AsIII) tolerance in Cyc07-expressing Arabidopsis, several up-regulated genes were identified by the differential display. VIM1, one of these genes, reported to function as an Ub-ligase and DNA methylase, increased As(III) tolerance when over-expressed in yeasts and Arabidopsis. VIM1-expressing Arabidopsis showed higher As(III) tolerance but

reduced accumulation of As(III). In addition, ubiquitinated proteins were decreased while 20S proteasome activity was enhanced in transgenic plants, which might be ascribed to the Ub-ligase activity of VIM1. Among putative As(III) transporters, NIP3;1 (putative AsIII importer) expression was reduced in VIM1-Arabidopsis, suggesting NIP3;1 is responsible for the decreased level of As(III). Since VIM1 was reported to play as DNA methylase, the methylation level was examined in the promoter region of NIP3;1. Interestingly, methylation at 3 CpG of NIP3;1 promoter in transgenic plants was 6-7 fold higher than in control plants, suggesting that NIP3;1 expression is decreased due to enhanced promoter methylation by the function of DNA methylase of VIM1. The knockout Arabidopsis of VIM1 (atvim1) exhibited opposite phenotypes of VIM1-Arabidopsis including lower As(III) tolerance, increased ubiquitinated proteins, reduced 20S proteasome activity, enhanced expression of NIP3;1, and reduced expression of putative As(III) exporter PIP2;2. Furthermore, the expression of putative As(III) exporter At4G08570 was higher in VIM1-Arabidopsis, but lower in atvim1 Arabidopsis. Taken together, it appears that VIM1 enhances As(III) tolerance by reducing As(III) accumulation through dual function of Ub-ligase and DNA Methylase in Arabidopsis.

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FRO3 Plays an Integral Role in Whole Plant Iron Homeostasis in Arabidopsis (1100-075)
Hall 2

Iron deficiency is a major nutritional problem for human populations throughout the developing world and the majority of people acquire iron primarily from plant sources. Additionally, iron bioavailability is a major limiting factor in about 30% of arable croplands worldwide. An improved understanding of iron uptake and homeostasis is necessary to help combat both issues. We are focused on understanding the role of a mitochondrially-localized ferric iron reductase (FRO3) in cellular iron dynamics and whole plant iron homeostasis. While FRO3 is expressed throughout the plant, its expression is greatest in the vasculature. Knockout of FRO3 causes a 50% reduction in mitochondrial iron content and also alters whole plant iron sensing. fro3 lines accumulate 1.2X as much iron as WT plants do, while showing an increased iron deficiency response compared to WT suggesting that while accumulating more total iron, they sense some level of iron deficiency. Furthermore, RNA-seq data suggests that fro3 lines have an altered genomic response to iron deficiency, and sense a greater iron deficiency than WT. These data show that loss of FRO3 disrupts Fe homeostasis and suggest that vascular mitochondrial iron content may play an important role in whole plant iron homeostasis.

Co-author(s): Erin CONNOLLY,
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Primary Poster Presenter: Brendon Juengst

Functional analysis of rice transcription factor OsERF106 under salinity stress (1100-068)
Hall 2

Rice Ethylene Responsive Factors (OsERFs) belong to a large gene family and involve in different plant growth, development and stress responses. Analysis of public microarray data and qPCR indicated that the expression of OsERF106 gene was up-regulated under salinity stress. However, the function of OsERF106 under salt stress is still unknown. We showed that OsERF106-overexpressing transgenic rice displayed retarded growth with a high level of reactive oxygen species and significantly decreased antioxidant enzyme activity, especially under salt stress. Moreover, the OsERF106-overexpressing rice line increased the expression level of ion transporter OsSOS1 and decreased OsHKT1; 4 gene expression, resulted in higher Na+ and K+ accumulation in rice shoots. The transcriptome analysis showed that OsERF106 down-regulated the gene expression levels of OsHKT1;4 and stress-related transcription factors OsbZIP16, OsWRKY42, OsWRKY45, while the gene expression of potassium transporters OsTPKb, OsHAK12 and chloride transporter OsCCC1 were up-regulated. Taken together, OsERF is highly suspected as a negative regulator of rice in response to salt stress. OsERF106 may change the oxidative stress state and Na+/K+ homeostasis to affect rice growth and salt stress tolerance.

Co-author(s): Hung-Chi Chen,
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Primary Poster Presenter: Men-Chi Chang

Identification of salt tolerant genes in rice by weighted-co expression analysis (1100-077)
Hall 2

Plant tolerance to salinity stress is a complex trait, which includes multiple gene networks and molecular pathways. The chromosome segment substitution line (CSSLs) of 'Khao Dawk Mali 105 (KDML105)' rice, containing various regions between RM1003 and RM3362 markers on chromosome 1 of doubled haploid line, was used in this research in order to identify the putative salt tolerant gene in rice. The CSSL16 rice showed salt tolerant phenotype under salt stress in seedling and vegetative stages, leading to the best productivity under salt stress condition. To identify the putative salt tolerant genes via transcriptomic approach, RNA-seq was used to investigate the transcriptomes under abiotic stress condition. The transcriptomes of CSSL16 treated with salt stress at seedling and booting stages were subjected to weighted co-expression network. A total of 57 genes showed the increase in connecting edges under salt stress, when compared to the data from the normal grown plants. Only 4 of them contain SNPs between CSSL16 and 'KDML105' rice and are located between RM1003 and RM3362 markers. The gene expression analysis revealed the consistent patterns of gene expression of BTBZ1,

ERD and LOC_Os01g64870. These genes have the high potential to contribute to salt-tolerant phenotype of CSSL16.

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Primary Poster Presenter: Supachitra Chadchawan

1:30 PM - 3:00 PM

Improving Arabidopsis salinity tolerance through cerium oxide nanoparticle scavenging of ROS and enh

Salinity is a widespread environmental stress that severely limits crop yield worldwide. Here, we demonstrate that applying designed cerium oxide nanoparticles (nanoceria) can augment plant ROS scavenging ability, modulate the activities of K+ efflux channels, improve K+ retention in leaf mesophyll cells, and thus enhance salinity stress tolerance in Arabidopsis thaliana (Col-0). Briefly, we found that the designed negatively charged poly acrylic acid coated nanoceria (PNC; hydrodynamic size, 10 nm) with low surface Ce3+/Ce4+ ratio have the unique capability of catalytically reducing levels of stress-induced reactive oxygen species (ROS) including hydroxyl radicals ($^{\cdot}OH$) that lack enzymatic scavenging pathways. Compared with plants without nanoparticles, plants embedded with these PNC exhibit an increase in photosynthetic performance such as quantum yield of photosystem II, carbon assimilation rates, and Rubisco carboxylation rates, and the biomass under abiotic stresses e.g. salinity, high light, heat, and chilling. Also, catalytic $^{\cdot}OH$ scavenging by PNC in Arabidopsis leaves showed about three-fold lower NaCl-induced mesophyll K+ efflux compared to control leaves upon exposure to salinity stress, indicating a significant improvement in mesophyll K+ retention, a key trait associated with salinity stress tolerance. Moreover, the ROS-activated plasma membrane nonselective cation channels (ROS-NSCC) were identified as the main $^{\cdot}OH$-inducible K+ efflux channels which are tuned by PNC. Our study demonstrates a plant nanobionics approach of augmenting plant ROS scavenging by PNC for understanding and improving plant tolerance against abiotic stresses.

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Primary Poster Presenter: Honghong Wu

Increasing Zn content, starch synthesis, and root growth by manipulating

phloem transport of Zn (1100-073)
Hall 2

Iron (Fe) and zinc (Zn) deficiencies are common worldwide, and cause millions of deaths annually. Soil fertilization or overexpression of metal transporters on the root has been the major efforts used to increase the content of these beneficial elements in edible portions of crops. To increase the uptake of metals from apoplast/xylem to phloem, transgenic potato plants were generated with AtIRT1, a Fe/Zn transporter. The construt was driven by the promoter of a phloem specific gene, AtSUC2. Transgenic potato plant was accumulated higher level of transcripts of AtIRT gene, the zinc content and starch content in potato tubers were much higher than those in wild type plants. Our results also indicated an increase sugar level from shoot to root in transgenic plant, which showed higher photosynthetic rate, and also higher sucrose content in leaf, phloem, root and tuber. We proposed that the increased carbon transport might be due to more active sink activity caused by more abundant supply of Zn in potato.

Co-author(s): Cankui Zhang,
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Primary Poster Presenter: Lin Chen

Iron treatment on sweet and non-sweet sorghums (1100-080)
Hall 2

Sorghum [Sorghum bicolor (L.) Moench] is an important multi-purpose crop that can be grown for grain, biofuel, and also sugar. Previous work identified a possible link between mutations in an iron transport gene and differences in sugar accumulation between sweet and grain sorghum. To further study the potential relationship between iron and sugar, we quantified phenotypic differentiation among sorghum genotypes under different iron treatments. Eleven genotypes, including four sweet sorghums, six non-sweet sorghums, and one wild S. bicolor subsp. verticilliflorum were grown hydroponically under three experimental conditions: control, no iron and excess iron. In the no-iron condition, young leaves started showing symptoms of iron chlorosis as early as 10 days after planting in three of the six non-sweet sorghum lines and in only one sweet line. After 13 days with no iron, the other three non-sweet sorghums and one other sweet genotype exhibited chlorosis in young leaves. The final two sweet sorghums did not show symptoms until day 14. There were also a significantly lower percentage of total chlorotic leaves in the sweet sorghums compared to the non-sweet sorghums. Plant height in the no-iron condition was significantly lower in 9 out of 11 genotypes when compared to plants grown in control or excess iron conditions, but was not significantly higher in excess iron conditions compared to the control. These results suggest that while iron deficiency universally restricts plant growth in all sorghum varieties, photosynthetic ability is more severely impacted in non-sweet sorghums. The slightly enhanced resistance to iron deficiency observed during early plant

development in certain sweet sorghums supports the hypothesis that increased iron transport could lead to higher sugar accumulation in these varieties.

Co-author(s): Elizabeth Cooper

Primary Poster Presenter: Kittikun Songsomboon

Over-expression of an R2R3 MYB Gene, GhMYB73, Increases Tolerance to Salt Stress in Arabidopsis (1100-069)
Hall 2

MYB family genes act as important regulators modulating the response to abiotic stress in plants. However, much less is known about MYB proteins in cotton. Here, we found that a cotton MYB gene, GhMYB73, was induced by NaCl and abscisic acid (ABA). Silencing GhMYB73 expression in cotton increased sensitivity to salt stress. The cotyledon greening rate of Arabidopsis thaliana over-expressing GhMYB73 under NaCl or mannitol treatment was significantly enhanced during the seedling germination stage. What's more, several osmotic stress-induced genes, such as AtNHX1, AtSOS3 and AtP5CS1, were more highly expressed in the over-expression lines than in wild type under salt treatment, supporting the hypothesis that GhMYB73 contributes to salinity tolerance by improving osmotic stress resistance. Arabidopsis lines over-expressing GhMYB73 had superior germination and cotyledon greening under ABA treatment, and some abiotic stress-induced genes involved in ABA pathways (AtPYL8, AtABF3, AtRD29B and AtABI5), had increased transcription levels under salt-stress conditions in these lines. Furthermore, we found that GhMYB73 physically interacts with GhPYL8 and AtPYL8, suggesting that GhMYB73 regulates ABA signaling during salinity stress response. Taken together, over-expression of GhMYB73 significantly increases tolerance to salt and ABA stress, indicating that it can potentially be used transgenic technology approaches to improve cotton salt tolerance.

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Primary Poster Presenter: Xiaoyang Ge

Phospholipase Dα1 mediates response to high magnesium stress in Arabidopsis (1100-081)
Hall 2

For plants, magnesium is essential macronutrient. As a cofactor of many vital enzymes, it is required for number of fundamental cellular processes such as energy metabolism and photosynthesis. Intracellular level of Mg2+ is tightly regulated and its deficiency or excess affects normal growth and development. Although there is a relatively good understanding of the physiological mechanisms of magnesium deficiency, much less is known about cellular responses to high magnesium condition.Phospholipase D cleaves ordinary phospholipids such as phosphatidylcholine releasing phosphatidic acid (PA) and free head group. The PA can then serve as signaling molecule. In Arabidopsis, there are 12 members of PLD

family. Several PLDs were reported to be involved in response to high salinity stress.Recently, we identified T-DNA loss-of-function phospholipase Dα1 mutant pldα1 that is hypersensitive to high magnesium condition. In that condition, pldα1 has shorter roots and lower fresh weight in comparison with wild type (wt). Expression of PLDα1 did not differ in magnesium treated plants but PLDα activity increased after Mg2+ treatment. The interaction between Mg2+, Ca2+ and K+ homeostasis was described. Therefore, we determined amount of these elements in magnesium treated pldα1. Interestingly, in 17-day-old seedlings grown for 10 days on 10 mM MgCl2 the magnesium and potassium content was significantly lower in pldα1 than in wt plants. The amount of Ca2+ did not differ. Finally, we found out, that expression of the CBL-interacting protein kinase 9 (CIPK9) which is involved in magnesium homeostasis and the high affinity K+ transporter 5 (HAK5) which is involved in potassium homeostasis are impaired in pldα1 under high Mg2+ condition.Based on these results it is possible to conclude that PLDα1 mediates response to high magnesium stress in Arabidopsis.This study was funded by the Czech Science Foundation (grant No. 17-00522S).

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Physiological and Biochemical Responses in Shrub Willow Genotypes to Nutrient Strength (1100-084)
Hall 2

Second to carbon, nitrogenis an essential element for plant growth and development and for the synthesis of secondary cell walls. As the need for alternative energy sources has risen, understanding how bioenergy crops uptake and metabolize excess nitrogen is necessary to achieve the maximum biomass attainable. In this study, we examined the effects of varying nitrogen treatments on both biomass accumulation and nitrogen containing metabolites (amino acids and polyamines) in shrub willow (Salix spp.) to evaluate their effectiveness as both bioenergy crops and agricultural buffers. Shrub willow cultivars were grown under differing nitrogen treatments and the harvested leaf tissue was used for analysis via high performance liquid chromatography. A particular shrub willow genotype, a native S. sericea, exhibited the greatest response in growth to increased nitrogen, when compared to other genotypes. Growth data and changes in metabolite profiles of amino acids and polyamines will be presented. The data will be used in selection of genotypes most suited for use as agricultural buffers, as well as what direction to take for genetic improvement.

Primary Poster Presenter: Michelle Serapiglia

POPEYE spatial dynamics play an essential role in the iron deprivation

response (1100-078)
Hall 2

Iron deficiency (-Fe) in plants causes physiological and developmental modifications that alter nutrient uptake, translocation, storage and metabolism. POPEYE (PYE) is a -Fe responsive transcription factor (TF) that positively regulates iron deficiency response by directly regulating genes essential for iron translocation, storage and redox status, as well as a host of other processes. PYE also form heterodimers with homologous proteins, bHLH104, ILR3 and bHLH 115, which also positively regulate -Fe response. Under -Fe conditions, mRNA encoded by PYE accumulates in the root pericycle, while the protein is localized to the nucleus in all root cell types, suggesting that PYE is a mobile protein. In this study we used scanning Fluorescence Correlation Spectroscopy coupled with pair correlation function to analyze PYE movement across cell types. We also characterized the physiological, transcriptional and ionomic effects of PYE mislocalization across various cells types within the root and found that PYE non-cell autonomously regulates physiological responses to iron deficiency. Our findings also indicate that PYE transcript and protein stability are affected by Fe availability, suggesting that PYE is post-transcriptionally regulated by Fe. Thus, the intercellular movement and tissue-specific localization of PYE is essential for its function and plays a vital role in metal acquisition.

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Primary Poster Presenter: Terri Long

Proteomic Analysis Reveals Proteins Involved in Seed Imbibition under Salt Stress in Rice (1100-141 (Screen 9))
Hall 2

Enhancement of salinity tolerance during seed germination is very important for direct seeding in rice. In this study, the salt-tolerant japonica landrace Jiucaiqing was used to determine the regulators that are involved in seed imbibition under salt stress. Briefly, the comparative proteomic analysis was conducted between dry (0 h) and imbibed (24 h) seeds with 150 mM NaCl. Under salt stress, the uptake of water increased rapidly before 24 h imbibition (Phase I), followed by a plateau of seed imbibition from 24 to 96 h imbibition (Phase II). We identified 14 proteins involved in seed imbibition, in which the majority of these proteins were involved in energy supply and storage protein. The early imbibition process was mediated by protein catabolism; the most of proteins were down-regulated after 24 h imbibition. Eleven genes in salt stress treated seeds were expressed early during the seed imbibition in comparison to control seeds. By comparison, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (BPM), glutelin (GLU2.2 and GLU2.3), glucose-1-phosphate adenylyltransferase large subunit (GAS8), and cupin domain

containing protein (CDP3.1 and CDP3.2) were near the regions of quantitative trait loci (QTLs) for seed dormancy, seed reserve utilization, and seed germination in Jiucaiqing. In particular, CDP3.1 was co-located in the region of qIR-3 for imbibition rate, and qGP-3 for germination percentage. The role of CDP3.1 was verified in enhancing seed germination under salt stress using T-DNA mutant. The identified proteins might be applicable for the improvement of seed germination under salt stress in rice.

Co-author(s): Enshun Xu

Primary E-Poster Presenter: Hui He

Pumping Iron: nutrient mobilization in legumes (Glycine max and Phaseolus vulgaris) (1100-074)
Hall 2

Legumes are the most important crops for humans. They provide protein, fiber and macro and micronutrients. Some models have been created in order to explain how different nutrients are assimilated and mobilized through the plant under different circumstances but we are still lacking a complete more detailed picture of those molecular mechanisms involved in the soil to seed journey. Here, we share some of our findings in two of our ongoing projects in two close-related legumes. First, we are using COMMON BEAN as a model to study iron homeostasis due to its high natural variation and because genetically is less complex than other legumes. By screening a mini core collection, we found the seed composition is highly variable between cultivars. Further analysis in specific varieties is focused in understanding what is driving these differences. On the other hand, SOYBEAN has been widely used to understand the symbiotic nodulation process and, in our Lab, we are interested in deciphering how iron and other nutrients are distributed during this process and the nutrient relationship between the nodule and the plant development and fitness.

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Primary Poster Presenter: Norma Castro

Salt accumulation beneath plants and depth to watertable affects the water relations and leaf mass o (1100-085)
Hall 2

This study examined the effects of salt accumulation in the root zone and depth to watertable on the growth and water relations of Atriplex nummularia. The experiment was conducted in Western Australia using a stand of A. nummularia. During the 2.2 years of this investigation the depth to watertable varied between 0.5 and 1.5 m and groundwater salinity (ECw) was 18.9–22 dS m-1. Calibrated EM38 surveys were conducted on 5 sampling dates over 2.2 years and were used to

estimate the soil salinity at 3 depths beneath the plants and 4 m away from the plants. Regression analysis showed that the salinity of the groundwater at the site increased with time (P = 0.002; R2 = 0.94). Comparisons of soils beneath plants and 4 m away showed 70% increases in EC1:5 at 0–25 cm, and 45% increases in concentration at 25–50 and 50–75 cm. Superimposed on these effects of plants, there were seasonal effects on soil salinity. Beneath the plants there was a significant negative relationship between increasing rainfall and average EC1:5 at 0–25 cm, but not 25–50 or 50–75 cm. In autumn, increasing ECah values were associated with increasingly impaired plant water relations, but in spring these were associated with improving water relations parameters. It is argued that the improved leaf growth at high ECah was associated with the development of shallower depths to watertable in spring. These results suggest that plants do not grow much in autumn because of high levels of salt accumulation in the root zone, but the plants resume growth in spring as the plants use the rain water in the upper soil and the shallow groundwater.

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Primary Poster Presenter: Hesham Alharby

The role of leaves in iron sensing and signaling in Arabidopsis (1100-082)
Hall 2

Iron (Fe) is an essential micronutrient for all living organisms and plants are the major source of Fe for humans and livestock. Fe deficiency in humans has been described as the most common nutritional deficiency affecting nearly 30% of the world's population. Fe deficiency also has a negative impact on plant development, crop yield, and seed quality. Understanding sensing and signaling regulation in plants will help in developing crops with higher nutritional value.In Arabidopsis, OPT3 has recently been identified as a component of the systemic network mediating Fe deficiency responses. opt3-2 mutants show a constitutive Fe-deficiency response and over-accumulate Fe in roots and leaves. Using RNA-seq, we demonstrated that opt3-2 roots display an activation of the major networks mediating Fe uptake. However, markers for Fe excess are exclusively induced in leaves, suggesting that Fe excess is properly sensed in leaves. In addition, we have found that the leaf vasculature responds more rapidly than roots to changes in Fe availability, suggesting that the vasculature is the primary site for sensing the Fe status of the whole plant. Our current experiments, including high-throughput protein-DNA interaction together with gene network analyses of leaf specific time-course RNA-seq data, are directed towards the identification of the transcriptional networks that coordinate the response to changes in Fe availability and the crosstalk to other nutrients.

Co-author(s): Mather Khan

Primary Poster Presenter: David Mendoza

Tomato Varieties Respond Differently to Aluminum Toxicity

Aluminum found naturally in the environment becomes toxic to crops at low pH levels. This aluminum toxicity can lead to stunted growth, shorter roots, and decreased fruit size. Tomatoes (Solanum lycopersicum) uses aluminum tolerance genes such as ALMT12, ALMT9 and MATE in order to release the metabolites malate and oxalate, which help the plant to combat the presence of aluminum. They bind to the aluminum before it has the chance to bind to the cell wall and cause damage. The focus in our study was to determine how different varieties of tomato respond to aluminum toxicity. Four varieties were grown for one week in a hydroponic system in the presence or absence of Al. After being grown in the presence of Aluminum, root length decreased as the concentration of aluminum increased. Furthermore, the amount of malate exudate increased with increasing concentration of aluminum. Preliminary qPCR data looking at genes shows up-regulation of ALMT12. This increased expression could help plants that are growing in toxic environments survive under Al-toxic conditions.

Co-author(s): Susan Bush

Primary Poster Presenter: Collin Modelski

Vascular plant One-Zinc finger (VOZ) transcription factors are positive regulators of salt tolerance (1100-079)
Hall 2

Soil salinity, a poses problem in agriculture, by limiting the productivity of crop plants. Plants responds by reprogramming gene expression via multiple signaling pathways that converge on transcription factors. In order to develop strategies to generation of salt-tolerant crops, it is necessary to identify transcription factors that modulate salt stress responses in plants. Here , we investigated the role of plant specific VOZ (VASCULAR PLANT ONE-ZINC FINGER PROTEIN) transcription factors (VOZs) in salt stress response. Transcriptome analysis in wild type, single, double mutant and VOZ2 complemented lines revealed that many stress-responsive genes are regulated by VOZs. Enrichment analysis for Gene Ontology terms in mis-regulated genes in voz double mutant not only confirmed known roles of VOZs and suggested a novel role for them in salt stress. To confirm VOZs role in salt stress, we analyzed seed germination and seedling growth of WT, voz1, voz2-1, voz2-2 single mutants, voz1-1 voz2-1 double mutant and a complemented line under different concentrations of NaCl. Interestingly only the double mutant exhibited enhanced sensitivity to salt stress as compared to WT, single mutants and a complemented line. Expression analysis showed that hypersensitivity of the double mutant was accompanied by reduced expression of salt-inducible genes. These results suggest that VOZ transcription factors act as positive regulators of several salt-responsive genes and that the two VOZs are functionally redundant in salt stress. Further, transcriptome analysis indicated VOZ's play significant role in metabolism of plant growth regulators.

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Primary Poster Presenter: KASAVAJHALA PRASAD

Ethylene modulates cortical microtubule reassembly in response to salt stress in Arabidopsis (1100-142 (Screen 11))
Hall 2

Regulation of cortical microtubule reorganization is essential for the survival of plant cells exposed to high salinity. In response to salt stress, microtubules undergo fast depolymerization followed by reassembly to form a new microtubule network that favors plant survival. Although this recovery response is essential for adaptation to salt stress in plant cells, the upstream regulatory mechanism for this process is largely unknown. In this study, we demonstrate that ethylene signaling facilitates salt-stress–induced reassembly of cortical microtubules, which is important for Arabidopsis survival under conditions of high salinity. In the presence of NaCl, suppression of ethylene signaling with Ag+ or with ethylene-insensitive mutants did not substantially affect microtubule depolymerization but significantly inhibited microtubule restoration. In particular, ETHYLENE-INSENSITIVE 3 (EIN3), a key transcription factor in the ethylene signaling pathway, plays a central role in ethylene-enhanced microtubule recovery under salt stress. In addition, we functionally characterized the microtubule-stabilizing protein WAVE-DAMPENED2-LIKE5 (WDL5) of A. thaliana and found that WDL5 promotes ethylene-mediated microtubule reassembly and plant salt tolerance. These findings indicate that ethylene participates in microtubule reassembly by upregulating WDL5 expression in response to high salinity. This study reveals a mechanism of ethylene signaling in plants that involves microtubule reassembly under salt stress.

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Primary E-Poster Presenter: Xiangfeng Wang

Stress-induced microtubule depolymerization in Chlamydomonas reinhardtii (1100-143 (Screen 13))
Hall 2

In flowering plants, such as Arabidopsis thaliana, cortical microtubules are depolymerized rapidly upon hyperosmotic stress conditions and are repolymerized gradually over the next several hours under persistent stress. Propyzamide-Hypersensitive 1 (PHS1) is the responsible tubulin kinase for the destabilization of microtubules. PHS1 genes are absent in non-plant organisms. Interestingly, PHS1 is not found in the genomes of red algae but are present in those of the Chlorophytes green algae with a fresh water habitat. In the fresh-water living unicellular green algae Chlamydomonas reinhardtii, cytoplasmic microtubules were destabilized upon

salt stress and this response required CrPHS1. Compared to wild-type strains, two T-DNA knockout alleles of CrPHS1 showed reduced growth arrest phenotypes under moderate salt conditions. These results indicate that PHS1-mediated microtubule destabilization contributes to the growth arrest in fresh-water living algae when the algal cells are challenged with salt stresses.

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Primary E-Poster Presenter: Lee Mei Ng

Abiotic: Water

Identification of new Arabidopsis protein kinase family that activates SnRK2 protein kinase in ABA a (1100-144 (Screen 7))
Hall 2

The plant hormone abscisic acid (ABA) plays a critical role in drought resistance responses. The core signaling components are snf1-related protein kinases (SnRK2s) which are activated by ABA-dependent inhibition of type 2C protein phosphatases (PP2Cs). Activation of SnRK2 protein kinases requires phosphorylation of the SnRK2 kinases themselves. It remains unclear whether the activation of SnRK2s is mediated by auto-phosphorylation or by other protein kinases and associated proteins in planta. Through a combination of a redundancy-circumventing genetic screen and biochemical analyses, we have identified functionally-redundant protein kinases that phosphorylate and activate the OST1/SnRK2 kinases in Arabidopsis. Mass-spectrometry revealed a specific trans-phosphorylation site in OST1/SnRK2.6 that is targeted by these kinases and required for strong SnRK2 activation. Reconstitution of full ABA-induced OST1/SnRK2.6 activation and S-type anion channel activation require these kinases, suggesting that they are a new member of the early ABA signaling core. Higher-order knock-out plants show not only reduced sensitivity to ABA but also strongly impaired osmotic stress-induced SnRK2 activation. Our results demonstrate that these protein kinases are required for ABA- and osmotic stress signaling through activation of SnRK2 kinases.

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Primary E-Poster Presenter: Yohei Takahashi

Uncovering how lateral root branching is regulated by water availability (1100-145 (Screen 2))
Hall 2

As plant roots grow through the soil they are faced by a variety of external signals such as heterogeneous water and nutrient availability. Plants are able to sense these signals and respond by altering root development to ensure roots continue to grow into areas where water and nutrients are present. My PhD has aimed to improve our understanding of how roots sense and respond to changes in water availability in soil. Using X-ray computed tomography (CT) scanning I have been able to non-destructively visualise root systems within soil and have observed that changes in water availability alter the branching pattern of roots. If water availability is higher on one side of the primary root than the other then branches will only form towards the wet side, a response called hydropatterning, and if the root experiences a temporary water deficit branching will stop completely, a response called xerobranching. Both are striking responses that illustrate how sensitive root development is to variations in water in the soil. In order to understand how water flow into the root may signal these developmental changes Arabidopsis thaliana knock out lines in water channels have been tested. Knocking out plasmodesmata related proteins can disrupt the hydropatterning response, suggesting water movement through the plasmodesmata is a necessity to pattern root branching in response to water. We have also modeled water movement during hydropatterning using the Model of Explicit Cross-section Hydraulic Architecture (MECHA) which highlights the importance of asymmetrical water movement during this response. These results provide interesting insights as to how water could be sensed during root growth.

Primary E-Poster Presenter: Emily Morris

Insertion of limited transpiration trait into soybeans to improve drought adaptation (1100-147 (Screen 6))
Hall 2

Insertion of limited transpiration trait into soybeans to improve drought adaptation

Co-author(s): Jason Gillman,
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Primary E-Poster Presenter: Avat Shekoofa

Multi-layer mucilage of Plantago ovata seeds: superior water-holding and gelling properties arise fr (1100-146 (Screen 1))
Hall 2

The production of hydrophilic mucilages found in angiosperms is a common adaptation for plants to arid environments. Mucilage is a mixture of polysaccharides extruded by seeds or roots upon hydration, which forms a gel-like capsule and retains water for plants. Due to its diverse range of rheological properties and a remarkable water holding capacity, seed mucilages are also highly sought-after materials as ecologically friendly options for applications in agricultural, pharmaceutical and food industries. In this study, I aim to establish the relationship between structures of complex arabinoxylans (AXs) from Plantago ovata seed mucilage and their gel forming properties and biological functions. I have discovered a three-layered architecture in P. ovata seed mucilage and explored the function of each layer in comparison with mutant plants. I have successfully separated individual AX species from a complex mixture in mucilage layers and demonstrated that two AX fractions exhibit distinctly different gel forming and melting properties, despite having identical primary structures. By using a combination of rheological techniques, small angle X-ray/neutron scattering, NMR and microscopy, I have uncovered that hydrogen bonding and structure of AX side chains are two key factors that drive gel formation. Furthermore, the results support the hypothesis that small variations in side chain motifs of otherwise chemically similar AXs from P. ovata seed mucilage have a great impact on their chain conformation and intermolecular hydrogen bonding, which leads to dramatic differences in rheological properties and water structuring. This research generates substantial new knowledge in molecular assemblies, rheological properties and biological functions of seed mucilage, which create new future possibilities instrumental in rational design of seed-coating material to enhance the abiotic stress tolerance of cash crops or novel additives to improve food quality.

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Primary E-Poster Presenter: Long Yu

RNA-Seq Transcriptome and Biochemical Analysis of the Resurrection fern, Pleopeltis polypodioides. (1100-148 (Screen 2))
Hall 2

Pleopeltis polypodioides is an epiphytic fern that can survive extended drought and resume growth upon rehydration. To gain insights into the molecular mechanisms by which this fern survives desiccation, we utilized RNA-Seq analysis to assess global gene expression in fresh, 50% dehydrated, and dry fronds. From three replicate RNA libraries of each condition, we de-novo assembled 202,500,520 Illumina reads in Trinity that generated 403,808 transcripts clustered into 209,107 genes. The quality of the De Novo assembly was examined with Bowtie2 v.2.3.4. About 86% of the original reads mapped back to the assembly, with most mapping as properly paired reads. 90% of the total transcription is represented by an E90-

transcript set of 61557 transcripts and E90N50 of 1731. The alignment of the reads to the reference (Trinity assembled) transcript and relative abundance of the expressed transcripts were estimated using RNA-Seq by Expectation Maximization, RSEM. We applied Trinotate to annotate De Novo assembled Pleopeltis transcriptome and detected differentially expressions using limma-voom. With a cutoff of FDR adjusted p-value <0.001 and fold change >4, a total of 502, 502, and 296 genes were differentially expressed between fresh and dry, fresh and partially dry, and partially dry and dry conditions, respectively. The RNA-Seq results suggest that dehydration induces upregulation of transcripts related to sugar metabolism, fatty acid biosynthesis, and ROS signaling. These changes were corroborated with biochemical studies on ROS and fatty acid contents. Similarly, reduced transcripts related to photosynthesis and cell wall maintenance correspond with vitality tests and ROS measurements. The combination of RNA-Seq information and biochemical assays provide valuable information on the essential components of desiccation tolerance. Future work will focus on quantifying candidate genes using RT-qPCR. Supported by NASA grant NNX10AP91G.

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Primary E-Poster Presenter: Susan John

A regulatory cascade involving transcriptional and N-end rule pathways in rice under submergence (1100-086)
Hall 2

Group VII ERFs play a pivotal role in plant response against submergence stress and are regulated by N-end rule proteolysis pathway which is believed to be the oxygen sensing mechanism. Arabidopsis ERFVIIs are degraded via N-end rule pathway under normoxia, but are stabilized under hypoxia to trigger downstream responses. In rice, Sub1A-1 is a member of the group VII ERFs and confers the majority of submergence tolerance to rice. However, despite having the canonical N-degron sequence, SUB1A-1 is able to evade N-end rule protease degradation under normoxia. Besides, Sub1A-1 is reported to response to several stresses, including dehydration or prolonged darkness. This raised an interesting question of how rice senses low oxygen stress from the others. We found that the other two ERFVIIs, ERF66 and ERF67, are regulated by the N-end rule pathway and that ERF66 and ERF67 genes are transcriptionally regulated by SUB1A-1 as well. Our studies showed that Sub1A-1 and ERF66/ERF67 form a regulatory cascade in response to submergence stress in rice. SUB1A-1 is the only known ERFVII protein escaping from the N-end rule pathway by far, so it is interesting to know how SUB1A-1 escapes the N-end rule proteolysis degradation. Our preliminary results suggest that the C-terminus of SUB1A-1 is important for escaping. With sequential

truncation of C-terminus of SUB1A-1, we identify a segment of the C-terminus of SUB1A-1 that is crucial to the escaping.

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Abscisic acid-activated protein kinase-like protein modulates drought stress response in soybean (1100-087)
Hall 2

Abscisic acid is the key hormone mediating responses and adaptation to abiotic stresses in plants. We previously identified an abscisic acid-activated protein kinase (AAPK) in broad bean and demonstrated that AAPK is a positive regulator that can enhance abscisic acid signaling. In this study, we analyzed the role of AAPK-like protein kinases in plant responses to drought stress. We studied the expression of AAPK-like protein kinase (AALK) genes in the root of soybean plants. Analysis of GmAALK overexpression lines shows that these transgenic plants exhibit enhanced drought tolerance, suggesting that the GmAALK is a positive regulator of drought tolerance. For further understanding the molecular mechanisms of GmAALK gene, RNA-Seq approach was utilized to profile the root transcriptomes under drought condition. Many critical genes involved in drought response have been identified as differentially expressed genes between control and drought conditions in control samples, and 2813 differentially expressed genes in the GmAALK RNAi-silenced samples under drought stress. The study reveals the dynamic transcriptome reconfiguration in soybean roots to acclimate drought stress and highlights the gene expression changes associated with signaling through GmAALK. These results will also provide genetic foundations for developing drought-tolerant soybean cultivars via manipulating kinase gene family.

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Adaptation mechanisms to drought stress in spring wheat (1100-094)
Hall 2

Here we compared drought response in two spring wheat genotypes: Drysdale was selected for high transpiration rate under drought (low Δ13C); and Hollis was selected for high yield and protein content under drought. As anticipated, the stomatal conductance was higher in Drysdale than in Hollis under drought conditions at PAR levels below 1500. Furthermore, despite similar grain yield under drought in field or greenhouse trials, their developmental programs differed. At the

whole plant level, Hollis suspended root and shoot growth during drought, but maintained high grain yield per spike, whereas Drysdale sustained root growth and production of spikes, but with lower grain content. Analysis of chlorophyll fluorescence demonstated dependence of the response on drought severity. Under moderate drought Hollis dissipated excitation pressure more efficient by NPQ and by photochemical (electron transport) quenching than Drysdale. However, under severe drought, Drysdale developed more photoprotective NPQ than Hollis and therefore was probably less vulnerable to photodamage as reflected by higher Ff/Fm. Analysis of the PSII and PSI photochemical quantum efficiency indicated higher electron flux in Hollis under control conditions followed by significant decline under severe drought. On the contrary, electron transport rate under normal conditions in Drysdale was lower, but then less affected by drought. This indicates the electron flux decline in Hollis resulted from increased NPQ and was not due to downstream alternative electron sinks like carbon fixation and photorespiration. ROS-scavenging also differed between genotypes, and was active in Drysdale under all conditions, but only activated by drought in Hollis. We conclude that distinct developmental and physiological mechanisms could sustain high yield under drought.

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Arabidopsis NIP2;1: A Lactic Acid Transporter With a Role in Plant Survival Under Low Oxygen Stress (1100-095)
Hall 2

In Arabidopsis thaliana oxygen deprivation, or hypoxia, occurs as a result of flooding leading to a genetic response through the expression of core hypoxia genes that encode fermentation and glycolytic enzymes as well as other proteins associated with an adaptive response. Included in these core hypoxia genes is AtNIP2;1 which encodes a member of the aquaporin superfamily of membrane channel proteins, specifically a subgroup called “Nodulin-like Intrinsic Proteins” (NIP). Unlike other aquaporins, NIP2;1 transports the protonated form of lactic acid as opposed to water or other solutes. This protein is hypothesized to play a role in the transportation and compartmentalization of lactic acid (toxic byproduct of lactic acid fermentation) during anaerobic respiration. NIP2;1 is a root-specific gene product that shows drastic induction (300-fold) under hypoxia challenge. During hypoxia, NIP2;1 localizes to the plasma membrane as well as to internal membrane compartments. Analysis of T-DNA insertional mutants show that knockout of the NIP2;1 gene significantly decreases the survival of plants challenged with extended periods of hypoxia. Upon 8 hours of hypoxia in conjunction with complete darkness, wild type plants display a more rapid recovery as well as a statistically significant higher survival rate. T-DNA nip2;1 knockout plants display a significant change in the transcript levels of several of core hypoxia genes and fermentation enzymes during hypoxia. The data suggest that NIP2;1, perhaps through its lactate transport function, may affect lactate and metabolite homeostasis in a manner that regulates

the expression of other core hypoxia response genes as part of the coordinated response of Arabidopsis roots to low oxygen stress. (Supported in part by NSF MCB-1121465).

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Comparative transcriptome analysis reveals conserved genomic characteristics of drought responsive g (1100-101)
Hall 2

Drought stress is one of the most important abiotic stresses that negatively impacts agricultural production through slowing plant growth and reducing crop yield. This study explores what genomic characteristics are conserved for drought responses across five plant species including Arabidopsis, soybean, poplar, rice and maize. Because most gene functions in plants have been characterized using the model species, Arabidopsis thaliana, comparative transcriptome analysis between Arabidopsis and other plant species is particularly important for functional annotation of genes in other plant species. We have developed a unified computational pipeline to investigate conserved drought responsive genes and regulatory sequences across these five species. Using this pipeline, we have identified 120 common gene families induced by drought in these five species. We compared regulatory sequences and transcription factor binding motifs of drought-induced genes and we detected four types of motifs conserved across these five species. To further understand how genetic variations in the regulatory sequences contribute to changes in gene expression, we analyzed published SNP data from 3000 rice genome project, and we identified candidate SNPs that potentially alter conserved, drought regulatory sequences in the promoter regions of a PP2C (protein phosphatase 2C) gene. The discoveries and computational pipeline developed in this study could be useful to annotate gene functions and understand underlying mechanisms of gene regulation in crop species under other abiotic and biotic stress conditions.

Primary Poster Presenter: Song Li

Complex Genetic Variation and Physiology of Anaerobic Germination in Rice (1100-104)
Hall 2

To improve food security in the developing world, the current trend in rice production is to shift from transplanting seedlings to direct sowing of seeds. Following heavy rains, direct-sowed seeds may need to germinate under flooded,

anaerobic conditions, but most rice genotypes cannot survive these conditions. To identify complex trait loci associated to anaerobic germination (AG), we integrated phenotypic germination information with a 700,000 SNP data base from the rice 3,000 genome initiative for genome-wide association studies (GWAS). Using 109,440 seeds, we quantified AG% in 2,700 (wet season) and 1,500 (dry season) rice genotypes and performed GWAS, followed by post-GWAS analysis that encompassed a generalized SNP-to-gene set analysis, metanalysis and a network dense module search. We determined that transcription factors linked to ethylene responses or genes involved in several metabolic processes are significantly associated with AG. SNP-to-gene, meta- and dense module network GWAS analyses identified genes that have shown changes in gene expression in response to AG in previous experiments. We found two significant gene-sets involved in sphingolipids metabolism, whose function in AG has not been characterized. In our network-GWAS analysis we evaluated the top 100 network modules; these modules showed genes involved in a wide variety of metabolic processes and found a fatty acid desaturase that also was significant in the SNP-to-gene set analysis. We determined that anaerobic germination percentages are highest among indica subpopulations, and AG is a polygenic trait with complex physiological differences among rice genotypes. We selected several genes of interest that have not been linked to AG before to perform further functional genomics analyses. Currently we are characterizing these genes' relationship to flooding in rice mutants by doing genetic, physiological and biochemical experiments.

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Drought tolerance: a key trait for yield performance in durum wheat genotypes in semi-arid regions (1100-108)
Hall 2

Wheat is one of the most important staple food crop of the world. Nevertheless, climate change has led to drastic reductions in wheat production over the last five years in Tunisia and other countries because of several drought events. Here, we focused on identifying yield traits that provide information on drought tolerant wheat genotypes. Keeping in view the importance of drought tolerant wheat cultivars, an experiment with two water regimes in field conditions was conducted in central Tunisia characterized by the semi-arid climate. Overall, canopy temperature (CT) was increased under drought in seven studied genotypes. Drought stress significantly decreased ($P<0.001$) shoot fresh weight, plant height and tiller number. Similarly, ear lenght was significantly reduced ($P<0.05$). Data of net photosynthetic rate (A) measured on flag leaf recorded highest values in the most productive genotypes. The treatment had a highly significant effect ($P<0.01$) on the 1000 kernel weight.

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Effect of drought stress on plant growth, photosynthesis, and proline metabolism in sugarbeet (1100-093)
Hall 2

Drought stress is one of the major abiotic stresses that causes yield and economic loss in crops worldwide. The stress limits plant growth and influences many physiological processes in plants, including proline metabolism. In sugarbeet, the effects of drought stress on plant growth and proline metabolism are not well characterized. To better understand and quantify the impact of water stress on plant growth and proline metabolism, greenhouse experiments were conducted to evaluate the effects of different levels of water stress on plant growth, leaf photosynthesis, proline content, and enzyme activities and gene expression of proteins involved in proline metabolism in leaves and roots of sugarbeet plants. Plants were grown in 15-liter pots for 18 weeks with supplemental light under a 16 h light/8 h dark regime and subjected to water stress for 1 or 2 weeks prior to harvest. Shoot and root growth, leaf photosynthesis, transpiration, stomatal conductance, and total chlorophyll were reduced by water stress. Proline content increased in leaves and roots in association with an increase in the activities of the proline synthesizing enzymes, pyrroline-5-carboxylate synthase (P5CS), pyrroline-5-carboxylase reductase (P5CR) and ornithine aminotransferase (OAT). Changes in gene expression of P5CS and OAT, but not P5CR, were enhanced by water stress. The activity of proline dehydrogenase (PDH) was not affected by water stress although PDH expression was upregulated in roots but not leaves. These results indicate that drought stress impacts plant growth and enhances proline synthesis in sugarbeet leaves and roots.

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EPICON: From Leaves to Roots to Microbes – How Sorghum and Its Microbiome Respond to Drought (1100-109)
Hall 2

EPICON research focuses on drought, important due to the increased frequency and severity of this abiotic stress with climate change. Both transcriptomic and epigenetic changes play major roles in regulating drought responses. EPICON's cohesive, high-resolution transcriptomic study was on sorghum [Sorghum bicolor (L.) Moench], a C4 cereal crop, noted for drought tolerance. This large-scale, multi-

year field experiment explores spatiotemporal responses under fully irrigated and two different drought stress regimes in replicate plots of two sorghum genotypes, differing in pre- and post-flowering drought responses. Drought was imposed in fields in California's Central Valley, where rare summer rainfall permits controlled drought conditions. Leaf and root samples were taken weekly over the plant's lifetime with the goal of understanding mechanisms functioning in acclimation to and recovery from pre- and post-flowering drought, using RNA-Seq, BS-Seq, proteomics, metabolomics, and histone profiling. A resulting data set of over 350 transcriptomes revealed 44% of expressed genes being significantly affected by drought. Roots showed greater transcriptional disruptions than leaves; samples from pre-flowering drought had more complex temporal changes than post-flowering drought; large differences were found between genotypes. To gain additional insights into drought responses, impacts of this abiotic stress were also studied in microbial populations, using shotgun metagenomics, metatranscriptomics, and metabolomics. Composition of fungal and bacterial communities, associated with these same plants, led to additional comprehensive data resources that will be available to the community to unravel the complex drought responses of plants and their field-associated microbial communities. Cumulative data is being used to devise models to better predict and control roles and interactions of transcriptional regulation, epigenetics and the microbiome in sorghum's response to drought.

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Flooding tolerance of three cabbage (Brassica oleracea var. capitate) genotypes (1100-089)
Hall 2

Cabbage (Brassica oleracea var. capitata), one of the most abundant vegetables, exist a huge market gap in summer production at lowland area in Taiwan, because high temperature and heavy rainfalls are two critical obstacles for cabbage production. Taiwan requires new cultivars that adapt climate changes to fill the gap of year-round production at lowland. However, cabbage breeding without molecular assisting costs at least 10 years for a single cultivar. To establish the basis for mapping quantitative trait loci (QTL) for our long-term objective, we firstly focused on flooding and developed quantitative phenotyping measurements to describe flooding tolerance. Multiple measurements have been utilized, including leaf damage index, growth of new leaves, growth of shoot apex, and flooding responses, such as hyponasty. 'Fuyodori' showed lowest leaf damage index, and highest growth of new leaf and shoot apex among three cultivars. Notably, '228' shown the lowest hyponasty response and largest shoot elongation during submergence. Here, we identified 'Fuyodori' is a relative tolerant cultivar, whereas '288' is a flooding intolerant cultivar. These tolerant and intolerant lines, 'Fuyodori' and '228' are two parent candidates for generating segregating population for mapping QTL related to flooding tolerance. Taken together, this study not only assessed flooding tolerance in three cabbage cultivars, but also provides a basis for further mapping QTL associated with flooding tolerance.

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Genetic variation among durum wheat genotypes in transpiration responses to vapor pressure deficit (1100-107)
Hall 2

Environmental constraints such as drought largely contributed to the launch of the Arab uprising in 2011 in the city of Sidi Bouzid known for the highest agricultural activity in the country. The price hikes in cereal is the major factor affecting the livelihood sustainability of small farmers in the region. This study evaluates the performance of forty-height wheat genotypes (Triticum durum Desf.) collected throughout Tunisia and differ in drought tolerance. For this purpose, two experiments were conducted. The first experiment focused on transpiration response (TR) to vapor pressure deficit (VPD). In the second experiment, two water availability regimes (normal irrigation and withholding irrigation) were applied to study transpiration response to soil drying. Four replicates of a randomized complete block design were used. The transpiration responses of wheat genotypes to changes in vapor pressure deficit under semi-controlled conditions showed significant differences in the slopes of the 48 durum wheat genotypes. The variation in the slopes classified the 48 genotypes according to the transpiration at VPD breaking points. Differences in segmented transpiration differed in the level of VPD where changes can be detected. Genotypes with lower transpiration at the breaking points were considered as water-saving. Comparison of means using Duncan's test

(5%) showed very highly significant differences among the genotypes. Transpiration varied between 19-42 mg m-2 s-1. The photosynthetic photon flux density (PPFD) was beyond 500 µmol m-2 s-1 during midday measurements. The fraction of transpirable soil water (FTSW) in the irrigated treatment was maintained at around 1.0 by watering the PVC pots daily to 80% of the field capacity (FC). After witholding irrigation, the soil water contents (FTSW) decreased steadily. After 12 days of treatment, FTSW declined to zero. Genotypic differences in the dynamics of FTSW were observed ($P<0.001$) under the drought.

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High-throughput Phenotyping and Genome-Wide Association Studies Reveal the Genetic Architecture of D (1100-100)
Hall 2

Abstract: Cotton (Gossypium hirsutum Linn.) is an important economic crop and often subjected to various undesired stresses in the course of its growth and development. Resistance to drought is a complex trait that is supposed to be regulated by a variety of genes. Without a comprehensive profiling of drought resistance (DR) -related traits, the knowledge of genetic basis for drought resistance in cotton remains limited. In order to investigate the genetic architecture for drought resistance, an automatic phenotyping platform (APP) was applied to image 110 phenotypic traits (i-traits) systematically either under normal water condition and drought stress (mild drought and severe drought) during seedling stage to generate 6 time points (as T1 to T6) across a natural population of 200 representative upland cotton accessions. Those i-traits included 36 morphological, 60 texture and 11 leaf traits. The value of DRC (drought resistant coefficient) and RCRW (recovery capability after re-watering) were used to evaluate the capability for the accessions under mild drought condition, severe drought condition and re-watering condition. Some traditional i-traits were acquired through APP, such as plant height and plant width, and novel i-traits could also be extracted, such as plant density and plant compactness which reflected the drought characteristics. Based on data from some selected i-traits under both standard watering condition and drought condition, the population of 200 upland cotton accessions could be divided into three sub-groups, whereby sub-groups I to III represented the cotton accessions that were affected severely or slightly by drought stress.

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Investigation of the drought-tolerance of foxtail millet (Setaria italica) landraces (1100-149 (Screen 12))
Hall 2

Due to the climate change, the frequency and severity of drought stress is increasing which causes the reduction of crop yields worldwide. It is urgent to develop the drought resistant crop cultivars in response to the prevalence of stresses in the future. Foxtail millet (Setaria italica), a drought-tolerant crop species, is a model plant for studying the responses to abiotic stresses. We used more than 150 S. italica landraces to test their responses to drought treatment. The hydroponic solution containing 18% polyethylene glycol (PEG) 6000 was applied to the 4-leaf stage seedlings for a week. Plant height, weight and leaf withering score were measured to evaluate their responses. The results suggested that most landraces showed medium-to-high tolerance to PEG6000 treatment while a few landraces were showed growth retardation and severe withering after treatments. Further physiological and genetic analyses will be carried out to investigate the mechanism involved in controlling the drought sensitivity of S. italica, which might provide useful information to improve the drought resistance in cereal crops.

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Lipid-mediated long-distance signaling in response to abiotic stress (1100-105)
Hall 2

To survive environmental fluctuations, plants must efficiently adjust their development both locally and distally. One component of the plant long-distance trafficking system, the phloem, is known to transport photoassimilates as well as (signaling) metabolites, nucleic acids, proteins, and lipids. We are investigating PHLOEM LIPID-ASSOCIATED FAMILY PROTEIN (PLAFP) and other predicted lipid-binding proteins (LBP), their interaction with the signaling lipids and their role in signaling in Arabidopsis thaliana. We propose that these LBPs act in long-distance, lipid-mediated signaling to systemically coordinate the plant’s response to stress. We have shown that PLAFP, as well as other predicted LBPs, respond to stress at the transcriptional level and that PLAFP binds specifically to phosphatidic acid (PA). This PLAFP-PA interaction could occur as part of a joint systemic signal, or during protein-membrane interaction at the local (origin) or distal (receptor) location, respectively. We have identified several putative PLAFP-interacting proteins, including two kinases that suggest a role of PLAFP-PA in signaling. Current studies investigate PLAFP-PA complex movement and the biochemical mechanism of the PA-PLAFP interaction. Preliminary results suggest movement of radiolabeled lipid, possibly facilitated by PLAFP. Future studies will employ optogenetics to investigate

PLAFP movement. To better understand the PLAFP-PA interaction, we will mutagenesis followed by lipid binding assays, crystallography and a FRET approach. An RNA-Seq analysis comparing PLAFP wild type, knock-down, overexpression, and complement lines indicates which developmental processes are coordinated by PLAFP-PA. Funded by USDA #MICL02414/ 04147 and NSF #1144391/1836680 to SHB and USDA-NIFA-NNF 2015-38420-23697 to AK.

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Molecular Cloning and Characterization of a Novel OsGRAS Gene in Rice (1100-098)
Hall 2

A novel GRAS-like transcriptional regulator gene (OsGRAS-like) (GIBBERELLIC ACID INSENSITIVE (GAI)、REPRESSOR of ga1-3 (RGA) and SCARECROW (SCR)) located at chromosome 3 were identified by association mapping strategy. It is highly related to root number, root length and drought stress tolerance at rice seedling stage. There are 23 single nucleotide polymorphisms were identified within the OsGRAS-like gene among different root morphology rice germplasms, including 11 SNPs could be used as the marker for root numbers. The tetra-primer amplification refractory mutation system PCR (Tetra-ARM) marker and In/Del marker were further developed. These molecular markers were verified using recombinant inbred lines cross from indica and japonica rice. Also, the morphology and thermal-image were observed with 21% PEG-6000 treatment. Besides, the expression profile were analyzed by quantitative real-time PCR during rice seedling development and stresses treatment. It showed that OsGRAS-like gene expression was positively correlated with shoot length and root length and was also related to drought tolerance at rice seedling stage. In conclusion, we identified a novel OsGRAS-like gene and used to functional marker for root morphology and drought tolerance in rice seedling.

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Quantifying relationships between water-logging, soil oxygen, and corn growth and development (1100-099)
Hall 2

Excessive soil moisture during early spring often complicates corn growth and development as plants are beginning to establish their canopy and root structures. Soil waterlogging occurs when rainfall exceeds a soil’s internal drainage ability, oversaturating the soil. Pore space previously containing air fills with water causing soil oxygen levels to decline. A 2-year experiment was conducted using an outdoor pot culture facility to quantify the effects of waterlogging duration on corn growth

and developmental traits. Two corn hybrids, Pioneer P2089 and Agrigold A6659, were grown in 15.2x30.5 cm PVC pots filled with a 3:1 sand to soil growing media mixture. All pots were irrigated with full-strength Hoagland's nutrient solution. Individual pots were plugged to prevent water from draining; thus, inducing waterlogged conditions. Treatments included 0, 2, 4, 6, 8, 10, 12, and 14 days of continuous waterlogging introduced when plants reached the V2 growth stage. Oxygen sensors inserted into a representative sample of pots continuously monitored soil oxygen levels throughout the experiment. Plants were harvested 15 days after the treatments were imposed, and all measurements were taken. Plant height, leaf number, and leaf area were measured; roots were washed, scanned, and analyzed using WinRhizo Pro software. Leaves, stems, and roots were separated and dried for weighing. As the duration of waterlogging increased, all plant growth parameters decreased. Plant height, leaf number, longest root length, and total root tips decreased linearly following an increasing duration of flooding. As waterlogging was imposed, soil oxygen levels rapidly declined until reaching 0% after approximately six days. Leaf area, root surface area, root volume, and all plant dry matter components decreased quadratically following an increase in the duration of waterlogging with the steepest decline occurring during the first six days.

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Primary Poster Presenter: Hunt Walne

Rapid, Genotype-independent Agrobacterium-based Sorghum Transformation Using Morphogenic Genes (1100-103)
Hall 2

Sorghum bicolor (L.) Moench, noted for its drought and flooding tolerance, is the fifth most important cereal crop worldwide and is used for food, feed and biofuels. Despite its importance, agronomic and nutritional improvements are still needed. Genetic engineering and editing can be used to learn the genetic basis for such traits, which could provide opportunities for crop improvement. However, genotype dependence of transformation and the time and effort to generate target tissues have slowed progress. Here we report methods with less dependence on genotypes and difficult-to-obtain target tissues. We utilized Agrobacterium, carrying the morphogenic genes, Wuschel2 (WUS2) and Ovule Development Protein2 (ODP2) [similar to Baby Boom (BBM)] in which expression is driven by a maize auxin-inducible promoter (Axig1pro) and maize phospholipid transferase protein promoter (Pltppro), respectively. CRE-mediated excision of WUS2 and ODP2 is required to avoid effects of developmental gene expression at later developmental stages. This approach leads to rapid formation of somatic embryos from surface cells of the scutellum of an immature embryo, avoiding a callus phase and reducing somaclonal variation. PCR was used to confirm presence of introduced genes. Time to generate T0 plants was shortened and success was achieved with RTx430, BTx623, fast-cycling SC187, and sweet sorghum variety, Ramada. We also achieved success using an alternative target tissue, mesocotyl leaf segments, prepared from young

seedlings, which eliminates the resource- and time-intensive generation of immature embryos. Using an Agrobacterium-based system, it also requires the morphogenic genes, WUS2 and ODP2 (BBM), driven in this case by the nopaline synthase (NOS) promoter and maize ubiquitin (UBIM) promoter, respectively. Transformation efficiencies from both approaches are being generated. These advances will speed gene function studies and lead to new strategies for improving sorghum and other crops.

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Root metaxylem as a novel target for improved drought tolerance in maize
(1100-106)
Hall 2

Developing more drought tolerant crops is needed to sustain the food security of our growing population in the face of climate change. We hypothesize that restricted conductance through smaller, more numerous root metaxylem vessels enhances maize drought tolerance by improving the overall economy of water use. We grew 385 accessions of the Wisconsin Diversity Panel (WiDiv) in the field under well-watered and moderate water stress conditions at the Apache Root Biology Center in AZ in 2016. Using shovelomics at anthesis and laser ablation tomography, we found substantial variation in root metaxylem phenotypes in the WiDiv. A phenomics study using a bulked segregant analysis and partition around medians clustering revealed that the "many-small" metaxylem phenotype and restricted hydraulic conductance was related with greater shoot growth under water stress. In greenhouse mesocosms using a subset of the WiDiv, we found that under water stress lines with smaller metaxylem vessels maintained greater photosynthetic rates and leaf water potentials than lines with larger vessels. To determine the genetic mechanisms underlying variation in metaxylem phenotypes, a genome-wide association study (GWAS) was performed with the WiDiv and a panel of 577,161 single nucleotide polymorphisms (SNPs) identified via RNA-seq. Several significant SNPs were identified under water-stress and well-watered conditions, and for the plastic response to drought. The genes where these SNPs reside are also highly expressed in maize root tissues, particularly in the stele. Ultimately, these candidate genes may become novel targets in maize molecular breeding programs.

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Primary Poster Presenter: Stephanie Klein

Suggestive roles of a gene encoding acetolactate synthase under hypoxic stress in Arabidopsis (1100-102)
Hall 2

In fungi, a substantial number of reports demonstrated that the increase in branched-chain amino acid (BCAA) level helps fungal cells survive hypoxic environment by acting as an alternative electron sink under a limited supply of oxygen. Acetolactate synthase (ALS) is the first enzyme in the biosynthesis of BCAAs such as valine, leucine, and isoleucine. We identified two homologous genes encoding ALS in Arabidopsis. We also analyzed the expression patterns of the genes in different environments and organs. Our results showed that a gene encoding ALS was strongly expressed in all organs and highly expressed under submergence and hypoxic stresses. To understand the biological role of the gene, we generated transgenic plants designed to overexpress the gene in Arabidopsis. We found that an ALS-encoding gene confers tolerance to hypoxic stress through phenotypic analysis of transgenic Arabidopsis. These results altogether indicate that ALS may play essential roles under submergence or hypoxia in Arabidopsis. In this research, we provide the first report on the possible role of ALS in the hypoxia or submergence response of Arabidopsis.

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Primary Poster Presenter: Geunmuk Im

Targeted modification of ethylene signaling improves grain yield under drought stress in maize (1100-088)
Hall 2

The phytohormone ethylene regulates plant growth and development as well as plant response to abiotic stress, such as drought, high temperature, shading and nutrient deficiency. Reducing ethylene biosynthesis or the signal transduction has been shown to increase drought tolerance in maize. When constitutively overexpressed under control of the maize UBIQITIN and GOS2 prompter, the maize ARGOS8 reduced plant sensitivity to ethylene, enhancing grain yield under both optimal and drought stressed conditions in multiple hybrids. Multi-year and -location field testing of GOS2 PRO::ARGOS8 events also revealed that some of the transgenic hybrids have reduced resistance to root lodging. We found that exogenously supplied ethylene precursor ACC promoted nodal root emergence in WT plants while reduced ethylene signaling in the transgenic plants delayed nodal root emergence. To mitigate this undesired phenotype, developmentally regulated promoters were tested for targeted transgene overexpression. Results showed that maize transgenic plants expressing ARGOS8 driven by the female inflorescence-preferred FTM1 promoter produced high grain yield while their resistance to root lodging remained unchanged relative to controls. This study demonstrates that selective modification of ethylene signaling at different developmental stages can

decouple improved grain yield from root lodging, suggesting that the strategy might be feasible for developing drought tolerance hybrids.

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Primary Poster Presenter: Jinrui Shi

The genetic architecture of osmotic stress tolerance in plants (1100-096)
Hall 2

Single cell models such as yeast have aided the identification of core osmo-sensory pathways in non-plant cells. To facilitate defining such pathways in plants, we have utilized high-throughput genetic screening methods in the alga, Chlamydomonas. We have recently found that mutants in putative osmosensory pathways in Arabidopsis are necessary for survival of Chlamydomonas after hyperosmotic shock indicating conservation across the plant lineage (Vilarrasa-Blasi et al., in preparation). Initial genome-scale mutant screens have identified hundreds of loci that are necessary for growth under hyperosmotic conditions including putative signal transduction components not previously associated with the osmotic stress response. Transcriptomic and proteomic profiling of Chlamydomonas under these conditions has enabled a systems level understanding of osmoregulation without the complications of a multicellular context. Characterization of these genes in Arabidopsis has revealed broad conservations of the newly uncovered pathways.

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Primary Poster Presenter: Jose Dinneny

The Involvement of Group 1 Late Embryogenesis Abundant Proteins in Desiccation Tolerance (1100-097)
Hall 2

In orthodox seeds, the acquisition of desiccation tolerance during the maturation drying stage of seed development provides species with the ability to survive extreme drought and cold during offseason temperatures. However, during maturation drying, crowding of intercellular contents because of loss of bulk cytoplasmic water may result in damaging intermolecular interactions. Several bio chemicals accumulate during maturation drying to prevent these damaging interactions, among them, late embryogenesis abundant (LEA) proteins. Generally LEA proteins are intrinsically disordered and hydrophilic and the 51 Arabidopsis

thaliana genes that encode them can be classified into 7 groups based on sequence motif. Our broad goal is to determine the role of LEA proteins in desiccation tolerance in seeds. Our specific objective is to compare the seed physiology of mutants lacking alleles for group 1 LEA proteins (which accumulate solely during seed development) with their wild-type isolines of A. thaliana. We compared dry weight and moisture content of mature seeds and found that whereas dry weight did not differ, moisture content was statistically significantly higher in the mutant seeds. The results suggest that Group 1 LEA proteins may facilitate water removal from hydrophilic molecular surfaces in the cell during maturation drying and thus are consistent with the Water Replacement Model of their function. Further research is being conducted to compare desiccation tolerance between developing mutant and wild-type seed.

Co-author(s): Sheila Blackman

Primary Poster Presenter: Joanne Thomson

The Nature of the Progression of Drought Stress Drives Differential Metabolomic Responses in Populus (1100-092)
Hall 2

The use of woody crops for Quad-level (1018 J) energy production will require use of marginal agricultural lands, where periods of water stress are frequent. Our previous research demonstrated that some Populus sp. genotypes have the capacity to increase dehydration tolerance by lowering the osmotic potential via osmotic adjustment, which allows turgor and growth maintenance under mild to moderate stress and facilitates growth recovery after stress relief. The aim of the study was to determine how the inherent genetic potential of a given clone interacts with the nature of the stress experienced to determine the degree of the biochemical response. A drought stress study on Populus deltoides 'WV94' was conducted in a greenhouse and the resulting metabolomic profiles of leaves were determined for plants subjected to cyclic mild (-0.5 MPa predawn water potential) drought vs cyclic severe (-1.26 MPa) drought after 2 or 4 drought cycles in contrast to well-watered controls (-0.1 MPa), and in contrast with plants subjected to acute drought, where plants were not rewatered, but were desiccated for up to 8 days. Extracts of leaf metabolites were silylated and analyzed by gas chromatography-mass spectrometry with electron impact ionization (70 eV). Organic solute accumulation under cyclic stress was moderate (1.20x) relative to well-watered controls and was largely constituted by soluble sugars, organic acids, and amino acids. In contrast, acute onset of prolonged, severe drought induced the greatest osmotic adjustment after 7 days (1.42x) with the greatest responses in higher-order salicylates and hydroxycinnamic acid conjugates of salicin; the populosides, playing a key role in drought tolerance of P. deltoides by lowering osmotic potential. Overall, the nature of drought onset, frequency of drought, and the severity of drought interacted to dictate the degree of osmotic adjustment and the nature of the organic solutes that accumulated.

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Primary Poster Presenter: Timothy Tschaplinski

The role of ABA-responsive elements in drought-inducible proline accumulation in barley (1100-090)
Hall 2

Proline is a compatible solute and has a significant role in drought response in plants. Our previous study showed that genetic variation in P5cs1 promoter between wild barley ISR42-8 and cultivar Scarlett was critical for the drought-inducible proline accumulation. In present work, we performed allele mining for P5cs1 promoter variation in sixty barley genotypes comprising wild accessions, landraces and cultivars. We found several haplotypes for the promoter variation including deletion alleles which resulted in the loss of ABA-responsive elements (ABREs) and MYB binding motifs. Loss of a single coupling element 3 in a wild barley HOR9840 caused a significant down-regulation of P5cs1 expression and reduced proline accumulation under drought compared to other haplotypes. Similar results were obtained when plants were subjected to external ABA treatment. Further, we identified and targeted four putative ABRE binding factors (ABFs) in barley using CRISPR-Cas9 system. We found four mutation events in two barley ABFs in the T1 generation. Selected T1 plants are grown in the greenhouse to obtain homozygous T2 mutants. In parallel, we purified recombinant proteins of putative barley ABFs to test the DNA-protein interaction using electrophoretic mobility shift assay to identify promoter elements in mediating P5cs1 transcription. As proof of concept, we measured the proline in Arabidopsis quadruple mutant (abf1abf2abf3abf4) and Col-0 upon 0 to 96 hours of ABA application. We observed significant up-regulation of AtP5cs1 and proline accumulation in both Col-0 and abf1abf2abf3abf4 after 48 hours. However, the shoot proline concentration in Col-0 was approximately three-fold higher after 72 and 96 hours of ABA application. These findings suggest the putative role of ABREs in regulating proline accumulation under drought. Characterization of barley ABF mutants and EMSA results will add further evidence to the proposed hypothesis.

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Primary Poster Presenter: Asis Shrestha

Two isoforms of trehalase with different subcellular localization produced from a single transcript (1100-091)
Hall 2

Trehalose is a non-reducing disaccharide which is well-known for its role in stress tolerance in microorganisms. While plants encode many trehalose biosynthesis-related enzymes, they only accumulate low levels of trehalose. These are insufficient for an osmolyte or stress protectant function, but consistent with a more sophisticated regulatory role for trehalose and its biosynthetic intermediate trehalose-6-phosphate. Interestingly, plants with reduced trehalose levels due to overexpression of trehalase (AtTRE1, hydrolyzing trehalose into two glucose molecules) showed increased drought stress tolerance in Arabidopsis thaliana (Van Houtte et al., 2013). The precise function of trehalose in drought stress tolerance and stress regulation of trehalase function in plants are not yet known. Moreover, AtTRE1 was reported to be a plasma membrane-bound protein with its catalytic domain oriented towards the apoplast, suggesting that it hydrolyzes extracellular trehalose (Frison et al., 2007). A. thaliana infection by Plasmodiophora brassicae (causing clubroot disease) results in trehalose accumulation and induction of AtTRE1 in infected organs (Brodmann et al., 2002), consistent with a role for the plasma membrane-anchored trehalase in the defense against trehalose producing and metabolizing pathogens. This leaves a puzzling question: how do plants metabolize endogenous trehalose? Here, we present the novel finding that in Arabidopsis two different isoforms of trehalase with different subcellular localization are produced from a single transcript. We are currently investigating their expression regulation and we are generating transgenic plants to characterize the exact functions of the two isoforms. The findings from this study provide new insight into the regulation of trehalase by different stress conditions and might provide new targets to enhance drought stress tolerance in plants.

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Applied: Biotechnology, Molecular Breeding

The impact of Brassica napus-Arabidopsis thaliana GL3 subdomain exchange on trichome production, gro (0200-056 (Screen 5))
Hall 2

GL3 is part of a regulatory complex that controls aspects of plant development, including trichome formation and anthocyanin production. Transformation of B. napus cv. Westar with A. thaliana AtGL3 genomic or cDNA constructs under the control of the constitutive 35S promoter produced lines highly abundant in trichomes. Both lines had comparable levels of trichomes, as well as other phenotypes including growth retardation, twisted/curled leaves and delaying flowering. Expression of the AtGL3 cDNA under the control of the cuticle-specific CER6 promoter did not produce high levels of trichomes nor any of the off phenotypes as observed with the 35S::AtGL3 cDNA construct. Expression of a B. rapa GL3 allele from a moderately hairy B. rapa line under the control of 35S promoter increased trichomes by 20%, whereas, expression of a B. rapa GL3 allele from a more hirsute accession increased trichome production by 80%. The two B. rapa GL3 proteins differ in only 5 amino acids (Ala>Glu242, Thr>His247, Leu>Phe339, Gly>Lys378 and Thr>Ile535). To investigate why the AtGL3 orthologue induces abundant trichome production while the B. napus or B. rapa GL3 orthologues do not, chimaeric GL3 proteins were generated. Constructs were created that exchanged amino acids 1-212 (involved in interaction of GL3 with MYB proteins), amino acids 212-401 (involved in interaction of GL3 with TTG1) and the carboxy terminal domain of the non-functional B. napus GL3 gene with those of the functional A. thaliana GL3 gene. When expressed in B. napus cv. Westar under the direction of the 35S promoter, the 1-212 and the 212-401 exchange constructs increased trichome production by 80%, but not to the level observed with AtGL3.

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ANTIFUNGAL AND PHYTOCHEMICAL ANALYSIS OF CURCUMA LONGA LINN. (TURMERIC) (0200-057 (Screen 11))
Hall 2

The effect of Curcuma longa Linn.(Turmeric) extract was carried out on the mycelium growth of fungi using direct poisoning method at the Laboratory of Plant Science and Biotechnology Department, Imo State University, Owerri, Imo State, Nigeria. Aqueous and ethanol extract were prepared from the Curcuma longa Linn. 0.5ml and 1ml of each of the extract were used to poison the media. Aspergillus niger (Van Tieghen.), Geotrichum candidum (Link.), Fusarium oxysporum (Schlecht.) and Rhizopus oryzae (Went & Prins. Geerl.) were isolated. For aqueous extract both at 0.5ml and 1ml concentration, Aspergillus niger showed the most percentage mycelium growth inhibition (91.10%) at 0.5ml and (96.70%) at 1ml being glaringly higher in percentage value occurrence than G. candidum (0.00%) at 0.5ml and (0.0%) at 1ml, F. oxysporum (75.00%) at 0.5ml and (75.00%) at 1ml and R. oryzae (0.00%) at 0.5ml and (0.00%) at 1ml. The ethanol test on the

extract of Curcuma longa Linn.(Turmeric) using all fungal isolated, showed that Aspergillus niger was as well the highest frequently occurring organism (73.30%) at 0.5ml and (74.45%) at 1ml than G. candidum (0.00%) at 0.5ml and (0.00%) at 1ml, F. oxysporum (45.00%) at 0.5ml and (52.50%) at 1ml and R. oryzae (84.40%) at 0.5ml and (87.80%) at 1ml. But the comparism between the two solution (aqueous and ethanol) against mycelia growth of fungi isolates, aqueous was confirmed the highest in the percentage mycelia growth inhibition. The result so far obtained are indicative of disease preventive properties of Curcuma longa Linn.(Tumeric).

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Turning a green alga red: engineering astaxanthin biosynthesis by intragenic pseudogene revival in C (0200-058 (Screen 5))
Hall 2

The green alga Chlamydomonas reinhardtii does not synthesize high-value ketocarotenoids like canthaxanthin and astaxanthin, however, a β-carotene ketolase (CrBKT) can be found in its genome. CrBKT is poorly expressed, contains a long C-terminal extension not found in homologues and likely represents a pseudogene in this alga. Here, we used synthetic re-design of this gene to enable its constitutive overexpression from the nuclear genome of C. reinhardtii. Overexpression of the optimized CrBKT extended native carotenoid biosynthesis to generate ketocarotenoids in the algal host causing noticeable changes the green algal colour to a reddish-brown. We found that up to 50% of native carotenoids could be converted into astaxanthin and more than 70% into other ketocarotenoids by robust CrBKT overexpression. Modification of the carotenoid metabolism did not impair growth or biomass productivity of C. reinhardtii, even at high light intensities. Under different growth conditions, the best performing CrBKT overexpression strain was found to reach ketocarotenoid productivities up to 4.5 mg L-1 day-1. Astaxanthin productivity in engineered C. reinhardtii shown here is competitive with that reported for Haematococcus lacustris (formerly pluvialis) which is currently the main organism cultivated for industrial astaxanthin production. In addition, the extractability and bio-accessibility of these pigments was much higher in cell wall deficient C. reinhardtii than the resting cysts of H. lacustris. Engineered C. reinhardtii strains could thus be a promising alternative to natural astaxanthin producing algal strains and may open the possibility of other tailor-made pigments from this host.

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A potential nanofiber platform for modulating abiotic stress in plant growth media (0200-010)
Hall 2

Electrospun nanofibers have been studied for potential applications for pest control, seed fungi protection, seed development, and bacterial and microbe introduction in the agriculture industry. However, to date, nanofibers have not been applied as a tool for sensing or inducing complex abiotic stress conditions. Therefore, designing a platform that mimics the complexity, frequency and intensity of stresses induced in plant growth media can potentially bridge the knowledge gap between lab and field stress conditions. In this study, we designed a nanofiber platform with three different compositions of Polyvinylidene fluoride (PVDF) and polyvinylpyrrolidone (PVP). PVDF is a widely applied material whose piezoelectric properties are highly desirable for converting abiotic stresses into electrical signals. These signals can then be used for computational modeling the intensity, fluency and frequency of stresses seen in the field. However, PVDF membrane is hydrophobic in nature, therefore PVP, a hydrophilic polymer is necessary to increase permeability of the platform. Here we present data characterizing the structure and stability of the nanofiber using X-ray photoelectron spectroscopy (XPS) and SEM analysis after plasma treatment. We also characterized the effect of nanofiber on Arabidopsis germination and growth. We found that while seedling root development was initially slower when grown on this nanofiber, there were no significant differences after 8 days, suggesting that this platform is plant friendly and can be potentially applied to agriculture. Future efforts include optimizing and functionalizing other nanofiber compositions.

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Primary Poster Presenter: Hsuan Chou

A Success Story of Grain Yield Improvement in Transgenic Maize (0200-001)
Hall 2

Maize is the most-produced crop for food, feed and industrial uses. Increasing maize grain yield has been a major focus of CORTEVA Agriscience™. Here, we

report that modulating expression of a maize MADS-box transcription factor gene under the control of a moderate-constitutive maize promoter results in maize plants with increased plant vigor, leaf biomass, leaf area, photosynthesis capacity and nitrogen utilization. These positive attributes create a significant increase in grain yield relative to the wild type controls that is consistent across years, environments, and elite germplasm. Molecular and biochemical characterization of the transgenic plants confirms that their enhanced agronomic traits are the result of elevated plant carbon assimilation and nitrogen utilization, which leads to enhanced crop performance.

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Primary Poster Presenter: Jingrui Wu

A Synthetic Peptide Encoded by a Random DNA Sequence Inhibits Red Light Responses During Seedling De (0200-007)
Hall 2

We have employed a reverse-chemical-genetics approach to identify a novel, synthetic plant growth regulator. Plants transformed with random DNA sequences produce synthetic peptides that affect plant biology. One specific peptide inhibits red-light-mediated photomorphogenic development in Arabidopsis thaliana. Seedlings expressing the PEP6-32 peptide exhibit longer hypocotyls and diminished cotyledon expansion when grown under red light. Other red-light-mediated seedling processes such as induction of Lhcb (cab) transcripts or loss of directional growth remained unaffected. Long-term responses, such as repression of flowering time, do not show defects in red light signaling or integration. A synthesized peptide applied exogenously induces the long-hypocotyl phenotype under red light in non-transformed seedlings. The results indicate that the PEP6-32 peptide causes discrete cell expansion defects during early seedling development in red light, mimicking weak phyB alleles. The findings demonstrate that new chemistries that control discrete facets of plant growth and development may be identified in

populations of plants expressing random DNA sequences giving rise to purely synthetic peptides.

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Primary Poster Presenter: Tautvydas Shuipys

Analysis of regenerative capacity in various potato cultivars (0200-014)
Hall 2

Potato (Solanum tuberosum L.) is the third most important food crop in the world. There are more than 4,000 potato cultivars, which have different physiological traits. However, regeneration or genetic transformation can apply to only several cultivars, since regeneration capacity of many potato varieties are largely unknown. In this study, we investigated regeneration capacities of 9 different potato cultivars: Bintje, ChuBaek, Desiree, Irish Cobbler, Red Norland, Red Pontiac, Russet Burbank, Superior, and Solanum phureja. We cultured leaf explants on a series of MS solid media containing phytohormones and vitamins to induce organogenesis. Shoot induction was started at 4 weeks and was observed to 8 weeks. Shoot regeneration capacity was the highest in Desiree (73.3%), but several other cultivars exhibited low or extremely low efficiency (e.g. Red Pontiac, almost 0%). We also measured rooting rate of regenerated shoots and found that cultivars showed different rooting capabilities (Desiree, 98%; ChuBaek, 50%). These data suggest that potato cultivars have very diverse regenerative capacities. Recently, many researchers have attempted molecular biological approaches to investigate the mechanism of regeneration. Our team also carried out stepwise expression analysis in the regeneration process of potato using significant genes that were found to be involved in callus induction or shoot regeneration. These studies will help improve the efficiency of crops or cultivars that are difficult to regenerate.

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Primary Poster Presenter: Ji-Sun Park

Assessment of abiotic stress-response regulators in alfalfa using RNAi

The livestock industry, which depends critically on our ability to grow forage crops in a highly productive manner, is an essential component of Canada’s agri-food sector. Of the perennial forages, alfalfa (Medicago sativa L.) is the most widely grown, with an estimated global cropping area of approximately 30 million

hectares. However, despite the many benefits of this species, adverse environmental conditions often have a severe negative impact on alfalfa production. Indeed, in Canada, alfalfa is often subjected to drought, saline soil, winter freezing and/or inappropriately timed frosts, as well as waterlogging, at various times throughout any given growing season. These factors are expected to become increasingly problematic in coming years due to climate change, as well as the use of intensive agricultural practices. As such, there is an imminent need to enhance the resilience of this crop to abiotic stress factors. The aim of this study is to therefore assess the effect of down-regulating a group of gene homologs in alfalfa that have previously been shown to act as negative regulators of stress response in other plant species. Six such homologs have been identified in alfalfa, and RNAi genotypes targeting each homolog have been generated and are currently under assessment. Such targets have the potential to be utilized downstream for the development of novel alfalfa cultivars with superior climate resiliency using either conventional breeding or genome editing platforms.

Chair and Concurrent Symposium Speaker: Surya Acharya,
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Primary Poster Presenter: Stacy Singer

Candidate gene analysis of chlorophyll content and leaf senescence using progeny from an interspecif (0200-008)
Hall 2

In plant, chlorophyll content and leaf senescence are associated with grain filling and yield. In this study, we developed introgression lines from an interspecific cross between O. sativa cv. Hwaseong and wild species O. graindiglumis. One of the introgression line CR7501, which showed stay-green phenotype, was selected and crossed with Hwaseong. For QTL analysis, 58 F3 and 38 F4 lines were analysed and QTL for chlorophyll content on chromosome 2, qCC2, was detected. To examine whether qCC2 is responsible for delayed senescence, dark-induced senescence assay was performed. CR2002 which has qCC2 O. grandiglumis locus showed delayed leaf yellowing and higher Fv/Fm value. In addition, endogenous expression levels of senescence marker genes and chlorophyll degradation genes in CR2002 were lower than in Hwaseong. qCC2 locus harbors the GW2 gene regulating grain width and weight. O. grandiglumis GW2 allele has 1-bp deletion of functional nucleotide polymorphism (FNP) on the 2nd exon, leading to increased grain size. GW2 encodes RING-type E3 ubiquitin ligase and this protein functions degradation of multiple target protein. To know whether GW2 is associated with stay-green phenotype, gw2-knockout mutant was examined in dark-induced senescence. gw2-knockout mutant showed delayed leaf senescence under dark condition with down-regulated expression of senescence associated genes. These results indicate that RING-type ubiquitin ligase GW2 possibly regulates leaf senescence.

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Primary Poster Presenter: Sang-Nag Ahn

CRISPR-mediated gene editing of lipase and lipoxygenase in rice to improve storage life (0200-040)
Hall 2

Oxidation of free fatty acids in the bran layer of rice (Oryza sativa) contributes to reduced shelf life of brown rice products. Lipase enzymes release free fatty acids from lipids in rice bran; lipoxygenases act on such fatty acids to produce rancid odor compounds. Several lipases and lipoxygenases expressed primarily in seed tissue are ideal targets for gene editing to eliminate their contributions to brown rice quality reductions. These include lipoxygenase-3 (LOX3) and a lipase designated L2. LOX3 has been mutated with TALEN-based gene editing and silenced by RNA interference, but targeting of LOX3 by CRISPR gene editing or of L2 via mutation or silencing has not been reported. LOX3 and L2 were individually and simultaneously targeted for mutation by CRISPR-Cas9 at two locations per gene in the model cultivar Nipponbare. Three T_0 lines with mutations in targeted regions of LOX3 were obtained, but to date, no seed has been recovered from these lines. Six lines with mutations in targeted regions of both LOX3 and L2 have been identified in the vegetative stage of T_0 plants. Seed was collected from two T_0 lines with mutations in the L2 gene. Resulting T_1 plants were screened for mutations and three distinct homozygotic lines were selected for grain production in the T_2 generation. Grain will be harvested at maturity and subjected to accelerated aging in rough rice and brown rice forms, and evaluated for lipoxygenase and lipase activities, free fatty acid content, conjugated diene formation, and seed longevity. Previous research has shown limited effects of LOX3 mutations on brown rice rancidity. With simultaneous knockout of L2, it is hoped that a synergistic effect will result in more drastic reductions in oxidation product formation.

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Primary Poster Presenter: Kathryn Haydon

Development and Validation of High-throughput PACE Markers in Cannabis sativa (0200-025)
Hall 2

Abstract: Cannabis sativa is a promising new crop grown for grain, fiber, and cannabinoids. High-throughput molecular markers for several major traits could be useful for breeding and production. Cannabis sativa is generally dioecious with no obvious differences in seed or early plant characteristics between sexes, but only

female plants are desired for cannabinoid production. The cannabinoid type (THC vs CBD) of Cannabis sativa is regulated by genes at a single non-recombining locus, but cannot be easily tested phenotypically before harvest, nor can it be reliably tested in male plants. Total cannabinoid content is also not testable before harvest or in male plants. While total cannabinoid content is multifactorial, the Aromatic Prenyltransferase (AP1) gene is known to be an important factor. We developed and validated low cost, high-throughput PACE markers for plant sex, cannabinoid type, and total cannabinoid content in Cannabis sativa. Using these markers, we found roughly equal distribution of genetic male to female plants across many dioecious cultivars, but some appeared biased towards males. We also found that some cultivars of hemp grown for seed, fiber, or CBD production had significant proportions of plants with active THCA synthase, which might contribute to non-compliant high levels of THC. Selection for a high activity AP1 gene was apparent in high-CBD cultivars. These markers will be useful in breeding and production of Cannabis sativa.

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Primary Poster Presenter: Jacob Toth

Development of a Gene-based Markers for the SUN Gene in Cultivated Tomato (0200-039)
Hall 2

Tomato fruit shape, which is the most visible characteristic among the other fruit trait, is considered to have a substantial influence on consumers. SUN is a gene to show a significant role in the elongated shape of various tomato varieties. SUN encodes a protein that is a positive regulator of growth resulting in elongated fruit and is hypothesized to alter hormone or secondary metabolite levels. However linked marker of SUN had been reported, in this study we found SNPs within SUN gene nucleotide sequence of the domestic breeding lines by re-sequencing and developed a derived cleaved amplified polymorphic sequence (dCAPS) markers. Developed dCAPS markers is helpful to discriminate of SUN gene variation. These molecular markers are expected to enhance the efficiency and accuracy of selection tomato fruit shape in breeding programs.

Primary Poster Presenter: Hyunjung Kim

Development of the second generation glyphosate-tolerant canola product MON88302 (0200-022)
Hall 2

The TruFlex Canola (MON88302) is a second generation glyphosate-tolerant canola productthat will provide growers with improved weed control and greater flexibility for glyphosateapplication. MON88302 contains one transgene for glyphosate tolerance. Through the use of aspecially engineered chimeric promoter that was designed to provide optimal expression of aglyphosate tolerance transgene, the expression of the CP4-EPSPS protein is enhanced in malereproductive tissues while maintaining high expression in other tissues. This enhancedexpression results in a wider window for glyphosate application in crop and protects yield.

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Primary Poster Presenter: Clayton Larue

Discovering genetic tools for flower color manipulation of Dendrobium orchids (0200-023)
Hall 2

In orchid cut-flower industry, flower color is the main factor that determines the commodity value. Orchid flower color is produced by two classes of pigments: anthocyanins and carotenoids. In orchids the largest variety of color is produced by anthocyanins. Dendrobium, the largest genus of the orchid family, display predominantly, purple, lavender and pink flowers due to cyanidin and peonidin accumulation. Blue delphinidin is absent in Dendrobium hybrids while orange pelargonidin is found in a few rare mutants. The enzyme dihydroflavonol 4-reductase (DFR) is a key enzyme that limit the color palette of flowers due to its substrate specificity. Petunia DFR cannot efficiently reduce dihydrokaepferol (DHK) to produce orange pelargonidin. However, in some plants such as Dendrobium, DFR and flavonoid 3'-hydroxylase competes for the same substrate, DHK. Our previous research demonstrated that enzyme competition for the common substrate DHK is the reason for the predominance of purple color in Dendrobium rather than the substrate specificity. Main objective of this research is to find the appropriate genetic tools to manipulate anthocyanin biosynthesis of Dendrobium towards orange pelargonidin production. We have isolated and cloned the DFR gene from Anthurium under the Cauliflower Mosaic Virus (CaMV) 35S promoter and nopaline synthase terminator. Resultant binary vector was inserted into petunia W80 mutant lacking DFR via Agrobacterium-mediated transformation. We compared the anthocyanin quality, quantity and flower color of the transgenic Petunia carrying Anthurium DFR with those containing Dendrobium and Antirrhinum DFR genes in the same Petunia host. Our results indicate the most robust orange color is

produced by Anthurium DFR compared to the DFRs of other two plant species. We are proposing to use a Dendrobium that is deficient in F3'H to reduce enzyme competition and transform it with Anthurium DFR in order to get robust orange colored Dendrobiums.

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Primary Poster Presenter: rasika Mudalige-Jayawickrama

Efficient production system of virus-tested apple plantlets using temporary immersion bioreactor (0200-029)
Hall 2

Efficient production of virus-tested apple plants is needed to meet the demand of the fruit tree industry as its fruit production has been decreased up to 30-50% by virus infection. In this study, shoot-tips (5mm in size) of in vitro grown apples were treated at the high temperature (32°C) or ribavirin 25 μg/ml to eliminate four viruses (ACLSV, etc.) and a viroid ASSVd from the virus infected apple trees. For the efficient production of virus-tested apple trees, in vitro M9 plantlets were cultured in temporary immersion bioreactor (TIB) system. TIB was treated with continuous immersion, for once per 3 hours (TIB-3) and once per 6 hours (TIB-6) for immersion time of the medium (control: solid & liquid culture). After 6 weeks of culture, the fresh weight of the harvested plants was around 182.6 mg in TIB-3 treatment, and the shoot length was about 2 times longer than those in the solid medium. The rate of root development was highest at 100% in TIB-6, whereas the rooting rate was lowest at 45.4% in liquid stagnant cultivation. Leaf area was the smallest in the plants grown in the control and frozen stagnant cultures, and well developed and wide leaves were observed in all bioreactor-grown plantlets. All bioreactor grown plantlets did not show hyperhydricity, and it is postulated to be the result of forced air ventilation in TIB system. Through this study, we prove that TIB can produce a large quantity of virus-tested plantlets than the conventional solid culture method, and it is an efficient system to produce healthy plantlets which are advantageous for acclimatization through the development of secondary xylems.Acknowledgment: This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(grant number 3105003-5).

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Primary Poster Presenter: So-Young Park

Engineered PPR10 RNA-binding protein for tissue-specific expression of recombinant proteins in potat (0200-044)
Hall 2

Non-green plastids are desirable for the expression of recombinant proteins in edible plant parts to enhance the nutritional value of tubers or fruits or to deliver pharmaceuticals. However, plastid transgenes are expressed at extremely low levels in the amyloplasts of storage organs such as tubers. Here we report a regulatory system consisting of a variant of the maize RNA binding protein PPR10 and a cognate binding site upstream of a plastid transgene encoding GFP. The binding site is not recognized by the resident potato PPR10 protein, restricting GFP protein accumulation to low levels in leaves. When the PPR10 variant is expressed from the tuber-specific patatin promoter, GFP accumulated up to 1.3% of total soluble protein, a 60-fold increase over 0.02%2, the maximum protein yield achieved to date in potato tuber. This regulatory system enables an increase in transgene expression in non-photosynthetic plastids without interfering with chloroplast gene expression in leaves.

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Primary Poster Presenter: Pal Maliga

Exploring the Proteome of Acourtia cordata roots producing the sesquiterpene benzoquinone, Perezone" (0200-003)
Hall 2

The roots of Acourtia cordata produce many compounds of terpene origin, including the sesquiterpene perezone. Perezone has several pharmacological activities and the antifeeding effects of the perezone derivatives against larvae of insect pests of agricultural importance and their phytotoxic activity were also reported. The present research was conducted to determine the protein composition and to identify differentially expressed proteins in plants that produce different amounts of perezone. The comparative proteomic analysis of the roots of A. cordata provided information to identify proteins expressed in this specific tissue with different levels of perezone production. More than 300 protein spots were reproducibly resolved in the two-dimensional gels electrophoresis, in which 1 spot was up-regulated and 3 spots were down-regulated in the group of high against low producers of perezona. These proteins spots were analyzed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and peptide mass fingerprint database searching. Some selected protein spots were identified: PP134_ARATH-Pentatricopeptide repeat-containing protein At1g80150, mitochondrial (Arabidopsis thaliana), XP_017604040.1-Predicted nodulation protein H-like (Gossypium arboreum) and XP_022021167.1-F-box/FBD/LRR-repeat protein At1g13570-like (Helianthus annuus). This is the first proteomic study reported for A. cordata that is a species without sequence and annotation of its genome, proteomic research with a model like this one is very challenging, however, the proteins identified to give a general view of the proteomic profile, specifically of the roots of this plant species. This work will open future research paths, especially in the non-model species that are also considered an important species in the group of medicinal plants.

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Primary Poster Presenter: Ma del Carmen Garcia Mendez

Factors Effect on Biomass and Bioactive Compounds Production in Air-Lift Bioreactor System and Rapid (0200-026)
Hall 2

Advance in bioreactor culture showed a useful method for both biomass production and bioactive compounds accumulation in adventitious root cultures of medicinal plant. In this study, various parameters such as different auxins types and concentration (IBA and NAA), MS medium salt strength (¼□2-fold strength of MS), and sucrose concentration (0□10%), were optimized in a 3-L balloon type bubble bioreactor (BTBB). The results show that IBA was more effect on root growth than NAA; low MS salt strength increased bioactive compounds but inhibited in root biomass. The most effectively on biomass and bioactive production were achieved on adventitious root culture in full-strength MS medium supplemented with 2 mg·L-1 IBA, 5% sucrose after 4 weeks of culture. The optimized culture conditions resulted in maximum root biomass (98.46 g·L-1 FW, 13.46 g·L-1 DW), and bioactive compounds (53.08 mg·g-1 DW phenolics and 25.10 mg·g-1 DW flavonoids). Furthermore, adventitious roots under different culture conditions (3 and 5% sucrose, flask, 5-L, 20-L bioreactor, and adventitious root samples were treated with elicitors (50 and 100 μM MeJA, and 50 and 100 μM SA), and natural roots were analysed by Fourier transform infrared (FT-IR) methods to determine whether metabolic fingerprinting for whole cell extract can be used to discriminate and compare metabolic equivalence in Polygonum multiflorum root samples. The FT-IR results showed that the whole metabolic pattern from adventitious roots under different culture condition was similar. However, adventitious root in pilot-scale bioreactors could be discriminated with the other. These results are useful for large-scale cultivation of Polygonum multiflorum adventitious roots for industrial purpose.

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Primary Poster Presenter: So-Young Park

Fine mapping of the qSCT12UK locus, a major QTL for seedling cold tolerance (0200-009)
Hall 2

Rice seedling is easily damaged to low temperature and results in yellow leaf, growth retardation, reduced tiller number, which can cause severe yield losses. Three hundred eighty-six RIL population was evaluated cold tolerance under

5~13℃ for 14 days in the growth chamber followed with recovery for 4 days. QTL analysis was performed with QTL IciMapping program. Three SCT (Seedling cold tolerance) QTL, qSCT12UK, qSCT11.1UK, qSCT11.2UK, were detected on chromosome 11and 12. Among these QTLs, qSCT12UK, a major QTL, on chromosome 12 showed 26.3 of LOD score with 25.5% of phenotypic variation. We developed 28 additional Indel markers to narrow down the position of qSCT12 UK. The position of the qSCT12 UK was delimited to 478kb region between InDel12-29 and InDel12-30. On the other hand, we selected 48 lines harboring qSCT12UK using MAS 686 BC2F8 BILs population. Selected 48 lines harboring qSCT12UK showed higher level of cold tolerance than Hanareum2. Finally, we selected 3 lines harboring qSCT12UK with good agronomic characters. This elite lines will be useful for development of super yielding rice in the temperate regions.

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Primary Poster Presenter: Sumin Jo

Functional analysis of soybean chloroplast vesiculation genes: progress and prospects. (0200-016)
Hall 2

Soybean is the most important crop in terms of cultivated area in the Mercosur area. Grown during summer, soybean is frequently exposed to drought periods which cause great economic losses. Identification of genetic components involved in stress responses will contribute to crop breeding for drought tolerance. We have identified two soybean genes encoding Chloroplast Vesiculation proteins (GmCV1/GmCV2) which are induced under stress. GmCV transcripts accumulate in roots under drought stress. CV proteins were previously described in Arabidopsis and rice as proteins that participate in chloroplast protein degradation during stress-induced senescence. AtCV silencing resulted in enhanced chloroplast stability and delayed senescence under stress. We have addressed the function of GmCV genes by the generation of constructs for silencing and generating knockout mutants of these genes using CRISPR/Cas9 system. Furthermore, we have generated constructs and transgenic plants for identification of GmCV subcellular localization and interactors. Analysis of CV1 and CV2 promoter activity upon treatments with salt, methylviologen, nitrogen or light deprivation and natural senescence showed that these genes were upregulated in response to stress treatments or senescence. There was a marked differential promoter activity

between these two genes in roots and aereal plant tissues, suggesting functional specialization between CV genes in soybean.

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Primary Poster Presenter: Andrea Fleitas

Genetic variation and development of a SCAR marker of anemone-type flower in chrysanthemum (0200-012)
Hall 2

An anemone shape featuring elongated and pigmented disk florets is an important target in breeding for flower shape in chrysanthemum. A good understanding of the inheritance of anemone-related traits and specific molecular markers are vital for accelerating the breeding progress. In this study, two segregating populations of anemone-type 'Nannong Xuefeng' × nonanemone-type 'QX096' and same anemone-type 'Nannong Xuefeng' × 'Monalisa' were employed to investigate the genetic variation of anemone traits, the anemone-specific markers were developed via the bulked segregant analysis (BSA) method by constructing two extreme bulks from each 10 anemone-type and nonanemone-type F1 lines, respectively. A moderate high level of variation coefficient (~ 24 to 57%) was obtained for most tubular floret traits relevant to the anemone type in both segregating populations. The morphology of the hybrids was intermediate between their parents, and some transgressive individuals were observed, indicating great potential for the selection of lines with the desirable anemone type. In the BSA, 4 sequence-related amplified polymorphism (SRAP) primer combinations were identified as informative between the bulks of the anemone and nonanemone F1 individuals. After cloning and sequencing, only one SRAP marker, i.e., M11E1_272, was successfully converted to the sequence-characterized amplified region (SCAR) marker SCAR168. This marker was validation in the two F1 populations demonstrated that SCAR168 could truly discriminate two types with a high coincidence ratio 87.86% ~ 92.5%. In addition, a general linear model (GLM) based association analysis ($P < 0.01$) revealed that the SCAR marker exhibited a positive effect on the anemone traits and explained 20.83% ~ 33.66% phenotypic variation. Results of the current study show the inheritance pattern of anemone traits, and the developed anemone-specific SCAR marker paves the way for the marker-assisted selection of flower shape in chrysanthemum.

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Primary Poster Presenter: Xiaodong Yang

High-speed regeneration via somatic embryogenesis in elite Indian banana variety Somrani monthan (AB (0200-027)
Hall 2

The present study reports successful regeneration system for an elite Indian Musa cv. Somrani monthan via somatic embryogenesis. Meristematic shoot tips were used as explant and cultured on MS medium supplemented with 6-BAP and IAA for scalp (cauliflower like compact buds) induction. The highest percentage (~ 60%) of homogenous differentiation of shoot tip explants into scalp was observed in MS medium supplemented with 100 µM of 6-BAP and 1 µM of IAA after 6 months of culture. The scalps were sub-cultured on MS medium supplemented with different compositions of 2, 4-D (1, 2, 3, 4, 5 and 10 µM) and zeatin (1 and2 µM) for embryogenic calli induction. The highest frequency (97%) of embryogenic calli induction was recorded in MS medium supplemented with 5 µM 2, 4-D and 1 µM zeatin after 6 weeks of culture. The histological and morphological studies suggest that, callus possessed high competence for embryogenesis and could be induced to form somatic embryos. Embryogenic cell suspension (ECS) was successfully established by culturing these embryonic calli in liquid medium. About 70% of the mature somatic embryos germinated and converted to plantlets with a regeneration capacity of 8.5 × 1000. After the gradual acclimatization and hardening, the plants were transferred to nursery with 82% of survival rate. The embryogenic callus and ECS obtained in this study are the most ideal explants for mass clonal propagation, germplasm conservation and genetic transformation for future research.

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Primary Poster Presenter: Anjana Rustagi

Identification of Heat-Induced Cysteine-containing Proteomes in Tomato Flower Buds Using iodo-TMT Pr (0200-031)
Hall 2

Yaoguo Qin, Hui Li, Theodore Thannhauser, Yong Yang, Suping Zhou*. For this study, the tomato cultivar ‘Micro-Tom’ plants were grown at 32/26oC (with a 12-h; day/night cycle) for the heat-treated plants and at 26oC constant for the non-heat treated ones. After two weeks of heat treatments, some flower buds turned brown and died. Death of flower buds was taken as the indication of heat stress (HS). Flower buds with no visible physical damage between 2.0-2.5mm in length were harvested. The HS-induced proteome profiling was performed on these flower buds using the cysteine-specific iodoacetyl isobaric tandem mass tags (iodoTMT) proteomics analysis. Proteomics analysis led to the quantification of 1234 proteins which were reported with two or more quantified peptides and 144 significantly changed proteins (SCPs) were identified. The SCPs passed the threshold values of

fold change >1.5 SD (Abundance ratio of HS treated/control:1.36-3.80) for up-regulated and <-1.5 SD (Abundance ratio of HS treated/control:0.69-0.38) for down-regulated proteins, at P ≤ 0.05. The 48 HS-down-regulated and 96 HS-up-regulated SCPs were organized into 39 gene ontology (GO) biological processes. These included gene transcription, protein translation, metabolic pathways and stress responses, among others. Many of the SCPs identified in flower buds were associated with molecular functions that enhanced thermo-tolerance in cells. This study is providing the first report of HS-induced proteomic changes in tomato flower buds prior to the microsporocyte stage. This project was funded by USDA-NIFA.

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1:30 PM - 3:00 PM

Identification of transcriptome-wide nut weight associated SNPs in Castanea crenata (0200-021)
Hall 2

Nut weight is one of the most important traits affecting chestnut grower's economic success. Due to the long juvenile phase in chestnuts, the selection of desired heritable characteristics at early ages is the major challenge for chestnut breeding. In this study, we identified single nucleotide polymorphisms (SNPs) for nut weight in chestnuts (Castanea crenata) through transcriptome-wide association analysis (TWAS). RNA-Seq data were generated from large- and small-nut trees using Illumina HiSeq2000 and identified 3,271,142 SNPs. A total of 21 putative SNPs were significantly associated for chestnut weight (FDR <10-5) from further analyses. We also carried out five machine learning (ML) algorithms, SVM, C5, k-NN, PLS, and RF, using 21 SNPs to predict the nut weight in another population. Consequently, the prediction accuracy of ML algorithms on chestnut weight was more than 68% in average. Taken all together, we suggest that these SNPs have potential to be used in marker-assisted selection for breeding large chestnut-bearing varieties.

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Primary Poster Presenter: Hyoshin Lee

Identifying Genome Editing Targets: Using Metabolism to Rationally Mine Genomics Data (0200-033)
Hall 2

Developing technologies to achieve step change increases in crop yield remains a critical unmet need in agriculture and a key challenge for future global food security. Yield10 is focused on achieving step change increases in the inherent yield

of major food and feed crops. This will likely require multiple gene modifications to increase photosynthesis and efficiently deliver the increased photosynthate to the desired organ, either harvestable seed or biomass depending on the crop. On some level, a plant phenotype or trait reflects altered metabolism due to genetic variation. Yield10 is leveraging our unique expertise in metabolic engineering to identify genes to achieve traits such as increased crop yield by using modeled metabolic changes to inform the mining of large-scale genomic data sets. This trait agnostic approach provides a unique and powerful method to identify smart gene targets.

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Identifying mutants having reduced seed coat fiber and increased protein content to improve seed mea (0200-018)
Hall 2

Pennycress (Thlaspi arvense) is an oilseed plant of the Brassicaceae family, native to Eurasia and naturalized to North America. Wild strains grow widespread throughout temperate regions of the world. Once commercialized, the oilseeds of elite pennycress varieties will provide additional income to farmers and agribusinesses thereby strengthening rural communities. Pennycress will also provide ecosystem services as a cover crop, reducing soil and nutrients runoff from otherwise vacant farmland. About two-thirds of the value of the pennycress seed is in the oil, which can be extracted by crushing and used as a biodiesel or biojet fuel feedstock. The remaining one-third value is the left-over seed meal, which can be used as a protein supplement in animal feed. However, the meal from wild pennycress strains is of relatively low quality due to the seed coat having high fiber content. Fiber is composed of lignin, cellulose, hemicellulose, and condensed tannins. To reduce pennycress seed coat fiber content, we used a forward genetics approach to screen through our EMS mutant populations for light-colored seeds indicative of reduced tannins, as well as a reverse genetics approach employing CRISPR genome editing to knock out function of the majority of genes in the flavonoid biosynthetic pathway responsible for producing condensed tannins (so-called TRANSPARENT TESTA genes). Here we will present data characterizing these mutants including identified effects on the agronomically-relevant traits seed coat fiber content, seed protein and oil content, and seed dormancy.

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Image analysis of agricultural traits in rice cultivar (0200-005)
Hall 2

Plant phenomics is a nondestructive analyzing methodology using image information of various phenotypes. In this study, rice was used as a model plant for application of phenomics approaches. Agronomic traits of rice were measured using parameters such as leaf area (LA), leaf width (LW), leaf color, projected plant height (PPH), convex hull (CVH), center of mass Y (COMY), compactness (COMP), and eccentricity (ECC) with a recombinant inbred lines (RILs) population derived from a cross between 'Milyang23' and 'Gihobyeo'. In seedling stage (2 and 4 weeks after sowing), two major growth related QTLs were discovered at semidwarf-1 (sd-1) region of chromosome 1 and loci of chromosome 12. In vegetative stage (6 and 8 weeks after sowing), growth related QTLs were detected at chromosomes 1, 2, 3, 7, 9, 11, and 12. Phytochrome B mutants (osphyb) was also investigated to analyze agronomic traits for drought stress. In recovery stage after drought stress, osphyb was more increased than WT in the leaf area (LA) and leaf width (LW) of RGB and water contents of Near infrared (NIR). It definitely well reflected drought resistance of osphyb. Besides, we are also trying to minutely detect nitrogen deficient traits through color classification of green color in leaf. We are gradually optimizing various traits analysis, and it will be widely applied for improving accuracy for crop breeding and phenotyping. * Corresponding author: Tel. 063-238-4658, Email: biopiakim@korea.kr

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Improve Agronomic Characters of Tomato with Site-directed Mutagenesis of SlBRI1 Phosphorylation Site (0200-042)
Hall 2

Brassinosteroids(BRs) play a crucial role in plant growth, development and stress resistance and the Brassinosteroids Insensitive 1(BRI1) is the bottle-neck component in BR signaling. Mutagenesis of Arabidopsis BRI1 phosphorylation sites implied the strong potential in agriculture. To mine the most potential of BR signal pathway in tomato the multiple phosphorylation sites of SlBRI1 mutated for mimic phosphorylation and non-phosphoryaltion displayed extensive modification in critical agronomic characters, which include stem diameter, the time of flowering , the number of fruits, the quality of fruits, the yield, as well as the heat resistance.

The study indicates that there are large potential to improve agronomic characters with mutagenesis of SlBRI1 phosphorylation sites in tomato.

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Primary Poster Presenter: xiaofeng wang

1:30 PM - 3:00 PM

Investigating the Phytotoxic Activity and Mode of Action of a New Bioherbicide

Spliceostatin C (sp C) is a natural product isolated from soil bacteria Burkholderia rinojensis. The chemical structure of sp C is similar to spliceostatin A (sp A) which was characterized as an anticancer agent and splicing inhibitor. Phytotoxicity analysis revealed that sp C exhibited strong phytotoxic activity against Palmer amaranth and several other weed species. Spliceostatin C also significantly affected the growth of Arabidopsis thaliana seedlings by triggering leaf bleaching with IC50 of 2.2 μM. To elucidate the inhibitory functions of sp C in plant, 7-day-old Arabidopsis seedlings were treated with sp C at the concentration of IC50, and RNAs were extracted for semiquantitative RT-PCR (RT-sqPCR) analysis. Our results showed that among 20 genes that were selected for the assays, five transcripts (tubulin alpha-5, mRNA splicing factor, SF3b14b, flowering focus M and circadian clock associated 1) underwent intron rearrangements such as intron retention and alternative 5′ or 3′ splicing site upon exposure to sp C. The expression levels of the rest of the genes were either increased or decreased significantly. To investigate the impact of sp C on gene expression further, we performed a global proteome profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. After exposure to sp C for 6 h, 145 protein isoforms were identified with fold change greater than 1.5 ($p \leq 0.05$), and among them, 134 were decreased and 11 increased. KEGG pathway analysis revealed that these proteins are associated with metabolic pathways, carbon metabolism, ribosome, and biosynthesis of secondary metabolites pathways. Further analysis of these proteins could provide insight into the mechanisms of inhibition of sp C in plant cell. Joanna Bajsa-Hirschel1, Zhiqiang Pan1, L.G. Boddy2, Michal Sabat2, Scott R. Baerson1 and Stephen O. Duke1

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Primary Poster Presenter: Joanna Bajsa-Hirschel

Isolation and characterization of lycopene ε-cyclase mutants for provitamin A biofortification (0200-024)
Hall 2

Vitamin A is an essential micronutrient for humans, the deficiency of which has been recognized as a public-health issue in developing countries. Generation of staple food crops (e.g. wheat) with high β-carotene (the most efficient provitamin A carotenoid) accumulation will contribute to long term alleviation of vitamin A deficiency. Grains of tetraploid wheat (Triticum turgidum ssp. durum) accumulate mainly lutein (containing one ε-ring and one β-ring), a non-provitamin A carotenoid competing with β-carotene (containing two β-rings) for the common biosynthetic precursor lycopene. Lycopene ε-cyclase (LCYe) catalyzes ε-ring cyclization of lycopene and directs lycopene to the lutein-branch of carotenoid metabolism. To reduce lutein accumulation and increase β-carotene levels in wheat grains, we attempted to block the LCYe activity by isolating mutants of the LCYe homoeologs (LCYe-A and LCYe-B) in tetraploid wheat. Loss-of-function Targeting Induced Local Lesions in Genomes (TILLING) mutants of LCYe-A and LCYe-B were identified and crossed to generate the lcye-a lcye-b double mutant. Our results on isolation and crossing, phenotypic and biochemical characterization, as well as gene expression analysis of the lcye single and double mutants will be presented.

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Primary Poster Presenter: Shu Yu

LARGE ROOT ANGLE1, encoding OsPIN2, is involved in root system architecture in rice (0200-032)
Hall 2

Roots play a central role in plant growth and development through providing anchorage and taking up nutrients and water from the soil. The root system architecture in the soil determines the scope of resources available to plants and responds to environmental condition. Root growth angle (RGA) is a vital constituent of root system architecture and is used as a parameter for variety evaluation in plant breeding. Large RGAs are now being deployed as targets in crop breeding programs for improving nutrient uptake efficiency in stressful soil environment. However, little is known about the underlying molecular mechanisms that determine root growth angle in rice (Oryza sativa). In this study, a rice mutant large root angle1 (lra1) was isolated and showed a large RGA and reduced sensitivity to gravity. Genome resequencing and complementation assays revealed that OsPIN2 is the gene responsible for the mutant phenotypes. OsPIN2 was mainly expressed in roots and the base of shoots, and showed polar localization in the plasma membrane of root epidermal and cortex cells. OsPIN2 plays an important role in mediating root gravitropic responses in rice and is essential for plants to produce normal RGAs. 3-D root system reconstruction using uCT imaging technique (a non-destructive imaging method) confirmed that the lra1 shows a shallow root growth

phenotype in soil. Taken together, our findings suggest that OsPIN2 plays an important role in root gravitropic responses and determining the root system architecture in rice by affecting polar auxin transport in the root tip.

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Primary Poster Presenter: Lingling Wang

Maize-Produced Porcine Epidemic Disease Virus Spike Protein Elicits Antibody Response in Pigs (0200-020)
Hall 2

Porcine Epidemic Diarrhea Virus (PEDV) causes severe diarrhea and mortality in newborn piglets. Existing vaccines based on inactivated virus provide only partial protection and more effective vaccines are needed. The PEDV spike protein has shown great promise in many studies as a subunit vaccine candidate. However, this candidate has been difficult to express as a recombinant protein. In this work, we have expressed several versions of the spike protien S1 subunit in transgenic maize. The highest levels of accumulation were obtained in constructs targeted to the endoplasmic reticulum or as fusions with the E. coli heat-labile enterotoxin (LTB) or a dendritic cell binding peptide. Oral administration of approximately 0.8mg per dose of the S1 maize material to pigs elicited serum neutralizing antibodies, suggesting that this approach may be practical for a new type of PEDV vaccine.

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Primary Poster Presenter: Erin Egelkrout

Metina: A Transcription factor involved in Iron Deficiency Tolerance in Arabidopsis thaliana (0200-030)
Hall 2

Iron (Fe) is vital micronutrient for living organisms. Plants are the principal source of dietary Fe. Fe deficiency leads to developmental defects and excess can cause toxicity. Plants tightly control the Fe homeostasis for optimal Fe absorption. In order to identify new key players in maintaining Fe homeostasis, the molecular components involved from Fe acquisition from root and transportation to sink are required to be studied comprehensively. To identify key players in Fe homeostasis, IRT1 (Iron Regulated Transporter 1) promoter-driven luciferase (PIRT1:LUC) system was used for genetic screening. This reporter system is activated under Fe-deficient conditions and repressed under Fe-sufficient conditions. EMS (Ethyl Methane

Sulfonate) mutagenesis approach was used to screen novel candidates. The idt1 (Iron Deficiency Tolerant 1) mutant was identified with constitutive IRT1/IRT1 expression. The Fe specific mutant idt1 is Metal Tolerance and Iron Accumulator (Metina) i.e. resistant to Fe deficiency, excess zinc (Zn), cadmium (Cd), copper (Cu), cobalt (Co), nickel (Ni) and lead (Pb) and accumulates more Fe. Quantitative analysis for Fe accumulation in idt1 shows that in excess Cd, Zn and other heavy metals the Fe content is higher. Transcriptomic analysis reveals that Fe deficiency signaling pathway including IRT1, FRO2 (Ferric Reduction Oxidase 2), FIT (Fer Like Iron-Deficiency Induced Transcription Factor) and bHLH100/101/38/39 is constitutively expressed in idt1. Optimal overexpression of IDTA320V in WT leads to "Metina" phenotype which results in enhanced protein localization in nucleus. Current data indicates that optimal expression of IDT can improve Fe bio-fortification in crops and counterbalancing the heavy metal toxicity which manifests its importance in phytoremediation..

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Primary Poster Presenter: Reena Sharma

Molecular and physiological characterization of cold stress-induced Lignin-forming peroxidase in swe (0200-002)
Hall 2

Peroxidases (PODs) are major enzymes regulating production and scavenging of reactive oxygen species by catalyzing the redox reaction between hydrogen peroxide (H2O2) and various substrates. The secretory class III plant PODs play key roles in plant growth and development under biotic and abiotic stress, and are involved in lignification by polymerization of monolignols. However, the functions of PODs under cold stress in sweetpotato [Ipomoea batatas (L.) Lam] has not been elucidated in detail. Tropical-origin sweetpotato plants are sensitive to low temperature. Previously, a strongly cold-induced Lignin-forming peroxidase gene (designated IbLfp) was identified from transcriptome analysis in cold-treated storage roots of sweetpotato. In this study, we isolated the IbLfp from sweetpotato for molecular characterization in transgenic sweetpotato plants. The expression level of IbLfp was the highest in fibrous roots than in other tissues of sweetpotato. Transcripts of IbLfp were highly increased under cold (4℃) and heat (45℃) conditions in both leaves and fibrous roots, whereas it was decreased under drought (30% PEG) and saline (200 mM NaCl) stresses in leaves. To investigate the physiological functions of the IbLfp in sweetpotato, the three transgenic sweetpotato plants (LP2, LP3 and LP8) with high transcript level of IbLfp by CaMV 35S promoter were generated for further characterization. POD activity reacted by oxidoreduction between H2O2 and guaiacol as a substrate were higher in LP plants than non-transgenic plants. LP plants showed enhanced tolerance to oxidative stress induced by methyl viologen (5 μM) using leaf discs. Further characterization of LP plants are under investigation in terms of stress tolerance of various abiotic stresses including low temperature and functions of IbLfp on lignification during the

formation process of storage roots in sweetpotato. The storage ability of LP plants under low temperature will be also tested using the storage roots.

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Primary Poster Presenter: Chan-Ju Lee

Optimization of maize microspore selection at the early stages of anther development (0200-015)
Hall 2

Root hairs (RH) are single cells that develop from a group of specialized epidermal cells referred to as trichoblasts while cells that lack them are called atrichoblasts. RH cell fate is regulated by a complex of transcription factors that promotes the expression of the homeodomain protein GLABRA 2 (GL2), which ultimately blocks the RH pathway by inhibiting ROOT HAIR DEFECTIVE 6 (RHD6). The suppression of GL2 expression triggers epidermal cells to enter into the RH cell fate program by the concomitant activation of RHD6 and a downstream series of TFs including ROOT HAIR DEFECTIVE 6 LIKE-4 (RSL4) and downstream target genes. Cell fate in the root epidermis is influenced by phytohormones like auxins and Brassinosteroids (BR). It has been shown that in the absence of BR, phosphorylated BIN2 (a Type-II GSK3-like kinase) promotes the inhibition of a protein complex leading to the down-regulation of the main RH repressor GL2. In this work, an arabinogalactan protein (AGP) mutant agp21 as well as β-Glucosyl Yariv (β-Glc-Y) treatment (that disturbs AGPs) and several mutants deficient in AGP modifications, all trigger an abnormal RH cell fate phenotype reminiscent of mutants with deficient BR responses. We have found that an O-glycosylated AGP21-peptide positively regulated by BZR1, impacts on RH cell fate by disturbing GL2 expression in a BIN2 dependent manner. Together, these results show that disruption of cell surface AGPs, and in particular AGP21, interfere in a specific manner with BR perception and BIN2 mediated responses on the RH repressor GL2 in root epidermal cells in Arabidopsis thaliana.

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Primary Poster Presenter: Kyung-Hee Kim

Orange protein with a single amino acid substitution enhances carotenoid contents in sweetpotato (0200-004)
Hall 2

In plants, carotenoids play essential roles in light-harvesting processes and protect the photosynthetic machinery from photo-oxidative damage. In our previous

studies, Orange gene (IbOr) from sweetpotato [Ipomoea batatas (L.) Lam] was isolated, which is involved in accumulation of carotenoids. IbOr protein with a holdase chaperone activity post-transcriptionally regulates phytoene synthase (PSY), an important enzyme in the carotenoid biosynthetic pathway. IbOr protects IbPSY stability, which leads to carotenoid accumulation and confers enhanced tolerance to heat stress at 47oC and oxidative stress in IbOr transgenic sweetpotato plants. In addition, IbOr interacts with oxygen-evolving enhancer protein 2-1 (PsbP), an extrinsic protein of the oxygen-evolving complex (OEC) of PSII, and the holdase chaperone function of IbOr can protect PsbP from heat-induced denaturation. In this study, substitution of a single amino acid (R96H) in a wild-type IbOr shows dramatically enhanced carotenoid accumulation by up to 30-fold in the transgenic sweetpotato calli. IbOr-R96H transgenic calli also showed enhanced tolerance to salt stress compared with IbOr-WT. To further explore the function of IbOr-R96H and its utilization to develop various industrial plants with enhanced carotenoid content and tolerance to abiotic stresses, transgenic sweetpotato plants overexpressing IbOr-R96H were successfully generated and are under characterization. We anticipate that IbOr-R96H transgenic sweetpotato plants will enhance production of carotenoids and various environmental stress tolerances for sustainable agriculture on marginal lands.

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Primary Poster Presenter: Chan-Ju Lee

Overexpression of OsPSTOL1 in wheat increases nutrient uptake and yield in low phosphate conditions (0200-011)
Hall 2

The protein kinase PHOSPHORUS STARVATION-TOLERANCE 1 (OsPSTOL1) has been shown to improve root biomass and grain yield in rice (Oryza sativa L.) under low phosphorus (P) conditions. However, the mechanism underlying OsPSTOL1 function and its role in other crop species is not known. In this study, we demonstrate that ectopic overexpression of OsPSTOL1 in spring wheat (Triticum aestivum L., cv. Fielder) leads to more vigorous growth and increased yield under replete and P limiting conditions. Transgenic lines displayed increased root growth and P uptake when grown in a sand-Green Grades Profile™ mixture. RNA-seq data from the roots and crowns under different P availability reveals alterations in core phosphate response as well as new insights into metabolism and root plasticity regulated by P availability and ectopic expression of OsPSTOL1. This work further elucidates the function of OsPSTOL1, and sets the groundwork for further development of low P tolerant crops.

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Physiological growth assessment of chloroplast-engineered tobacco to optimize foreign protein yield (0200-045)
Hall 2

Plant genetics is rapidly evolving into a tool with the potential to solve many of today's problems. Crops have already been engineered with improved tolerance to environmental stresses, stronger resilience to pests, and enhanced nutritional quality. Another less common application of this technology harnesses engineered plants as production platforms for high-value products, like the proteins used in industrial manufacturing, medical treatments, and animal feed additives. In particular, the unique, quasi-bacterial biochemistry of the chloroplast has spurred interest in chloroplast-engineering as a method of yielding massive amounts of recombinant proteins. However, recombinant protein production is burdensome on the host plant and can impede host plant growth. Little is known regarding how plants accommodate heterologous protein expression or how to fine-tune growth conditions to optimize foreign protein accumulation. Here, we present a comprehensive study of how a recombinant cellulase alters growth, hormone metabolism, and resource use throughout the life of the chloroplast-engineered tobacco line, TetC-cel6A. We also study the effects of altered ammonium nitrate and enhanced carbon dioxide on heterologous protein synthesis and plant health. Through these trials, we have found that chloroplast-engineered tobacco can be a cost-effective means of producing large quantities of high-value proteins as long as the plant's unique resource needs are recognized and met.

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Plant-Made Antibody Purification by Aqueous Two-Phase Separation (0200-019)
Hall 2

Monoclonal antibody (mAb)-based drugs have revolutionized the treatment of a broad-ranged diseases. Plant-based production platforms are a viable and less expensive option for producing these valuable biologics. However, the purification of mAbs using the current industrial standard, Protein A affinity chromatography, remains the major bottleneck for large-scale production and accounts for most of the production cost. Moreover, there is not an efficient bulk separation step for plant-made mAbs, resulting in the fouling of Protein A resin and compounding the financial burden of the chromatography step. Here, we explored the utility of a hydrophobin - protein A fusion protein (H-PA) in purifying mAbs. Our results showed that an anti-West Nile virus (WNV) mAb (E16) and H-PA were efficiently expressed separately in Nicotiana benthamiana. The plant-derived H-PA molecule retained its surfactant-like behavior. When plant extracts containing the mAb and

H-PA were mixed, H-PA was found to bind the mAb specifically and the H-PA/E16 complex was efficiently separated from most plant host proteins by aqueous two-phase separation (ATPS) with an inexpensive detergent (Triton X-114). Recovery of the plant-made mAb through ATPS is comparable to Protein A affinity chromatography. A flow cytometry-based functional assay demonstrated that E16 retained its specific WNV antigen recognition after ATPS. Co-expression of E16 and H-PA in the same plant was also investigated with competing and non-competing viral-based expression vectors. Our preliminary data indicate that co-expression of the mAb and the H-PA fusion has potential to streamline mAb production and purification in plants, albeit further optimization is required. Taken together, our results suggest H-PA-based ATPS can be utilized as a more efficient and cost-effective bulk separation step in plant-made antibody purification than those being used currently.

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Primary Poster Presenter: Collin Jugler

Rapid and efficient large-scale Agrobacterium-mediated site-specific integration in elite maize inbr (0200-035)
Hall 2

Here we report the implementation of a rapid and efficient Agrobacterium-mediated site-specific integration (SSI) system for maize product development. A total of 163 DNA constructs each containing 1-3 trait gene cassettes were inserted into SSI landing sites at the same genomic location in two elite maize inbreds. Over 7500 SSI events were regenerated to the plantlet stage with a transformation efficiency of ~7%. Characterization of the SSI event structure in these regenerated plants by qPCR identified 58% contained the desired single-copy DNA insertion at the targeted site and with no detectable vector backbone sequence transferred. The transformation efficiency across donor constructs with 1, 2 and 3 trait gene cassettes was similar. Through further process optimization on one inbred, the transformation efficiency was increased from 9.1% to 11.7% when the transformation procedure was truncated from three to two selection steps. This reduced the duration of transformation from embryo infection to the transfer of a plantlet to soil from 115 days to 96 days. In summary, this project has demonstrated reliable Agrobacterium-mediated site-specific integration transformation technology in maize inbreds.

Primary Poster Presenter: Terry Hu

Rapidly domesticating pennycress as an oilseed-producing winter cash cover crop that does not requir (0200-017)
Hall 2

Pennycress (Thlapsi arvense L.) is an emerging oilseed crop closely related to rapeseed canola and Arabidopsis that holds considerable agronomic and economic

potential in producing seed oil as a liquid biofuels feedstock and seed meal as an animal feed. Pennycress has a diploid genome and possesses a unique combination of attributes including extreme cold tolerance, rapid growth, over-wintering growth habit, and a natural ability to produce economically-relevant amounts of seeds high in oil and protein. Pennycress could generate billions of liters of oil annually throughout the U.S. Midwest without displacing food crops or requiring land use changes. For example, pennycress can be grown on ~35 million acres of U.S. Midwest farmland that rotates each year between corn and soybeans. Much of this land otherwise lays fallow, resulting in nutrients loss into streams and soil erosion – two urgent problems which pennycress can help mitigate as a winter cover crop. This presentation highlights our efforts employing EMS mutagenesis and CRISPR gene editing along with bioinformatics to rapidly improve pennycress agronomic traits including 1) optimized seed oil fatty acid composition to improve food/feed and fuel properties, 2) reduced seed glucosinolate content to make the oil and meal edible/palatable, 3) reduced seed coat fiber content to improve the nutritional value of the meal and to allocate more metabolite to oil and protein, and 4) reduced seed pod shatter to limit pre-harvest seed loss.

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Primary Poster Presenter: John Sedbrook

1:30 PM - 2:30 PM

RipTide™ High Throughput NGS Library Prep for Genotyping in Populations

High throughput genotyping technologies are required for large-scale population genetics. Evolutionary biology studies, human disease research and large-scale agricultural breeding programs all lend themselves to technologies that are able to provide more information at lower cost. Over the past decade, genotyping technology has transitioned from PCR-based SNP assays to microarrays, and is now shifting toward high-throughput genotyping by sequencing (GBS). The RipTide High Throughput Rapid DNA Library Prep allows for the preparation of NGS libraries from up to 960 individually barcoded samples in a few hours with automation. When combined with low coverage sequencing and imputation-based genotype analysis, the result is an order of magnitude greater information at a significantly reduced cost. Here we present data on 96 Zea mays (maize) samples consisting of 4 parent populations and 92 recombinant inbred lines (RILs). For each sample, hundreds of thousands to millions of haplotype markers, including SNVs and structural variants, are accurately detected. A minimum of 95% complete coverage of direct and imputed markers is obtained for each RIL. The approach can be applied to any species, regardless of genome size or GC content. In this study, a median of >1 million markers were genotyped by sequencing on an Illumina HiSeq 4000 instrument for an estimated cost of library construction and sequencing of < $25 per sample.

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Primary Poster Presenter: Take Ogawa

Screening cotton chromosomal substitution lines for tolerance to 2,4-D herbicide (0200-038)
Hall 2

Weed management strategies in cotton often rely on herbicides, due to their simplicity, ease of application, and effectiveness. However, due to the widespread adoption of glyphosate-resistant crops, numerous herbicide-resistant weeds have evolved. With glyphosate rendered ineffective, growers and companies are now in need for novel herbicide resistance genes. Given that most glyphosate-resistant weeds can be controlled with 2,4-dichlorophenoxyacetic acid (2,4-D), we have screened and identified cotton germplasm with novel tolerance against it. Previous studies have identified wild cotton species as a reservoir of novel genetic variations. Unfortunately, the application of conventional varietal breeding methods to interspecific introgression has resulted in little tangible success. An alternative breeding method, chromosome substitution (CS), involves first introgressing interspecific germplasm into an upland cotton genetic background and then screening Upland CS lines to discover novel variants. Here, we report the discovery of a novel Upland cotton germplasm as potential sources of genes for 2,4-D tolerance. A total of 47 CS lines of G. barbadense (CS-B), G. tomentosum (CS-T), and G. mustelinum (CS-M), in the genetic background of Gossypium hirsutum Texas Marker-1 (TM-1) were screened for resistance to a field-recommended rate (1.12 kg ai ha-1) of 2,4-D in a completely randomized design with sub-sampling (r=3). Injury from 2,4-D applied at 2 weeks after seedling emergence ranged from 25-100% at 21-28 days after treatment (DAT). Five CS lines including CS-T04-15, CS-B12, CS-B15sh, CS-T04, and CS-B22sh exhibited the lowest injury levels, possibly indicating the presences of 2,4-D tolerance gene(s). Field studies to re-test 2,4-D tolerance levels of the five CS lines are currently underway. Findings from this study could help discover novel 2,4-D tolerant cotton germplasm that ultimately improves weed management options in upland cotton cultivation.

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Primary Poster Presenter: Te Ming Tseng

Sequence, Assembly and Annotation of Bayer Crop Science's Maize Inbred Line LH244; a New Resource fo (0200-036)
Hall 2

Access to elite, transformable germplasm is required to design and maintain transformation pipelines. Product pipelines typically use transformable germplasm to initially introduce the novel variant, which is then crossed into broad, diverse germplasm lines relevant to the geographies where the product will be grown. Effective transformation pipelines are valuable for product development in the Ag industry but are also important for serving the scientific community by enabling basic science research through gene and pathway discovery and characterization. Bayer Crop Science, in collaboration with NRGene and the University of Wisconsin, reports the release of the LH244 inbred maize transformation line germplasm and assembled reference genome to academic research communities. The germplasm will be released to public seed stock centers and the assembled, annotated genome and a physiological description of the line will be published, and resources for efficient transformation will be available to the University of Wisconsin Crop Innovation Center. LH244 is a commercially relevant inbred line that is readily transformable, thus making it a complete resource for genomic and genetic exploration. In this talk, we will share insights into the unique features of the LH244 genome, transformability and physiology that make it a foundation resource for the maize genetics community.

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Primary Poster Presenter: Phil Taylor

ShGPCR1 confers increased tolerance to drought, salinity and cold stress in sugarcane (0200-006)
Hall 2

Sugarcane and energy cane (Saccharum spp. hybrids) are prominent sources of sugar-based ethanol and lignocellulosic biomass feedstocks globally. Abiotic stresses, including drought, salinity and cold result in significant yield-losses (>50%) and are major impediments to attain maximum sugar, biomass and biofuel yield potential in these elite feedstocks. G-protein coupled receptors (GPCRs) constitute the principal component of the conserved G-protein-mediated signaling pathway and are responsible for transmembrane signal transduction of diverse extracellular stimuli during plant growth and development, and abiotic stresses. In the present study, we identified and isolated a GPCR ortholog (ShGPCR1) from sugarcane and energycane and characterized its role in abiotic stress tolerance. Analysis of the ShGPCR1 amino acid sequence revealed characteristic features of a GPCR such as the seven transmembrane spanning domains, an extracellular N-terminus involved in ligand binding, and an intracellular C-terminus that binds to heterotrimeric G-protein. ShGPCR1 also contains a putative GTP binding site similar to other GPCRs in Arabidopsis (GTG1). Steady-state expression of ShGPCR1 is up-regulated in response to salinity, drought and cold-stress treatments in sugarcane. Overexpression of ShGPCR1 in transgenic sugarcane conferred tolerance to drought, salinity and cold stress when compared to non-transgenic plants. The stress-tolerance phenotype correlated with activation of drought, salinity and cold stress-responsive marker genes, such as dehydrin (DHY), ethylene-responsive transcription factor (ERF3) and cold binding factor (CBF3), respectively. Further work to determine the biochemical targets of ShGPCR1 will elucidate the mechanism of action.

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Primary Poster Presenter: MANIKANDAN RAMASAMY

Synthetic Sequences For Expression Of Insect Control Traits (0200-034)
Hall 2

Effective expression of a gene is important in both research and applied applications to provide a unique phenotype in corn. The primary tool to controlling expression properties is through the use of promoters which provide the main switch to when, where and to what level a gene will be expressed. Identifying a native plant promoter that can meet specific expression needs can be challenging but developing synthetic promoters may offer an approach to overcome these issues and tailor expression to what is needed. We have used a strategy that combines segments of different tissue-preferred promoters that together creates

unique expression properties. The ability to modify and modulate expression provides an exciting new opportunity for plant gene expression.

Primary Poster Presenter: Scott Diehn

The CmTCP20 gene regulates petal elongation growth in Chrysanthemum morifolium (0200-028)
Hall 2

Chrysanthemum morifolium is one of the most popular ornamental species worldwide, with high ornamental and economic value. Petal size is an important factor that influences the ornamental value. CmTCP20 is a member of TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTORs (TCPs) gene family, which is closely associated with the growth and development of plants. Our previous study found that the expression of CmTCP20 was obviously down-regulated during chrysanthemum petal elongation, but its function in petal elongation has not yet been revealed. We show here that the overexpression CmTCP20 in Arabidopsis and chrysanthemum leads to similar phenotypes, including larger flower buds (or inflorescences) and longer petals. Interestingly, ectopic expression in Schizosaccharomyces pombe yeast cells showed that CmTCP20 could repress cell division and promote cell elongation. Moreover, the yeast two-hybrid, BiFC and pull-down experimental results indicated that CmTCP20 may regulate petal size via interacting with CmJAZ1-like and inducing downregulation of CmBPE2 gene expression. This study preliminarily clarifies the function of CmTCP20 on chrysanthemum petal elongation, providing the basic theory for improving the ornamental characteristic of chrysanthemum.

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Primary Poster Presenter: Yunxiao Guan

The Validation of Methods Based on PCR for the Detection of Newly Approved GM Events in South Korea (0200-013)
Hall 2

In this study, we validated event-specific qualitative detection methods for the newly developed genetically modified (GM) crops in order to strengthen the label management for GM foods. Two GM events (sugar cane CTC175-A and potato SPS-Y9) were selected for the qualitative detection method. For the validation of the qualitative detection method, real-time polymerase chain reaction (RT-PCR) and polymerase chain reaction (PCR)(only potato SPS-Y9) were performed for

specificity, sensitivity and repeatability. As a result, the qualitative detection for sugar cane CTC175-A and potato SPS-Y9 showed specificity for other crops or other GM events and the limit of detection (LOD) of the qualitative detection method for two GM events were 0.05%. This study may imply that detection method can be established for GMO analysis.

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Primary Poster Presenter: YOUNG RAE CHO

The Wisconsin Crop Innovation Center (WCIC): Enhancing Public Sector Plant Transformation Capacity (0200-041)
Hall 2

Crop plant transformation remains a bottleneck for global efforts to advance crop functional genomics research and genomics-based crop improvement. The combination of genotype specificity, high complexity, and low efficiency of transformation protocols, variable responses of target tissues, lack of automation, intellectual property-related restrictions, with an overall lack of capacity at the national and international levels constricts hypothesis testing in crops. To increase public sector plant transformation capacity the Wisconsin Crop Innovation Center (WCIC), part of the University of Wisconsin – Madison, opened in January, 2017. This 100000 ft2 state-of-the-art facility is staffed with dedicated researchers with more than 150 years of combined experience in plant molecular biology and transformation striving to deliver new public sector innovations and processes to improve genotype independent transformation. In addition to elite soybean and LH244, B73 and other ex-PVP maize varieties, the WCIC plant transformation portfolio includes alfalfa, barley, cannabis, cassava, chickpea, cowpea, cucurbits, dry bean, poplar, potato, sorghum, switchgrass, tobacco, tobacco chloroplast, and wheat. Projects start with consultation and design in the Molecular Technologies Department which uses our Golden Gate mediated assembly platform to build your overexpression, amiRNA, RNAi, or CRISPR/Cas9 plasmids, which is subsequently delivered to the Production Department. ddPCR based transgene copy number measurement, as well as gene expression analysis, is available. The Molecular Technologies Department, Transformation Production Team, and Transformation R&D and Automation Team look forward to engaging with external researchers and collaborators who are seeking larger scale transformation and editing projects in a variety of plant species. Plan your plant transformation project today with mwpetersen@wisc.edu.

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Primary Poster Presenter: Ray Collier

Topolin cytokinins for micropropagation of elite tropical timber wood tree species (0200-043)
Hall 2

The demand of tropical elite trees for timber wood production is rising. Seeds are often difficult to obtain and the value of seedlings cannot be validated on short term. Therefore in vitro mass propagation is a good alternative. Nevertheless, a lot of species remain recalcitrant for the classical cytokinins such as benzyladenine. Modern cytokinins of the topolin family promise breakthroughs, as they follow different metabolic pathways. A number natural occurring meta-topolin derivatives such as mT, mTR, MemTR and MeoTR were compared with benzyladenine during micropropagation phase of Dalbergia retusa, Tabebuia guyacan, and Tectona grandis. Morphological observations were complemented by high-resolution multispectral imaging. The different reactions of the shoot explants will be discussed and put into perspective.

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Primary Poster Presenter: JEAN-PAUL REYNOIRD

Triton: A New Fusarium Wilt Resistant Cantaloupe Variety (0200-037)
Hall 2

Fusarium wilt is a fungal disease caused by Fusarium oxysporum that infects many plant species including cantaloupe. Crop rotation and chemical methods of tackling this disease have been proven ineffective. However, the R genes Fom-1 and Fom-2 in melon confer resistance to fusarium wilt, and thus the use of resistant cultivars is a powerful method of disease control. We have developed a new cantaloupe cultivar with resistance to fusarium wilt and improved flavor quality, known as 'Triton.' 'Triton' was created through traditional plant breeding methods that selected for melon lines with genotypic resistance as well as vigor and aesthetic qualities. Phenotypic fungal screens were performed to confirmed resistance to the disease. Following the confirmation of resistance to fusarium wilt and powdery mildew, another common melon disease, a Tuscan-type melon was introduced into the background for an improved flavor profile. Presently, 'Triton' is undergoing field trials and will be released for commercial sales in early 2020. Concurrently, the Fom-2 LRR region from several resistant and susceptible lines, including 'Triton,' was sequenced and multiple single nucleotide polymorphisms (SNPs) between the two phenotypes were identified. Genotypic markers were designed utilizing these SNPs to distinguish between resistant and susceptible lines on a molecular basis. Sixteen total markers were made from eight SNPs, and one susceptible marker was found to be the most accurate. These new molecular markers will provide efficient genotypic screening capabilities for future varietal development.

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Primary Poster Presenter: Mishi Vachev

A synthetic biology approach to modifying cotton cell walls and enhancing plant-based natural fibres (0200-059 (Screen 2))
Hall 2

The distinct properties of natural and artificial fibres drive consumer choice and end use. Natural fibres derived from plants and animals, such as cotton and wool, are biodegradable and renewable and are popular due to their breathability. In contrast, artificial fibres are made from non-renewable and non-biodegradable fossil fuels that can pollute the environment. Nevertheless, artificial fibres remain popular due to their low cost and functional properties such as elasticity. The broad aim of this project is to develop cotton fibres – specialised highly cellulosic cell walls surrounding a hollow lumen – with enhanced and novel properties, giving them the potential to replace artificial textiles in some applications. To do this, we are taking a synthetic biology approach and developing a gene and protein domain toolbox for targeting and retaining novel proteins and other molecules in plant cell walls. We are investigating the effects of transgene expression and protein targeting in three test plant species: transient expression in Nicotiana benthamiana as well as stable expression in Arabidopsis thaliana and cotton. In the short term, this study will provide insight into the functionality of promoters, signal peptides and other DNA/protein motifs as well as processes occurring during cotton fibre development. It will also improve our understanding of the relationship between cell wall components and the physical properties of cotton fibres. In the long term, this project aims to: 1) broaden consumer choice by providing fibres with new and unique properties; 2) reduce chemical and microplastic pollution by providing renewable and biodegradable plant-based alternatives to artificial fibres; 3) future-proof the cotton industry by developing high value differentiated products.

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High temperature alters flavonoid profiles in indoor-grown lettuce (0200-061 (Screen 6))
Hall 2

Indoor farming is an alternative technology for modern agriculture to produce maximum yield with high quality in a small area since most of growing parameters could be strictly controlled. The technology benefits people with limited land in

cities, reduces transportation costs, and minimizes uses of pesticides. Short life crops with short shelf life such as leafy vegetables are commonly the target in indoor farming. Lettuce (Lactuca sativa), being consumed globally, is considered as a good source of fiber, iron, folic acid, vitamin C, anthocyanin and polyphenols that have a positive effect on the prevention of cardiovascular disease (Beverage crops, 2018). Growing lettuce in indoor farm is widely adopted in many countries, however, consuming huge amounts of electricity remains as a major concern. Indoor-grown lettuce is usually cultivated at cool temperature to enhance vegetative growth and prevent early flowering. Higher temperature setting is one of the cost effective strategy. Nonetheless, various lettuce cultivars exhibited growth aberrant and altered phytochemicals at high temperature that might affect taste and consumer perception. Our study focuses on metabolic and transcriptomic changes of lettuce at different temperatures in order to identify key markers for temperature responses. Around thirty feature IDs, especially flavonoid compounds were identified as key metabolites in response to temperature. Transcriptomic data revealed relationship between stress response genes and secondary metabolites production. In addition, bitter compounds belonging to sesquiterpenes exhibited unique profiles among cultivars at two different temperatures. These findings help us to improve knowledge on flavonoids biosynthesis in lettuce and offer new tools for modern lettuce breeding.

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Primary E-Poster Presenter: Kulaporn Boonyaves

Plant Produced Bispecific Monoclonal Antibody Neutralizes Dengue-2 and Zika Viruses and Forgoes Anti (0200-060 (Screen 4))
Hall 2

Flaviviruses such as the dengue virus (DENV) and Zika virus (ZIKV) continue to be a health burden in tropical and subtropical regions of the world. The DENV and ZIKV co-circulate and generally cause self-limiting febrile illnesses, however they can progress to severe illnesses such as Dengue Shock Syndrome and plasma leakage (DENV) and microcephaly in newborns (ZIKV). DENV exhibits an infection mechanism known as antibody dependent enhancement (ADE) of infection, which is triggered by a secondary infection with a heterologous DENV serotype, thereby resulting in the most severe forms of dengue illness. Currently, FDA approved vaccines do not exist for DENV and ZIKV, calling for the development of effective and safe therapeutics. Monoclonal antibody-based therapies have been shown to be effective therapies for the treatment of cancers. In this study, we developed a bispecific monoclonal antibody (bsmAb) derived from a DENV neutralizing antibody and a ZIKV neutralizing antibody. The bsmAb was produced in a glycoengineered Nicotiana benthamiana tobacco plant line which produces mammalian-like glycosylation profiles that have been shown to produce homogeneous N-linked glycans. Functional assays of our bsmAb demonstrated that it bound to its intended targets and neutralized both DENV2 and ZIKV. Furthermore, our results indicated

that the bsmAb did not enhance the infection of DENV2 or ZIKV via the ADE mechanism. Producing therapeutic proteins in plants is a superior alternative to traditional means of therapeutic protein production for several reasons such as, speed of production, lower overall production cost, and the ability to glycosylate proteins in an on-demand and tailor-made fashion. Our results showcase glycoengineered plants as an effective system in producing various mammalian glycans to increase the safety and efficacy of therapeutics against medically important co-circulating viruses, thereby overcoming the potential risk of ADE, which is a major impediment in

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Primary E-Poster Presenter: Adrian Esqueda

Applied: Plants and Human/Societal Health

Baby Ginger Attenuates Lipid Droplet formation in 3T3 Adipocytes (0200-050)
Hall 2

Ginger (Zingiber officinale) are new emerging niche crops for small farmers in Virginia. Currently, locally grown ginger are not fully mature, and are marketed under 'Baby ginger.' Immature baby ginger is more perishable, less fibrous, and pungent when compared to fully mature imported products. It is also not known if phytochemical profile and health benefits of the "immature" ginger are different to "fully mature" produce. We conducted research to determine the phenolic content and anti-oxidation properties of ginger at different harvesting time and to test the effect of baby ginger on lipid droplet formation in 3T3 adipocytes. Ginger samples at different stages of maturity were harvested every two weeks starting from October 15, 2017 until January 15, 2018. Our data indicate that ginger has the highest content of phenolic compounds and superior anti-oxidation activity when harvested early (immature baby ginger); however, the concentration of phenolic compounds and its anti-oxidation activity were progressively reduced up to 50% as ginger matures. Furthermore, the data indicate that baby ginger at early stage of harvesting was able to reduce oil droplet accumulation by 25%-40% in 3T3-L1 adipocytes in a dose dependent manner, possibly via inhibition of de novo fatty acid synthesis. The effects of ginger on fat synthesis appear to be mediated through down-regulating CEBP-beta, Phosphoenol pyruvate carboxy kinase (PEPCK), and Acetyl CoA carboxylase (ACC) genes expression. Our results suggest harvesting of ginger at appropriate (early) time to optimize or maintain the qualitative and quantitative levels of biological active compounds. The data also suggest that a regular use of ginger can potentially affect lowering incidences of obesity and obesity-related complications, a growing concern in the state of Virginia.

Furthermore, increase ginger consumption will help to develop local and regional farm economy.

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Primary Poster Presenter: Haiwen Li

Characterizing the antimicrobial and anticancer activities and several associated bioactive compound (0200-049)
Hall 2

Commonly called the Mexican prickly poppy, *Argemone mexicana* is a stress-resistant member of the Papaveraceae family of plants that has been used in traditional medicine for centuries by indigenous communities in Mexico and Western parts of the United States. This plant has been used to treat a wide variety of ailments, including skin diseases and intestinal infections, with reported antimicrobial and anticancer properties. However, these properties are poorly understood, with no associated bioactive compounds yet identified. Herein, we describe the germination conditions of *A. mexicana* and preliminarily characterize the antimicrobial and anticancer activities of different parts (seeds, leaves, inner vs. outer roots) of the plant. We show that when comparing 1 mg of each sample normalized to background solvent alone, the *A. mexicana* methanol outer root and leaf extracts possess the strongest antimicrobial activity, with greatest effects against the gram-positive bacteria tested, and less activity against the gram-negative bacteria and fungi tested. Additionally, we report that when using the MTT colorimetric assay, the outer root and leaf methanol extracts and the seed hexane extract have pronounced inhibitory effects against T84 human colon cancer cells. Using normal-phase column chromatography and subsequent mass spectrometry analysis of the outer root and leaf methanol fractions, we have begun to chemically characterize several candidate antibacterial compounds. These preliminary results warrant further research into defining the bioactive chemicals produced in the roots, leaves and seeds of *A. mexicana* and are especially significant given the growing global concern of antibiotic-resistant 'superbugs' and lack of new antimicrobial and anticancer drug discovery.

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Drought increases accumulation of Phyto-antioxidants and Limits Retrieval of Salmonella on Greens (0200-046)

Hall 2

Introduction: Antioxidant compounds such as phenolics were reported negatively associated with surface colonization of Salmonella enterica. Moreover, extreme weather events frequently subject crops to periods of drought, which constitutes plant stress that induces the accumulation of antioxidant compounds. Purpose: Evaluate the effect of drought on S. enterica colonization of lettuce and kale.Methods: Four-week-old lettuce cultivar 'Mascara' or 2-week-old kale cultivar 'Improved dwarf' were subjected to drought for 6 days or regular watering (control) in a greenhouse (23°C, 16h L:8h D). Salmonella Newport adapted for rifampicin was grown overnight on tryptic soy agar (TSA) at 35°C and ~106 S. Newport were inoculated onto the adaxial side of the third true leaf of plants. Inoculated leaves were clipped 24 h post-inoculation, washed in 30 ml 0.1% peptone water, and serially plated onto TSA with rifampicin. The antioxidant capacity and total phenolic and flavonoid contents were measured from ground leaf tissue of lettuce and kale. Total anthocyanin and estimation of glucosinolates were measured in lettuce and kale, respectively.Results: The retrieval of S. Newport from drought-stressed lettuce was 2.42±0.26 log CFU/ml of rinsate compared to control at 1.19±0.16 log CFU/ml ($p<0.05$). Drought significantly limited the growth of S. Newport on kale to 1.4±0.2 log CFU/ml of rinsate compared to control at 2.4±0.3 log CFU/ml. Drought-treated lettuce plants showed higher antioxidant capacity ($p=0.07$), total phenolic, flavonoid and anthocyanin contents ($p<0.05$) compared to control lettuce plants. Kale under drought treatment yielded a higher antioxidant capacity ($p<0.05$), total phenolic content and glucosinolate content estimation ($p<0.05$) and total flavonoid content ($p=0.08$). Significance: These data suggest that leafy greens that are responding physiologically to drought stress may provide a less favorable environment for S. enterica colonization.

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Primary Poster Presenter: xingchen liu

Genetics of natural variation in provitamin A and vitamin E levels in maize grain (0200-047)
Hall 2

Carotenoids (vitamin A) and tocochromanols (tocopherols and tocotrienols; vitamin E) are lipid-soluble isoprenoids with antioxidant and essential nutrient functions in human beings and animals. Vitamin A deficiency remains prevalent through much of Asia, Latin America and sub-Saharan Africa, and coincides in part with regions in which maize is a staple. Improving the levels of these nutrients in crops such as maize, through plant breeding—a process termed biofortification—has been found to be a sustainable and cost-effective solution to ameliorate such deficiencies. To dissect the genetic loci underlying natural variation in maize grain carotenoids and tocochromanols, we conducted joint linkage-genome wide association studies and RNA-seq expression analyses in the 05000-line U.S. maize nested association mapping panel. For carotenoids and tocotrienols, the largest-effect QTL were found to be underlain by genes with a priori roles in biosynthesis and retention of these

nutrients, including prenyl group synthesis. For tocopherols, the major tocochromanol in maize embryos, the majority of variation was attributed to two homologs encoding protochlorophyllide reductase. While maize grain is nonphotosynthetic, small levels of chlorophyll were detectable in developing embryos, supporting along with other evidence the proposal that a novel chlorophyll biosynthetic cycle provides the tail group (phytol) for tocopherol biosynthesis. Overall, more than half of the identified QTL were eQTL. Most of these also exhibited pleiotropy, in the form of highly correlated QTL allelic effect estimates across traits—not unexpected given that these nutrients are produced sequentially in biosynthetic pathways with limited branching. These findings have increased our understanding of the accumulation and retention of carotenoids and tocochromanols in maize grain and potentially other cereals, within an analytical framework that is readily integrated with genomics-assisted breeding strategies.

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Primary Poster Presenter: Christine Diepenbrock

Impact of LED Supplemental Lighting on Accumulation of Carotenoids on Greenhouse Tomato (Lycopersico (0200-048)
Hall 2

Lycopene is the major carotenoid pigment found in tomato (Solanum lycopersicum) a known potent antioxidant of nutritional importance in the diet. Several factors influence accumulation of lycopene in tomato including duration and intensity of solar radiation. Use of supplemental lighting during winter production of tomato occurs in northern climates to offset the differences in the daily amount of photosynthetically active radiation (PAR). Light emitting diode (LED) lights provide

an alternative to high pressure sodium lamps because they offer a narrower wavelength of lights ideal for plant growth and development and emit less heat. Exposure to narrowed red to blue ratios of light have been found to affect biomass accumulation, plant morphology, nutrient uptake, and pigment concentration in a range of greenhouse crops. In this study, three greenhouse tomato varieties were treated with 90:10 red/blue ratio and 86:14 red/blue ratio respectively, with 0600 W LED light treatments for 16 hours/day. At harvest, yield, soluble solids and carotenoids were determined. According to our preliminary results, fruit yield increased 3-fold compared to the control among both light treatments. Soluble solids and carotenoid values were comparable to the control ranging from 5.5-7.0% and 62-77 ug/g FW respectively. Therefore, supplementation with LED lighting benefitted yield while appearing not to negatively impact accumulation of sugars and carotenoids, which are important attributes to fruit quality.

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Plant Extracellular Vesicles as Nanovectors of Cancer Therapies (0200-051)
Hall 2

We hypothesize that plant extracellular vesicles (EVs) can be used to deliver combinations of specific biological therapeutics to malignant tissue in humans, which would enable both targeted delivery of therapeutics and application of precision medicine. Targeted delivery and precision medicine are emerging as two distinctly different strategies to treat cancer. Targeted delivery utilizes nanotechnology to concentrate high doses of medicine to malignant tissue, sparing the body from side effects. Though liposomal- and carbohydrate-conjugated medicines have been successful in the clinic, current targeted delivery treatments are expensive and can be inaccessible to the patient. Precision medicine customizes treatment for each specific patient, often based on the genotype of their tumors. Precise combinations of medication have been effective when used in this manner, but roadblocks can bar oncologists from prescribing previously untested combinations of drugs. In plants, EVs function in plant-microbe communication, in which they are transferred in an inter-kingdom manner from the plant to plant pathogens, and likely vice versa. EV’s naturally contain specific transmembrane proteins and small RNAs, including miRNAs, siRNAs and tiny RNAs (10-17 nt long).We aim to: 1) engineer EV’s for preferential uptake by cancer cells (in vitro and in vivo) by display of cancerphilic ligands on the surface of EV’s, and 2) understand how natural cargo is loaded into EV’s in plants in order to engineer EV’s to contain biological therapeutics such as siRNA, miRNA, and fusion proteins for the treatment of precise cancer disease subtypes. Plant EV’s also address the current shortcomings of targeted delivery and precision medicine, in that they are inexpensive and easy to produce, and contain a combination of therapeutic elements within one medicine. Plant EV’s have the potential to revolutionize both targeted delivery and precision medicine, two burgeoning fields of cancer therapy.

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Biochemistry and Metabolism

Structures of Xyloglucan Xylosyltransferases revealed how simple steric rules define patterns of nat (0500-086 (Screen 6))
Hall 2

We have obtained the crystal structures of Arabidopsis xyloglucan xylosyltransferase 1 (XXT1) without ligands and in complexes with the substrates, UDP and cellohexaose. XXT1 initiates side-chain extensions from a linear glucan polymer by transferring the xylosyl group from UDP-xylose during xyloglucan biosynthesis. XXT1, a homodimer and member of the GT-A fold family of glycosyltransferases, binds UDP analogously to other GT-A fold enzymes. Structures and the properties of mutant XXT1s are consistent with a SNi-like catalytic mechanism. Distinct from other systems is the recognition of cellohexaose by way of an extended cleft. Steric conflicts in the acceptor binding cleft disallow XXT1 alone to produce the complete xylosylation patterns observed for native xyloglucans. Homology modeling of XXT2 and XXT5, the other two xylosyltransferases involved in xyloglucan biosynthesis, reveals the presence of an empty pocket in XXT5 that is large enough to encompass the xylose of a partially xylosylated glucan chain. The structural organization of three XXTs, unraveled in our study, support the existence of an organized multi-enzyme complex involved in the xyloglucan synthesis and explain how the particular XXXG pattern is synthesized. Results from computational docking suggest subunit interfaces of the homodimer XXT1 and the heterodimer XXT2-XXT5 are similar; however, different surfaces of the XXT1 homodimer and the XXT2 subunit in the XXT2-XXT5 heterodimer can interact to form a linear trimer of dimers in which the XXT1 homodimer occupies the central position, thus confirming our experimental observations. We propose a model of a multi-enzyme complex organization to produce the specific xylosylation patterns of the native xyloglucan in which the high substrate specificity of each of the XXT is mediated by steric constraints within their acceptor substrate active site cleft. This model significantly extends our limited understanding of polysaccharide biosynthesis in Golgi

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The entry reaction of the plant shikimate pathway is under highly-complex effector-mediated regulati (0500-087 (Screen 14))
Hall 2

The plant shikimate pathway directs bulk carbon flow to support biosynthesis of aromatic amino acids (AAAs) and numerous natural products including phytohormones, cofactors, pigments, phytoalexins, lignin, and more. These aromatic phytochemicals play critical roles in plant physiology and adaptation, and also provide essential nutrients, medicine, and industrial materials to the human society. In microbes, the shikimate pathway is feedback inhibited by AAA effectors at the first enzyme, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DHS). Although the DHS-catalyzed step is also thought to be regulated in plants, effectors that regulate plant DHS have not been identified for decades . Here, we generated recombinant enzymes of all three DHS isoforms of Arabidopsis thaliana (AthDHS1, AthDHS2, and AthDHS3) and conducted their biochemical characterization. Only the AthDHS2 isoform, but not AthDHS1 or AthDHS3, was negatively regulated by tyrosine or tryptophan, whereas phenylalanine had no effects. Chorismate, the final product of the shikimate pathway, strongly inhibited the activity of all three AthDHS enzymes, which was counteracted by a further downstream intermediate, arogenate. Caffeic acid and its derivatives, key intermediates of the phenylpropanoid pathway, were also effective inhibitors of AthDHS enzymes, uncovering a potential regulatory link between the shikimate and phenylpropanoid pathways. DHS activity detected from leaf crude extracts were inhibited by chorismate and caffeic acid, but not by any of AAAs, which appears to be due to the loss of the AthDHS2 AAA-sensitivity in the presence of AthDHS1. These findings reveal unique and highly-complex regulatory mechanisms of the entry reaction of the plant shikimate pathway and provide foundational knowledge to control the production of AAAs and diverse natural products in plants.

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Regulation of S-Nitrosation of Arabidopsis S-Nitrosoglutathione Reductase (GSNOR) by Thioredoxins an (0500-088 (Screen 10))
Hall 2

Nitric oxide (NO) is a short-lived gas that acts as a signaling molecule in all higher organisms, including plants. Despite the clear involvement of NO in multiple plant processes, including germination, root growth and fertility, a basic understanding of the mechanisms by which NO exerts its effects on systems critical for plant growth and development is lacking. Reversible S-nitrosation of critical protein cysteines due to reaction with nitric oxide (NO) and its derivatives is a redox-dependent posttranslational modification that impacts these plant physiological processes. Regulation of NO-levels in planta is predominantly achieved by reaction of reactive nitrogen species (RNS) with glutathione (GSH), thereby forming S-

nitrosoglutathione (GSNO), the principal NO reservoir. Mutation of Arabidopsis S-nitrosoglutathione reductase (GSNOR) leads to higher intracellular concentrations of S-nitrosothiols, confirming that the reduction of GSNO by the enzyme is a major route of GSNO catabolism in plants. GSNO-breakdown is believed to help sustain cellular redox poise both by curtailing RNS-bursts and by regenerating GSH. GSNOR contains evolutionary-conserved cysteine residues that are prone to S-nitrosation by different NO donors, leading to a partial loss of enzyme activity that could be recovered by reducing agents in vitro. Protein nitrosation was further confirmed by intact mass spectrometry, for which signals consistent with mono-, di- and tri-nitrosation were observed. In addition, GSNOR denitrosation analysis catalyzed by small oxidoreductases will be addressed. These data implicate a mechanism for RNS signaling by modulating redox-dependent posttranslational modifications of certain proteins. Reduced GSNOR activity is predicted to result in the accumulation of GSNO, itself an agent of protein S-nitrosation. By allowing GSNO to accumulate, inhibition of GSNOR may facilitate more robust NO signaling that regulates plant growth and developmental processes in plants.

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0500-031 - Comparative transcriptome and metabolome analysis suggests bottlenecks that limit seed and oil yield (0500-031)
Hall 2

Camelina sativa has attracted much interest as an alternative renewable resource for biodiesel, other oil-based industrial products and a source for edible oils. Its unique oil attributes attract research to engineering new varieties of improved oil quantity and quality. To increase oil yield, we engineered Camelina by co-expressing the Arabidopsis diacylglycerol acyltransferase 1 (DGAT1) and yeast glycerol-3-phosphate dehydrogenase 1 (GPD1) genes under the control of seed-specific promoters. Transgenic plants exhibited a higher-percentage seed oil content, a greater seed mass, and overall improved seed and oil yields, relative to wild-type plants. To further improve seed and oil yields, we utilized metabolite profiling, in conjunction with transcriptome profiling during seed development in WT and transgenics to examine potential rate-limiting step(s) in the production of building blocks for TAG biosynthesis. Our approach revealed several key genes/gene networks associated with significant changes, especially in the storage/retention of lipids and in pathways affecting carbon conversion efficiency in seeds. Further, metabolite analysis indicated major metabolic switches in the levels of glycerolipids, amino acids, sugars, and organic acids, especially the TCA cycle and glycolysis intermediates. From the transcript and metabolite analyses, we conclude that TAG production is limited by (1) utilization of fixed carbon from the

source tissues supported by the increase in glycolysis intermediates and the flux into pentose phosphate pathway (PPP), and decreased expression levels of transcription factors controlling fatty acids synthesis; (2) TAG accumulation is limited by the activity of lipases/hydrolases that hydrolyze TAG pools supported by the increase in free fatty acids and monoacylglycerols (MAGs). The integration of omics approaches is useful in understanding the regulation of TAG biosynthesis and identifying bottlenecks for metabolic engineering, and based on t

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A division of labor in the structural diversification of flavone conjugates in rice (0500-020)
Hall 2

In grasses, soluble flavone C-glycosides and O-conjugates (both soluble and cell wall lignin-bound) represent major flavonoid metabolites produced in vegetative tissues. Interestingly, these structurally similar compounds are biosynthesized via two independent pathways. We previously demonstrated that two CYP93G enzymes in rice, i.e., rice flavanone 2-hydroxylase (OsF2H/CYP93G2) and flavone synthase II (OsFNSII/CYP93G1), channel flavanone precursors towards biosynthesis of flavone C-glycosides and O-conjugates, respectively. Also, we identified 2 CYP75B enzymes that function as flavonoid B-ring hydroxylases (FBHs) with designated roles to produce these flavone conjugates in rice. Our previous in vitro enzyme assay study showed that CYP75B3 catalyzes 3′-hydroxylations of various flavonoid substrates including flavones, whilst CYP75B4 shows apigenin 3′- and chrysoeriol 5′-hydroxylase activities. Here, we further generated CYP75B3 single knockout and CYP75B3-CYP75B4 double knockout rice mutants by CRISPR/Cas9-mediated genome editing and analyzed them along with a T-DNA insertion mutant of CYP75B4. Metabolite and cell wall structural analyses of these mutants revealed that CYP75B3 is the sole FBH involved in the biosynthesis of flavone C-glycosides, whereas CYP75B4 alone is sufficient to catalyze both 3′- and 5′-hydroxylations to generate flavone O-conjugates including soluble tricin metabolites and tricin-lignins abundant in cell walls. We also examined the gene expression patterns of CYP75B3 and CYP75B4, and demonstrated that they are well co-expressed with OsF2H and OsFNSII, respectively. Thus, CYP75B3 and CYP75B4 represent two different pathway-specific enzymes recruited together with OsF2H and OsFNSII for generating flavone C-glycosides and O-conjugates, respectively. As the OsF2H-CYP75B3 and OsFNSII-CYP75B4 pairs are likely conserved in other grasses, their co-evolution might have led to the widespread occurrence of flavone metabolites in many grasses nowadays.

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A Fortuitous Discovery of β-Glucosidase from Corn Seed in The Purification of Recombinant Expansin f (0500-033)
Hall 2

Plant β-glucosidases are involved in numerous important functions, for instance ABA metabolism, cell wall formation and stress resistance. Even though different isoforms of β-glucosidase from maize seedlings, coleoptiles, leaves and even root have been studied, β-glucosidase derived from corn seeds has not been reported. Our original goal was to produce and purify recombinant expansin from transgenic maize seed. After extraction and purification steps including crude extraction, ammonium sulfate precipitation, cation and anion exchange column purification, relatively clean and enriched protein samples were obtained by following enhanced cellulase deconstruction of cellulose. However, these purified protein samples show significant cellulolytic activity on both cellobiose and 4-Methylumbelliferyl beta-D-cellobioside indicating that the purified protein samples could contain β-glucosidase activity instead of recombinant expansin. After further molecular weight characterization based on Native/SDS-PAGE and protein identification based on LC-ESI-MS/MS, we believe that β-glucosidase is the active constituent in the purified protein sample. Later enzymatic characterization indicated that the active protein has an optimal pH value of 5.0 and an optimal reaction temperature of 60 degrees Celsius.

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Primary Poster Presenter: Hong Fang

A novel HPLC-MS/MS method for Identification of Low-Molecular-Weight Thiols in Brassicaceae species (0500-022)
Hall 2

Thiols are a class of highly reactive compounds characterized by a nucleophilic -SH group and they represent the main form of reduced sulphur in plants, as protein thiols or low molecular weight (LMW) thiols. They play a primary role in maintenance of cellular redox homeostasis and in plant stress response. In particular, LMW thiols can readily form complexes with toxic compound and deactivate them; they can participate in enzymatic and redox reactions, modifying the redox state of sensitive molecules in plants. A huge diversity of LMW thiols exists, as evidenced by chromatographic analyses, but many of them are still unknown. Since their concentration in plants is very low, their identification represents one of the major challenges.In this work, a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method has been

developed for the identification of unknown thiol compounds in leaves of different plants representing Brassicaceae family from the Botanical Garden of Padova, after their extraction and derivatization with the label 4,4'-Dithiodipyridine (DTDP). By the generation of three characteristic fragments produced by Thiols-DTDP complexes, a list of mass/charge (m/z) ratio was obtained for each matrix using the precursor ion scan mode, with a Kinetex C18 column coupled to an HPLC-QqQ triple quadrupole mass spectrometer. Unknown thiols were identified with the exact molecular mass and molecular formula determined using high-resolution mass spectrometer (HRMS) Quadrupole-Time of flight (QTOF).Results indicate that extracts from different Brassicaceae species had distinct thiol compositions or profiles, with several species-specific compounds. Given the significance of thiols in biological systems, identification and definition of these novel LMW thiols could open prospective studies aspiring to understand their influence on plant metabolism and beyond.

Primary Poster Presenter: Silvia Millan

A Novel Maize Glycosyltransferase is Required for Carbon Export from Source Tissues (0500-028)
Hall 2

As autotrophs, plants must transport the carbon that is fixed in the photosynthetic source tissues, such as leaves, to the non-photosynthetic sink tissues, such as roots or reproductive tissues. This process, known as carbohydrate partitioning, is essential for plant growth and survival, and requires coordinated action by many enzymes and transporters.Here we describe a mutant with carbohydrate partitioning defects, including reduced growth, reproductive defects, and carbohydrate hyperaccumulation in leaves. We identified three alleles of the causal gene, which were all single amino acid mutations that mapped to a putative glycosyl transferase on chromosome 9. Little is known about the biochemical function of the predicted protein, which we show to be a Golgi resident, but preliminary analyses of cell wall chemistry identified altered carbohydrate linkage characteristic of changes in arabinoxylan chemistry, suggesting Cpd7 it may function in cell wall biosynthesis or remodeling.To further characterize these mutants, we conducted a morphological analysis that revealed ectopic lignin deposits in the phloem of mature leaves. These deposits occurred in a developmentally progressive pattern. We hypothesize that these lignin deposits might perturb long distance transport of sucrose, interfering with source-to-sink carbon transport. Radiotracer experiments revealed decreased basipetal transport of sucrose in source leaves, suggesting that perturbed long-distance sugar transport underlies the cpd7 phenotype. Future efforts will be aimed at further elucidation of the link between cell wall composition and carbohydrate partitioning.

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A Quantitative Mass Spectrometry Approach to Understanding Cellulose Synthase Complex (CSC) Composit (0500-036)
Hall 2

Cellulose, the most abundant biopolymer on the planet, is the primary load-bearing polysaccharide component of plant cell walls, and its biosynthesis is catalyzed at the plasma membrane by the cellulose synthase complex (CSC). Seed and non-seed plants both independently evolved from a common ancestor to contain CSCs with a rosette structure and 6-fold symmetry. In the model seed plant Arabidopsis, hetero-oligomeric CSCs contain three functionally distinct cellulose synthase A proteins (CESAs). Conversely, Physcomitrella patens, a model non-seed plant, contains seven CESA isoforms that span two clades. The CESA composition of non-seed plant CSCs is currently unclear. To address this question, we pursued a quantitative proteomic approach. We generated solubilized extracts from wild-type Physcomitrella (Gd11) and HA-tagged transgenic PpCESA3 strains. Each of these samples were separately subjected to immunoaffinity chromatography on anti-HA magnetic beads, and the resulting anti-HA eluates of three replicate wild-type control or HA-PpCESA samples were independently labeled with a unique isobaric tag. Quantitative mass spectrometry analysis was performed to identify protein-protein interactions between several of the PpCESA isoforms in the resulting mass spectrometry samples. In the PpCESA3 IP experiments, PpCESA3, 8, and 6/7 were identified with an abundance enrichment of 10.9, 7.8, and 6.2 respectively in comparison to wild-type, suggesting that these subunits reside in a stable CSC, and these results were further supported by targeted Western blot analysis of IP fractions as well as Bimolecular Fluorescence Complementation and Split-Ubiquitin Yeast Two Hybrid analyses. Similar analysis of P. patens CESA5 indicated that this CESA isoform exists as a novel homo-oligomeric CSC. These data suggest that non-seed plants, such as Physcomitrella, display hetero-and homo-oligomeric CSCs, which further supports the hypothesis of CSC convergent evolution.

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Alteration of seed protein and oil content in soybean by fast neutron induced mutagenesis (0500-029)
Hall 2

Soybean has been subjected to genetic manipulation by various approaches such as breeding, mutation, and transgenesis to produce value added quality traits. Among those approaches, mutagenesis through fast neutrons radiation is intriguing because it yields a variety of mutations, including single/multiple gene deletions and/or duplications. Characterizing the seed composition of the fast neutron mutants and its relationship with gene mutation is useful towards understanding oil and protein traits in soybean. From a large population of fast neutrons mutagenic plants, we selected ten mutants based on a screening of total oil and protein content using near infra-red spectroscopy. The mutant 2R29C14Cladecr233cMN15 (nicknamed as L10) showed the highest protein and lower oil content compared to wild type, followed by three other lines (L03, L05, and L06). We have physically mapped the position of the deletion or duplications of genes in each mutant using comparative genomic hybridization (CGH). All ten lines had one or more deletions and/or duplications. We selected the L03 mutant for detailed proteomic analysis because it exhibited 55% protein while only showing a homozygous deletion encompassing few genes. A proteomic profiling of the wild type and L03 revealed 3,502 proteins, of which 206 proteins exhibited increased abundance and 214 decreased abundance. Among the abundant proteins, basic 7S globulin increased four-fold, followed by vacuolar-sorting receptor and protein transporters. The differentially expressed proteins were mapped to the global metabolic pathways. A higher enrichment in ribosomal, endoplasmic reticulum, protein export and purine metabolic pathways were observed. A shift of carbon metabolism towards amino acid formation was also observed. The deletion of the sequence-specific DNA binding transcription factor along with 22 other genes may have caused a cascade effect on protein synthesis, resulting in an increased amount of 7S globulin.

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An Oxalyl-CoA Decarboxylase is important for oxalate catabolism in Arabidopsis (0500-008)
Hall 2

Considering the widespread occurrence of oxalate in nature and its broad impact on a host of organisms, it is surprising that so little is known about the turnover of this important acid. In plants, oxalate oxidase is the most well studied enzyme capable of degrading oxalate, but not all plants possess this activity. Recently, an Acyl Activating Enzyme 3 (AAE3), encoding an oxalyl-CoA synthetase, was identified in Arabidopsis. This enzyme has been proposed to catalyze the first step in an alternative pathway of oxalate degradation. Since this initial discovery this enzyme and proposed pathway have been found to be important to other plants as well as yeast. In this study we identify an oxalyl-CoA decarboxylase (AtOXC) that is capable of catalyzing the second step in this proposed pathway of oxalate

catabolism. This enzyme breaks down oxalyl-CoA, the product of AtAAE3, into formyl-CoA and CO2. AtOXC:GFP localization suggested that this enzyme functions within the cytosol of the cell. An Atoxc knock-down mutant showed a reduction in ability to degrade oxalate into CO2. This reduction in AtOXC activity resulted in an increase in the accumulation of oxalate and the enzyme substrate, oxalyl-CoA. Overall, these results suggest that AtOXC catalyzes the second step in this alternative pathway of oxalate catabolism.

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Primary Poster Presenter: Paul Nakata

Biochemical Characterization of Aspartate Aminotransferases in Plants (0500-027)
Hall 2

In addition to functioning as a component of proteins in all living organisms, the amino acid aspartate also participates in a variety of plant metabolic processes, including the assimilation of nitrogen via a transamination reaction. This reaction is catalyzed by the enzyme aspartate aminotransferase (AAT), of which five differentially-localized isoforms exist in model plant Arabidopsis thaliana. While the general reaction mechanism of these enzymes is known, they have yet to be fully characterized structurally or kinetically. In this research, comparisons of AAT amino acid sequences to that of the related prephenate aminotransferase (PAT) revealed the critical residues for the AAT reaction and its substrate specificity. Steady-state kinetics were then implemented to analyze the feedback regulation and substrate specificity of the five isoforms and of several site-directed mutants. In addition, x-ray crystallography was used to further characterize these enzymes and to better understand the mechanistic underpinnings of the reaction performed.

Primary Poster Presenter: Daniel Berkovich

CAM Biodesign: Engineering Crassulacean Acid Metabolism into Arabidopsis to Improve Water-Use Effici (0500-003)
Hall 2

Crassulacean acid metabolism (CAM) is a specialized photosynthetic mode to increase a water-use efficiency (WUE) that exploits a temporal CO2 pump with nocturnal CO2 uptake and concentration to reduce photorespiration to improve the adaptability of plants to hotter and drier climates. CAM species, with their inverted stomatal behavior, display water demands that are typically 3- to 6-fold less than of comparable C4 and C3 photosynthesis species, respectively. Thus, introducing the CAM pathway into C3 photosynthesis plants (CAM Biodesign) is expected to confer enhanced photosynthetic performance and WUE. Detailed functional analysis of the individual genes encoding C4 enzymes in common ice plant including McβCA2,

McPPCK1, McPPC1, McNAD(P)-MDHs, McNAD(P)-MEs, McPPDK, and McPPDK-RP of both the carboxylation and decarboxylation modules and cognate circadian clock-controlled promoters are required to reconstitute the appropriate temporal expression of the CAM pathway enzymes in the C3 model Arabidopsis. Furthermore, developing an effective multi-gene assembly tool for the large number of C4 enzyme gene cassettes is necessary to ensure proper expression of each CAM gene cassette in the target species. Current steps achieved to date for CAM Biodesign will be summarized including subcellular localization and phenotypic analysis of overexpressing 14 individual ice plant C4-cycle genes, mesophyll-specific, circadian clock-controlled promoter mining, vector set construction for multi-gene circuit assembly, and the phenotypic effects of engineering a four-component carboxylation module in Arabidopsis.

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Primary Poster Presenter: John Cushman

Carotenoids accumulation in stone fruits (0500-019)
Hall 2

Stone fruits (Prunus sp.), including peach, plum and apricot, exhibit wide range of fruit colors. Carotenoids are the main pigments contributing to their yellow and orange hues. Carotenoids are tetraterpene molecules produced by all photosynthetic organisms. They serve as pigments in many fruit and flower tissues, and some of their degradation products are volatiles, providing distinctive aromas. In addition, some carotenoids are essential components of our diet and carotenoids are known as agents helping in protection against various chronic diseases. We have characterized the carotenoid content and composition of stone fruits harvested from germplasm collections in Israel and the USA, representing wide genetic variability: ~120 apricot accessions and ~70 Japanese plum accessions from the Newe Ya'ar germplasm collection in Israel, as well as ~270 yellow peach accessions from collections in Clemson University and Prunus National Clonal Germplasm Repository, Davis, CA in the USA. Our results demonstrate that although peach, apricot and Japanese plum are genetically closely related, their fruit accumulate very different carotenoids. While apricot accumulates large amounts of the first intermediates of the carotenoid biosynthesis pathway, such as phytoene, phytofluene and even cis-isomers of lycopene, as well as beta-carotene, Japanese plum contains mainly beta-carotene, and the main carotenoid found in peach fruit is Violaxanthin, an end product of the carotenoid biosynthesis pathway. The data obtained will be used in combination with genomic characterization of the different Prunus accessions, to better understand the genetic factors controlling carotenoid accumulation in stone fruit. Elucidating the causes of variability in carotenoid profiles within each species and the differences in the carotenoid composition among peach, plum and apricot, could help in breeding new cultivars with desired carotenoid profiles, attractive colors and elevated nutritional value.

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Primary Poster Presenter: Tal Isaacson Lustig

1:30 PM - 3:00 PM

Characterization of a Complete Acylsugar Biosynthetic Pathway in Nicotiana acuminata (0500-021)

Sugar esters (acylsugars) are defensive, trichome-localized metabolites produced primarily within the Solanaceae family. Depending on the species, up to five acyl chains are esterified at various positions on the sugar core, typically sucrose. Due to acyl chain length and branching variation, tremendous acylsugar structural diversity exists within the Solanaceae. This diversity is better characterized in the Solanum genus relative to other lineages. To gain insight into acylsugar diversity outside Solanum, we characterized acylsugar profiles and the underlying biosynthetic pathway in the Nicotiana genus. Acylsugar profiles were screened from leaf dips using LC- and GC-MS, and acyl chain types were mapped onto a Nicotiana phylogeny. Most acyl chain types are present in all Nicotiana species, however some are restricted to closely-related lineages. For example, a unique acyl chain type, tiglic acid, was found in a single Nicotiana species (Nicotiana acuminata) and represents up to 80% of all acyl chains on the acylsugars in two of the four N. acuminata accessions screened. NMR structural determination of two purified acylsugars from N. acuminata showed that they had a total of four and five acylations on a sucrose core, of which two were tiglic acid. A comparative RNA-seq analysis of trichomes and stem tissue from a high tiglyl-acylsugar producing accession revealed that homologs of acylsugar biosynthetic genes in tomato were highly enriched in the trichomes. Enzyme assays with four trichome-enriched BAHD acyltransferase enzymes together with acyl-CoAs and sucrose produced tetraacylated sucrose, suggesting that these four enzymes represent a complete acylsugar biosynthetic pathway. Future work will test the activity of these enzymes with in vivo acyl-CoAs as the in vitro enzyme assay-produced tetraacylated sucroses did not match those found in planta. Further analysis will determine the biochemical mechanism underlying high tiglyl-acylsugar production in N. acuminata.

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Primary Poster Presenter: Craig Schenck

Characterization of a novel enzyme involved in phosphatidylcholine synthesis in plants and yeast (0500-024)
Hall 2

Phosphatidylcholine (PC) is the major lipid in eukaryotic membranes and has a central role in overall cellular lipid metabolism. In plants, PC also acts as a carrier of acyl groups exported from the plastid and as a substrate for a large number of fatty acid modifications, including the production of polyunsaturated fatty acids. The recently discovered acyl-CoA:glycerophosphocholine acyltransferase (GPCAT) activity in yeast provides a novel route of re-synthesising PC via lysophosphatidylcholine (LPC) after its complete de-acylation. We have identified the gene encoding GPCAT in yeast by screening the yeast deletion strain collection and by homology search also identified and cloned GPCAT genes from plant species. Database searches reveal a wide distribution of GPCAT homologues in eukaryotes, but the genes have not been annotated for any function in any organism and do not show any significant homology with any known acyltransferase or transferase. Biochemical studies of GPCAT from both yeast and plants expressed in yeast show that all enzymes utilise acyl-CoA to acylate GPC, forming LPC. All enzymes show broad acyl specificities. In vivo experiments of yeast GPCAT, shows that the enzyme is the major GPC acyltransferase and that its activity affects the PC species profile.

Primary Poster Presenter: Ida Lager

Characterization of a protein family involved in the metallocenter biosynthesis in the chloroplast (0500-013)
Hall 2

A number of metal-dependent proteins are imported into the chloroplast. The import process of a nascent polypeptide necessitates that each protein accurately folds and binds its correct cofactor once in this organelle. As a result, the machinery required for ensuring the biosynthesis of metallocenters (such as transporters and metallochaperones) must be present in the chloroplast. Here, we describe the phylogenomics-guided discovery and biochemical characterization of a subfamily of chloroplast-localized metal-binding GTPases (belonging to the COG0523 family and the G3E superfamily) that we predict are required for the maturation of metal-dependent proteins in the chloroplast. We have purified members of the land plant, green algal and bacterial families and demonstrated that these proteins possess the same low intrinsic GTPase activity that is stimulated by the presence of divalent cations. Based on a comparative genomic analysis inclusive of conserved gene neighborhood detection and co-occurrence profiles, we have predicted putative targets for these GTPases, purified them and characterized their metal-dependent activity. Moreover, we have developed a metal-transfer assay to test the interaction between the GTPase and its client protein in vitro. These assays show a clear metal-dependent activation of the clients upon metal release from the GTPases during GTP hydrolysis. These combined results present for the first time evidence for the delivery and transfer of metal ions to client metalloproteins in the chloroplast by members of the COG0523 family.

Co-author(s): Crysten Blaby-Haas

Primary Poster Presenter: Miriam Pasquini

Characterizing the role of CYP72A enzymes in maize environmental stress responses (0500-025)
Hall 2

Biosynthesis of many protective secondary metabolites that are induced by environmental stresses require cytochrome P450 enzymes (CYPs). Plant genome sequencing has revealed the presence of thousands of CYP genes with an average of about 0300 genes per plant. The CYP72A subfamily appears to have members in all angiosperms and provides the potential for diverse biochemical functions in each plant species. There are eleven genes for CYP72A enzymes in maize in clusters on chromosomes 3 and 8. Aphid and/or caterpillar feeding induces gene expression for some of the CYP72As. Most of the maize CYP72A sequences have orthologous partners in sorghum and several have orthologs in rice and Brachypodium. Phylogenetic relationships and gene expression data provide a hierarchy of relative contributions of each of the CYP72A enzymes in maize under a variety of environmental stresses. Using this hierarchy to guide our genetic experiments, we isolated transposon insertion mutants in maize. We are using mass spectrometry for metabolic profiling of differences between mutant and wild type plants to explore the role each enzyme plays in plant stress responses. Combinations of abiotic and biotic stresses reveals the subtle differences between wildtype and single CYP72A mutant plants. We have also modeled the three-dimensional structures to examine interactions between each CYP72A structure and potential plant metabolites. These data contribute to a better understanding of the metabolic potential of the CYP72A subfamily in plants.

Primary Poster Presenter: Leeann Thornton

Comparing Metabolism Across Plant Species using the Plant Metabolic Network (0500-016)
Hall 2

Plant metabolism is of immense economic and ecological importance. The Plant Metabolic Network (PMN) is an online database of curated and computationally predicted information about the enzymes, compounds, reactions, and pathways that make up plant metabolism, presenting them online in a searchable, interconnected format. Established in 2008, the network has added new species, metabolic data, and features throughout its decade of history. The latest release, PMN 13, contains metabolic networks from a hundred species, from single-celled algae to monocot and dicot crop species and model organisms. The release also adds new features for cross-species comparison and gene co-expression analysis. To explore how groups of plants compare metabolically and what pathways and compounds distinguish each group, we performed cross-species analyses of the data in PMN. Presence-absence matrices of the compounds, pathways, and

reactions in each species were used for multiple correspondence analysis and hierarchical clustering, revealing groups of plant species by similarities in their metabolism. Brassicaceae, Poaceae, and green algae appeared as particularly distinct groups. To explore the metabolism that results in these distinctions more deeply, we performed a study referred to here as a metabolism-wide association study (MWAS). A generalized linear model was used to find compounds, pathways, and reactions that strongly associated with different phylogenetic, life-style, or trait-based groups, such as classification as annual or perennial, woody or herbaceous, and wild or crop. A species' phylogenetic relationships were a stronger determinant of its metabolic content than non-phylogenetic traits, and many known clade-specific pathways and compounds were successfully found to be associated with their clades from the data. These techniques can highlight areas where understanding can be improved by more research and can uncover hidden patterns in the evolution of plant metabolism.

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Primary Poster Presenter: Charles Hawkins

Delay Tomato Fruit Ripening and Arabidopsis Leaf Senescence by Sound Waves (0500-004)
Hall 2

Sound is ubiquitous in nature. Recent evidence supports the notion that naturally occurring and artificially generated sound wave contribute to the plant robustness. We previously observed delayed ripening in tomato(Solanum lycopersicum) fruit exposed to 1kHz sound wave by regulating the expression of ethylene biosynthesis-related gene through controlling the expression of RIN and HB-1, which encoding transcription factors involved in ethylene biosynthesis. To investigate whether RIN and HB-1 directly activate the transcription of ACS and ACO. We performed transcriptional activation analysis in Arabidopsis thaliana leaf protoplasts, transiently expressing RIN and HB-1 and using reporter constructs with promoters of the tomato ACS and ACO genes. The RIN and HB-1 induced expression of these genes decreased, but the HB-1 induced expression of some genes increased after sound wave treatment. In addition to check retard tomato fruit ripening, we also examined delayed leaf senescence of ethylene mutant Arabidopsis treated with sound waves in sealed container filled with ethylene gas. Leaf senescence is a developmentally programmed event, but the initiation and progression of leaf senescence are affected by plant hormone including ethylene. To investigate the effect of sound wave on leaf senescence, we analyzed the morphological change of ethylene mutant Arabidopsis seedling using triple response assay. Sound wave treated ethylene mutant Arabidopsis seedling were highly elongated but no apical hook observed. According to the results, the expression of ethylene biosynthesis-related gene is regulated by sound wave, as a result, it seem to be delayed Arabidopsis leaf senescence and tomato fruit ripening.

Co-author(s): JinA Kim,
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Primary Poster Presenter: MiJeong Jeong

Differential defense programming in monolignol pathway mutants of Medicago truncatula (0500-030)
Hall 2

Attempts to modify lignin content and/or composition by genetic modification often result in negative growth effects. Although several studies have attempted to address the basis for such effects in individual transgenic lines, no common mechanism linking lignin modification with perturbations in plant growth and development has yet been identified. To address whether a common mechanism exists, we have analyzed transposon insertion mutants resulting in independent loss of function of five enzymes of the monolignol pathway, as well as one double mutant, in the model legume Medicago truncatula. These plants exhibit growth phenotypes from essentially wild-type to severely retarded. Extensive phenotypic, transcriptomic, and metabolomics analyses, including structural characterization of differentially expressed compounds, revealed diverse phenotypic consequences of lignin pathway perturbation that were perceived early in plant development, but were not predicted by lignin content or composition alone. Notable phenotypes among the mutants with severe growth impairment were increased trichome numbers, accumulation of a variety of triterpene saponins, and extensive but differential ectopic expression of defense response genes. No currently proposed model explains the observed phenotypes across all lines. We propose that the severity of the final growth phenotype in monolignol pathway mutants of Medicago is in large part the result of the extent of re-allocation of resources into defense pathways.

Primary Poster Presenter: Chan Man HA

Enhancing Soybean Oil Composition – A Cas9 Multi-Edit Approach (0500-035)
Hall 2

To improve the healthfulness and stability of soybean oil - the fatty acid composition of the oil within the seed must be addressed. Commodity soybean oil has a profile that is high in the polyunsaturated fatty acids, linoleic (C18:2) and linolenic (C18:3) acid. Although polyunsaturated fats are generally considered healthier than saturated fats, the presence of additional double bonds also decreases the oil stability during high-heat frying. Decreased stability can lead to an increase in free radical generation which is an unwanted side effect of the frying process. By shifting the oil profile in the seed from polyunsaturated fatty acids to the monounsaturated oleic acid (C18:1) both healthfulness and stability can be achieved. In this research, we discuss the biochemistry and modifications that led

to the generation of a high oleic soybean and the Pioneer™ Plenish® product in addition to new efforts to create a CRISPR-Cas9 gene edited trait. Additionally, we discuss the best transformation method, events, and data needed to create a product that unlocks value for both consumers and the farmers on a large scale.

Primary Poster Presenter: Andrew Foudree

Enhancing the efficiency of photorespiration through optimization of catalase temperature response (0500-011)
Hall 2

Photorespiration loses carbon dioxide primarily from enzymatic decarboxylation of glycine. As temperature increases, photorespiration likely releases additional CO2 from non-enzymatic decarboxylation of glyoxylate or hydroxypyruvate by hydrogen peroxide generated in peroxisome. It has been suggested that catalase plays an important role in protecting plants against the non-enzymatic decarboxylation by decomposing H2O2. The kinetic characterizations of catalase in higher plants have not been systematically evaluated, since the heterologous gene expression of catalase is recalcitrant to bacterial expression system. Here, we employed a cell-free wheat-germ expression system to express catalase genes and assayed for their catalytic activities. To optimize the carbon recycling efficiency of photorespiration in response to rising temperature, we characterized the in vitro kinetics of catalase from diverse species, including important bioenergy crops and species adapted to high temperatures under a range of physiologically relevant temperatures. These enzyme kinetics are being incorporated into a reaction-kinetic model of photorespiration, to determine if a more thermostable or otherwise more optimal catalase isoform can reduce excess CO2 loss in silico. In parallel with these simulations, these catalases are being transformed into Arabidopsis thaliana mutant lacking the native catalase gene to test the efficiency of photorespiration (e.g. CO2 compensation point, gross oxygen fluxes and CO2 assimilation) using in vivo gas exchange approaches. This work will help us to better understand the efficiency of photorespiration under current and future climates and determine how we can improve the response of photorespiration to temperature for increasing crop productivity.

Co-author(s): Berkley Walker

Primary Poster Presenter: Han Bao

Enrichment of erucic acid in the seed oil of crambe is potentially hampered by DGAT specificities (0500-023)
Hall 2

Crambe (Crambe hispanica subsp. abyssinica) is an industrial oil crop and has a high content (>55 %) of erucic acid (cis-Δ13-22:1, 22:1) in the seed oil. Erucic acid and its derivatives, mainly erucamide, are included in a wide range of industrial applications, e.g. as slipping agent for plastic films, lubricant, and quenching oil.

Crambe seed oil consists of triacylglycerol (TAG), a glycerol backbone esterified with three fatty acids. Several acyltransferases are required to transfer acyl-groups to the backbone, which results in the formation of TAG in the Kennedy pathway. Crambe as other Brassicaceae seldom store beyond 2/3 of 22:1 in their seed oil due to the reduced capability of one of their endogenous acyltransferases, LPAAT, to utilise 22:1-CoA. Transgenic crambe expressing LdLPAAT, BnFAE, and an RNAi construct suppressing the expression of endogenous desaturase manage to circumvent the endogenous inferior LPAAT performance towards 22:1, and significantly increases the levels of abundant di-22:1-DAG, the precursor of trierucin. The 22:1 content in these transgenic crambe lines increases to 73 %, but further increase seems to be hampered by other bottlenecks. The enzyme responsible for the final acylation step in TAG synthesis is diacylglycerol acyltransferase (DGAT), and it is likely one of the rate-limiting steps in the formation of TAG with three erucic acids. We identified eight forms of DGATs in crambe and characterised them in microsomal preparations of yeast expressing the enzymes using a wide range of acyl-CoA and DAG species. Using the trierucin precursors, di-22:1-DAG and 22:1-CoA revealed a much reduced enzymatic activity when compared to more favourable acyl-CoA and DAG species. Our results indicate that formation of trierucin by crambe DGATs is a limiting step for further increasing the levels of 22:1 in the previously developed transgenic crambe lines due to their poor abilities to acylate di-22:1-DAG.

Co-author(s): Sten Stymne,
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Primary Poster Presenter: Simon Jeppsson

Evolution of a gene cluster that shapes acylsugar acyl chain diversity in Solanaceae species (0500-014)
Hall 2

Glandular trichomes of the Solanaceae species produce protective acylsugars for insect defense. These specialized metabolites are mixtures of sugar aliphatic esters with acyl chains varying in carbon numbers and branching patterns. We observe phylogenetically-associated variation in acyl chain length across the family: Nicotiana, Petunia and Salpiglossis species accumulate acylsugars with short acyl chains (carbon number, C≤8), whereas species of Solanum and other close genera make acylsugars with long acyl chains (C≥10). We identified a gene cluster in tomato with tandem duplications of BAHD acyltransferase, acyl-CoA synthetase (ACS), and enoyl-CoA hydratase (ECH) genes. CRISPR-Cas9 ablation of two trichome expressed genes in the cluster (ACS30 or ECH80) affects long chain containing acylsugar in the cultivated tomato. This is likely by disrupting acyl-CoA metabolism: these are the donor substrates for acylsugar biosynthesis. ACS30 and ECH80 were co-opted from primary metabolism and are targeted to the mitochondria. This contrasts with the well-studied ACSs or ECHs, which generate long chain acyl-CoAs from lipid biosynthesis (mainly in chloroplasts) or fatty acid breakdown (mainly in peroxisomes). Syntenic analysis of this gene cluster in the

tomato genome revealed a homologous region on chromosome 12, which encodes SlASAT1 – the core acylsugar biosynthetic enzyme. Comparative genomic analysis led to evolutionary reconstruction of the gene cluster across the family. Gene duplication, gene transposition, and pseudogenization facilitated emergence of this gene cluster in the Solanum. These events presumably shaped the phylogenetically-restricted distribution of long chain containing acylsugars in the Solanaceae. Analysis of this system is providing insights into evolution of specialized metabolism by co-option of primary metabolic enzymes, emergence of cell type specific gene expression and furthering our understanding of mechanisms by which gene clustering arises.

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Primary Poster Presenter: Pengxiang Fan

FAT-PTM: A database for examining post-translational modification data in the context of proteins an (0500-038)
Hall 2

Post-translational modifications (PTMs) are critical regulators of protein function, and nearly 200 different types of PTMs have been identified. Advances in high-resolution mass spectrometry have led to the identification of an unprecedented number of PTM sites in numerous organisms, potentially facilitating a more complete systems-scale understanding of how PTMs regulate cellular behavior. While databases have been created to house the resulting data, most of these resources focus on individual PTM types, do not consider quantitative PTM analyses, or do not provide tools for the visualization and analysis of PTM data. Here, we describe the Functional Analysis Tools for Post-Translational Modifications (FAT-PTM) database (https://bioinformatics.cse.unr.edu/fat-ptm/), which currently supports 8 different types of PTMs and over 49,000 PTM sites identified in large-scale proteomic surveys of the model organism Arabidopsis thaliana. FAT-PTM currently supports tools to visualize protein-centric PTM networks, quantitative phosphorylation site data from over 10 different quantitative phosphoproteomic studies, PTM information displayed in protein-centric metabolic pathways, and groups of proteins that are co-modified by multiple PTMs. Additionally, tools to identify PTMs that are disrupted due to natural variation-based single nucleotide polymorphisms and to examine the conservation of PTM sites in multiple species will be discussed. The FAT-PTM database provides a robust platform to share and visualize experimentally supported PTM data, develop hypotheses related to target proteins, or identify emergent patterns in PTM data for signaling and metabolic pathways. Overall, these unique tools may lead to a more comprehensive understanding of PTM regulatory processes in Arabidopsis and potentially be used to identify critical signaling regulatory points and associated PTM sites that could be manipulated to control plant metabolism, growth, or development.

Co-author(s): Ian Wallace,
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Primary Poster Presenter: Edward Cruz

Finding targets and downstream components of trehalose 6-P signalling (0500-089 (Screen 5))
Hall 2

Trehalose 6–phosphate (Tre6P), the precursor of trehalose, is an essential signal metabolite in plants, linking growth and development to carbon metabolism. The sucrose-Tre6P nexus model postulates that Tre6P is both a signal and negative feedback regulator of sucrose, forming part of a mechanism to maintain sucrose levels within an optimal range. Induced short-term increases in Tre6P during the day led to a decrease in sucrose content by diverting photo-assimilates away from sucrose to generate carbon skeletons and fixed nitrogen for amino acid synthesis, mediated via post-translational activation of nitrate reductase and phosphoenolpyruvate carboxylase. However, the exact mechanism and downstream components by which Tre6P exerts its effects on plant growth and development are still mostly unknown. SNF1-related protein kinase 1 (SnRK1) plays a fundamental role in regulating cellular responses to energy limitation and carbon starvation and evidences for potential interaction between the SnRK1 and Tre6P pathways have been accumulating. These include: (i) inhibition of the in vitro activity of SnRK1 in young developing tissues by Tre6P, (ii) partial overlap in pattern of gene expression in response to constitutive changes of Tre6P and SnRK1, (iii) demonstration of a direct interaction between Tre6P and SnRK1α1 in vitro using Micro Scale Thermophoresis. These findings have led to a hypothesis that the effects of Tre6P on plant development are largely mediated through inhibition of SnRK1. However, the relationship between Tre6P and SnRK1 in developing tissues may be more complex than postulated by this hypothesis. We therefore aim at understanding this relationship further by comparing short-term metabolic and gene expression responses of transiently induced SnRK1α1, SnRK1α2 and heterologous TPS. Additionally, we utilize a unique SnRK1-specific reporter lines to monitor changes in SnRK1 in vivo activity in response to a transient increase in Tre6P levels.

Primary E-Poster Presenter: Omri Avidan

Functional characterization of anandamide hydrolyzing enzyme in Physcomitrella patens (0500-017)
Hall 2

The discovery of a mammalian endocannabinoid, anandamide (AEA or NAE 20:4) in Physcomitrella patens but not in higher plants prompted our interest in characterizing its metabolism and physiological role in the early land plants. Anandamide acts as an endocannabinoid ligand in the mammalian central and peripheral systems and mediates various physiological responses. Endocannabinoid

signaling is terminated by a membrane-bound fatty acid amide hydrolase (FAAH). Using in silico analyses, we identified nine orthologs of human and Arabidopsis FAAH in P. patens (PpFAAH1 to PpFAAH9). Predicted structural analysis revealed that all the nine PpFAAH contain characteristic amidase signature sequence with a highly conserved catalytic triad and share a number of key features of both plant and animal FAAH. These include a membrane binding cap, membrane access channel, substrate binding pocket and as well as potential for dimerization. Among the nine, gene expression for PpFAAH1 and PpFAAH9 was enhanced with exogenous AEA treatment. Cloning and heterologous expression, followed by radiolabeled in vitro enzyme assays revealed that PpFAAH1 activity was optimal at 37 °C and pH 8.0. Furthermore, PpFAAH1 showed higher specificity to NAE 20:4 than to other N-acylethanolamines such as NAE 16:0. Highest in planta amide hydrolase activity was noted in microsomes of gametophytes, suggesting the possibility for membrane localization of active FAAH. Interestingly, when FAAH1 was overexpressed, the moss cultures not only showed reduced growth but their transition from protonema to gametophyte was inhibited, which was rescued by exogenous AEA. Unlike overexpressors of AtFAAH1, which showed enhanced growth and hypersensitivity to abscisic acid, PpFAAH1 overexpressors showed tolerance to abscisic acid. Together, these data suggest that the occurrence of anandamide and distinct properties of PpFAAH1 in early land plants have physiological implications that are different from that of higher plants.

Co-author(s): Haq Imdadul

Primary Poster Presenter: Aruna Kilaru

Functionality of the FaPAL6 gene promoter of Fragaria x ananassa in response to UV-C light (0500-001)
Hall 2

The strawberry (Fragaria x ananassa) is a crop of great acceptance and economic importance around the world. It is rich in a broad range of bioactive phytochemicals related to the prevention of neurodegenerative and cardiovascular diseases as well as to many types of cancer. From these compounds, the flavonoids stand out for their high antioxidant activity. It is known that UV-C light induces flavonoids accumulation in the strawberry fruit, and this response is related to the FaPAL genes expression. It is also known that strawberry has 6 FaPAL genes (1 to 6) and previous studies in our lab shown that FaPAL 2 and FaPAL 6 gene expression are specifically induced by UV-C light. These results suggest that the promoters of these genes are responsible for the UV-C induction. The analysis of these promoters is important to understand the molecular mechanism through which UV-C light stimulates the flavonoid biosynthetic pathway in strawberry. The main objective of this work is to isolate and clone the promoter region of FaPAL6 gene, and to evaluate its functionality in response to the UV-C irradiation. For this purpose, we identified an upstream region of the FaPAL6 coding sequence (HM6418223.1), and using PCR procedures we isolated a 1262 bp fragment (PrFaPAL6). It was cloned into the pBI121 vector (CaMV 35S-GUS) in substitution of the CaMV 35S promoter.

This construction (pBI121-PrFaPAL6-GUS) and the original pBI121 vector, were introduced into Agrobacterium tumefaciens (LBA4404). Agroinfiltration procedure was used to achieve transient gene expression of GUS reporter gene, in post-harvest strawberry fruits. GUS activity was widely visualized by histochemical staining using X-Gluc in control fruits holding just the pBI121. The fruits treated with the pBI121-PrFaPAL6-GUS construct are being analyzed after UV-C light treatment to check the functionality of the PrFaPAL6 promoter.

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Primary Poster Presenter: Jesús Alonso Garduño-Hernández

Gene expression in Papaver bracteatum in response to hormonal elicitors (0500-039)
Hall 2

Elicitation plays an important role in regulating the synthesis of secondary metabolites in medicinal plants. The relationship between alkaloid biosynthesis and gene expression were identified by evaluating the effect of three different hormones on alkaloid production in suspension cultures of Papaver bracteatum. Biosynthesis of benzylisoquinoline alkaloids (BIAs) is initiated by inducible factors in opium poppy. Accumulation of gene transcripts alkaloids for tyrosine/dopa decarboxilase (TYDC), berberine bridge enzyme (BBE), salutardinol acetyltransferase (SAT) and codeinone reductase (COR) was observed in suspension culture of P. bracteatum. Gibberellic acid (GA), indole butyric acid (IBA), and indole-3-acetic acid (IAA) were applied as hormonal elicitors in the suspension cultures with three different doses and two timings along with the control. Induction of morphine alkaloid in the suspension culture of P. bracteatum was identified. Significant increases in the mount of morphine alkaloids were achieved by 20 mg/l of IAA after 48h treatment. Comparative analysis of transcripts showed that the expression of COR increased significantly, however TYDC, BBE and SAT did not indicate any significant changes compared to the control. Since 48h treatment could induce more morphine alkaloids compared to 24h treatments, the timing of hormonal elicitation has a significant effect.Key words: Papaver bracteatum, gene expression, Indole-3-acetic acid, Indole butyric acid and Gibberellic acid.

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Primary Poster Presenter: Marjan Behzadirad

GlcNA.UTs are important for protein N-glycosylation and ABA-mediated salt sensitivity in Arabidopsis (0500-002)
Hall 2

The N-acetylglucosamine-1-P uridylyltransferases (GlcNAc1pUTs) catalyze the biosynthesis of UDP-N-acetylglucosamine (UDP-GlcNAc), which is an essential

amino sugar for glycosylation in organisms. Two GlcNAc1pUTs, which are encoded by GlcNA.UT1 and GlcNA.UT2, respectively, have been identified in Arabidopsis. Since the single mutant glcna.ut1 and glcna.ut2 showed no obvious phenotype and the double mutant was lethal, transgenic plants (named iU1 lines) were created by expressing double-stranded partial sequence of GlcNA.UT1 in the glcna.ut2 mutants to define GlcNAc1pUTs function. The iU1 lines showed no obvious phenotype when grown on normal conditions, but exhibited delayed seed germination and enhanced postgermination developmental arrest when grown on medium containing 200 mM NaCl or 400 mM mannitol. The iU1 seedlings were also impaired in UDP-GlcNAc biosynthesis and the protein N-glycosylation under salt stress. Microarray analysis revealed that the expression of genes involved in salt, ER stress responses, and ABA biosynthetic and signaling pathways were affected. ABA levels in the salt-treated iU1 seedlings were also increased. Treatment of Fluridone, an ABA biosynthetic inhibitor, was able to rescue the delayed germination and developmental arrest phenotype of iU1. Disruption of N-glycosylation using tunicamycin treatment or mutants of N-glycan biosynthesis induced developmental arrest phenotype, but not germination delay, under salt stress conditions. Collectively, our data indicate that depletion of GlcNA.UTs results in defects in UDP-GlcNAc biosynthesis and protein glycosylation, which are associated with ABA-mediated salt hypersensitivity.

Co-author(s): Hwei-Ling Shen,
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Primary Poster Presenter: Ya-Huei Chen

Glycolytic Enzymes Are Involved in the Modulation of Autophagic Flux under Carbon Starvation C (0500-026)
Hall 2

To maintain cellular metabolic status, higher eukaryotes have evolved diverse strategies to control the balance between nutrient storage and utilization. Under nutrient-deficient conditions, cellular organelles and macromolecules are degraded by autophagy for nutrient recycling, and autophagic flux is tightly regulated depending on metabolic conditions. Photo-autotrophic plants produce organic carbon compounds through photosynthesis and consume them during nighttime as nutrients. Decade-long study has shown that the autophagy pathways and autophagy-related proteins are highly conserved in yeast, mammals, and plants. However, upstream signaling of autophagy and regulatory mechanisms of autophagic flux are poorly understood in plants. Here, we report specific phenomenon in plants; autophagic flux is controlled by a part of glycolytic enzymes that are closely linked to photosynthesis. This regulatory mechanism depends on ATG1 kinase complex activity and it is also modulated by nutrient availability. Considering that plants produce organic carbon compounds via photosynthesis, these mechanisms may provide a novel strategy to coordinate autophagic activity with carbon nutrient availability.

Co-author(s): Hyun-Sook Pai

Primary Poster Presenter: DUHWA LEE

Identification of a Native Polysaccharide Inhibitor of Cellulase from Transgenic Maize Seed (0500-034)
Hall 2

The maize seed expression system is a powerful platform for recombinant protein production. It has been utilized for high-yield production of industrial cellulases such as endo-1,4-β-D-glucanase (E1) and 1,4-β-D-glucan cellobiohydrolases (CBHI & CBHII). However, some preliminary data indicate that a concentrated E1 crude extract contained an inhibitory compound that could significantly reduce the cellulolytic efficiency. After further investigation, this inhibitory compound was detected even in purified E1 samples. With steps of purification and analysis, the inhibitory compound was determined to be thermostable polysaccharides with a molecular size over 10 kDa and accompanied only recombinant E1 but not CBHII. Even though the strict column purification process will remove water-soluble non-protein molecules, the inhibitory polysaccharides still remain. A possible inference is that E1 as a cellulolytic enzyme can bind to the inhibitory polysaccharides, carry them through all purification steps and finally release them when denatured by heat.

Co-author(s): Kendall Hood,
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Primary Poster Presenter: Hong Fang

Identification of a pathway for disposal of metabolite damage products formed by the respiratory int (0500-010)
Hall 2

Cellular thiols such as cysteine and glutathione spontaneously and readily react with the respiratory intermediate fumarate, resulting in the formation of stable S-(2-succino)-adducts. Fumarate-mediated succination of thiols increases in certain tumors and in response to glucotoxicity associated with diabetes. Therefore, S-(2-succino)-adducts such as S-(2-succino)cysteine (2SC) are considered oncometabolites and biomarkers for human disease. 2SC has not been detected in plants suggesting that they have a mechanism to prevent its accumulation. Recently, a pathway for 2SC disposal was discovered in Bacillus subtilis, but this pathway is only present in firmicute bacteria. A comparative genomics analysis identified two putative alternate pathways for 2SC disposal in prokaryotes; the enzymes of one of these pathways have close homologs in plants. The predicted plant-pathway is initiated by an acetyltransferase (At2g39000 or At4g28030) that acetylates 2SC. A glutathione-S-transferase-like enzyme (At5g44990 or At5g44000) then cleaves acetylated-2SC into succinate and N-acetylcysteine. Biochemical and genetic characterization of this pathway is ongoing. This pathway, if confirmed, represents a metabolite damage control system that could have

applications in improving stress tolerance and metabolic engineering in plants. This work also nicely exemplifies the use of cross-kingdom comparative genomics to predict the function of unknown genes in plants.

Primary Poster Presenter: Thomas Niehaus

Identifying Roles of Higher Inositol Phosphates in Plant Physiology and Phosphate Metabolism (0500-032)
Hall 2

Inositol pyrophosphates (PP-InsPs) are unique messenger molecules recently discovered to confer Pi availability and energy status in a variety of organisms however their role in plant metabolism is unknown. PP-InsPs are rapidly synthesized and degraded by a group of evolutionarily conserved enzymes, which makes them difficult to study. To delineate the roles of these molecules in plant metabolism, our lab has generated a suite of Arabidopsis thaliana mutants and transgenics by removing and overexpressing genes for these evolutionarily conserved enzymes. This work outlines characterization of a transgenic overexpressing a yeast gene and how artificial modulation of PP-InsPs affect plant response to exogenous Pi.

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Primary Poster Presenter: Catherine Freed

INVESTIGATING THE BIOCHEMICAL AND PHYSIOLOGICAL SIGNIFICANCE OF RIBOSOMAL PROTEIN S6 PHOSPHORYLATION (0500-037)
Hall 2

mRNA translation is the most energy intensive process in the cell and is under tight regulation. Translational control is indispensable to maintaining homeostasis, cell-proliferation, growth, and development. Hence, understanding the molecular basis and mechanisms of translational control is crucial. We are interested in understanding the role of translational regulation in plant growth and development using Arabidopsis thaliana as a model system. One of the major pathways implicated in translational control is the TOR-S6 kinase pathway. Several TOR and S6-kinase substrates have been identified to regulate translation. Ribosomal protein of the small subunit 6 (eS6) is a component of the 40S subunit of the eukaryotic ribosome and a prominent target of S6 kinase. eS6 is highly conserved among all eukaryotes and has been shown to undergo phosphorylation at multiple sites in its carboxy-terminal tail. The phosphorylation is regulated in response to various environmental stresses such as the level of sucrose, cold, heat shock, hypoxia and light conditions. By monitoring the oscillation dynamics of Phospho-eS6 we

observed that eS6 phosphorylation is jointly controlled by the circadian clock and light conditions. The integration of the two signals is unconventional, because, in contrast to other clock outputs, the light-driven cycle of eS6-P is out of phase with the clock-driven cycle. However, the biochemical significance of eS6 phosphorylation in translational control remains unknown. Our current efforts aim to understand the functional significance of eS6 phosphorylation in response to different intracellular and extracellular cues. We have performed mutagenesis to generate a series of phospho-mutant alleles for both of the paralogs of eS6, and we are preparing to test the potential roles of eS6 phosphorylation in translation regulation. Further, we hope to integrate our findings, to gain novel insights into the regulation of the translational apparatus in plants.

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Primary Poster Presenter: Anwesha Dasgupta

Involvement of 5 catalytic Arabidopsis beta-amylases in leaf starch metabolism and plant growth (0500-012)
Hall 2

Hydrolysis of transitory starch in Arabidopsis thaliana is carried out in large part by members of a family of nine beta-amylases, five of which are likely to be catalytically active: BAM1, -2, -3, -5 and -6. Of these five, four (excluding BAM5) are located in plastids and may play a role in starch hydrolysis. Understanding the specific roles of these enzymes has been challenging because single gene mutants often lack phenotypes, suggesting functional redundancy, and because tissue extracts usually contain multiple BAM activities. To address these problems, we constructed a set of quadruple mutants each expressing only one of the five catalytically active BAMs (B1-Q…) and the quintuple mutant lacking all five BAMs (B-Null). When grown under a 12/12 hr photoperiod the B-Null plant grew slowly, had no detectable beta-amylase activity, and accumulated large amounts of starch. B2-Q, B5-Q and B6-Q were all indistinguishable from B-Null indicating that BAM2, -5 and -6 do not contribute significantly to diurnal leaf starch metabolism. In contrast, B3-Q was indistinguishable from WT plants. B1-Q grew as well as WT but accumulated some starch. Total extractable beta-amylase activity from above-ground tissues of each mutant revealed that BAM1, -2, -3, -5 and -6 accounted for approximately 14%, 4%, 70%, 9% and 2% of the WT activity, respectively. There have been numerous reports of transcriptional regulation of BAM genes in response to photoperiod and abiotic stress, so with these mutants we tested the effects of these treatments on the activity of each BAM. In general, activities were not strongly affected by the treatments. Remarkably, activities of BAM1 and BAM3 were nearly constant over a 24-hr photoperiod and did not change appreciably in plants between 5 and 10 weeks of age. These results suggest that regulation of starch metabolism depends on molecules other than catalytically active BAMs.

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Primary Poster Presenter: Claire Ravenburg

1:30 PM - 3:00 PM

Polyamines alter primary root growth by modulating biotin synthesis (1100-002)

Hall 2

Cadaverine, a polyamine produced by plants and microbes, modulates root architecture by decreasing primary root growth, promoting lateral root development, and altering root skewing and waving. To identify genes involved in cadaverine response, a forward genetic screen was carried out in Arabidopsis thaliana. One of the identified hypersensitive mutations was mapped to a nonsynonomous polymorphism in the biotin-synthesis BIO3-BIO1 gene, affecting the catalytic pocket of DAPA synthase. Treatment with exogenous biotin suppressed the inhibitory effect of cadaverine on primary root growth in wild-type seedlings, and it alleviated cadaverine hypersensitivity of bio3-bio1 mutant, suggesting the biotin synthesis pathway is a target for cadaverine action. Arabidopsis BIO3-BIO1 enzyme was expressed in E. coli, affinity-purified and tested in in vitro enzymatic reactions leading to DTB synthesis, using KAPA as a substrate. Both putrescine and cadaverine were found to function as efficient amino donors. However, cadaverine appeared to somewhat inhibit the reaction when added together with putrescine. These preliminary data suggest that both putrescine and cadaverine can function as amino donors, but cadaverine is either catabolized less efficiently, or more strongly retained in the binding site of the enzyme, relative to putrescine thereby inhibiting the reaction. Biotin is an important molecule used as a co-factor in a number of carboxylation and decarboxylation reactions involved in central metabolism. Quantification of biotinylated proteins in cadaverine-treated seedlings showed reduced amounts of BCCP1, a subunit of Acetyl-CoA carboxylase. A lipidomic analysis revealed significant changes in the lipid profiles of cadaverine-treated seedlings relative to the control. We propose that cadaverine controls root growth by inhibiting biotin synthesis, thereby affecting central metabolic pathways. This works is supported by a UW-Madison HATCH grant.

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Concurrent Symposium Speaker: Shih-Heng Su

Structure-function analyses of two plant meso -diaminopimelate decarboxylase isoforms reveal that a (0500-007)

Hall 2

Meso-diaminopimelate decarboxylase catalyzes the final reaction in the L-lysine biosynthetic pathway, the decarboxylation of meso-diaminopimelate. It is the only known pyridoxal-5-phosphate -dependent decarboxylase that catalyzes the removal of a carboxyl group from a D-stereocentre. Currently, only prokaryotic orthologs have been kinetically and structurally characterized. Here we report the first in depth structural analyses of two eukaryotic meso-diaminopimelate decarboxylases from the plant species Arabidopsis thaliana. We have captured the enzyme in an asymmetric configuration, with one ligand-bound monomer and the other in an apo form. Based on our structural analyses, we suggest a mechanism whereby molecular interactions within the active site transduce conformational changes to the active site loop. These conformational differences may provide clues as to how the enzyme selects for the D-stereocentre of the substrate meso-diaminopimelate, to facilitate the synthesis of L-lysine.

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Primary Poster Presenter: Andre Hudson

The aldoxime metabolism is linked with the phenylpropanoid production in plants (0500-015)
Hall 2

Plants produce diverse secondary metabolites. Although each metabolite is made through its own biosynthetic pathway, plants coordinate multiple biosynthetic pathways simultaneously. Recent studies have shown a crosstalk between glucosinolate metabolism and phenylpropanoid production. Glucosinolates are defense compounds made from various amino acids. Phenylpropanoids such as lignin are made from phenylalanine through the phenylpropanoid pathway. The study with Arabidopsis mutants having a defect in the indoleglucosinolate biosynthetic enzyme revealed that the accumulation of indole-3-acetaldoxime (IAOx) or its derivatives affects the phenylpropanoid production. The mechanism behind the crosstalk involves increased expression of genes encoding F-Box proteins responsible for the degradation of phenylalanine ammonia lyase (PAL) which functions at the entry point of the phenylpropanoid pathway. Given that aldoximes are precursors of various compounds in addition to glucosinolates and the phenylpropanoid pathway is present in most plants, it is possible that this mechanism is conserved throughout plant kingdom. We examined the impact of aldoxime metabolism on phenylpropanoid production in Camelina sativa by overexpressing Arabidopsis thaliana CYP79B2 which encodes IAOx producing enzyme. We found that overexpression of AtCYP79B2 affects plant growth and phenylpropanoid metabolism. The transgenic plants display characteristic high auxin morphology and reduced phenylpropanoid contents and PAL activity. From phylogenetic study, we identified a total of 459 non-redundant proteins containing kelch-motif(s) in Camelina sativa and found that the expression of a set of KFBs involving in PAL degradation is increased in the transgenic lines. The results

suggest that the aldoxime accumulation negatively influences on the phenylpropanoid production through the transcriptional activation of KFBs responsible for PAL degradation in Camelina sativa.

Primary Poster Presenter: Jeongim Kim

Tree peony species are a novel resource for production of alpha linolenic acid (0500-006)
Hall 2

Tree peony is known worldwide for its excellent ornamental and medical values, but recent reports that their seeds contain over 40% α-linolenic acid (ALA), an essential fatty acid for humans drew additional interest of biochemists. To understand the key factors that contribute to this rich accumulation of ALA, we carried out a comprehensive study of oil accumulation in developing seeds of nine wild tree peony species. The fatty acid content and composition was highly variable among the nine species; however, we selected a high- (P. rockii) and low-oil (P. lutea) accumulating species for a comparative transcriptome analysis. Similar to other oilseed transcriptomic studies, upregulation of select genes involved in plastidial fatty acid synthesis, and acyl editing, desaturation and triacylglycerol assembly in the endoplasmic reticulum was noted in seeds of P. rockii relative to P. lutea. Also, in association with the ALA content, transcript levels for fatty acid desaturases (SAD, FAD2 and FAD3), which encode for enzymes necessary for polyunsaturated fatty acid synthesis were higher in P. rockii compared to P. lutea. We further showed that the overexpression of PrFAD2 and PrFAD3 in Arabidopsis increased linoleic and α-linolenic acid content, respectively and modulated their final ratio in the seed oil. In conclusion, we identified the key steps that contribute to efficient ALA synthesis and validated the necessary desaturases in P. rockii that are responsible for not only increasing oil content but also modulating 18:2/18:3 ratio in seeds. Together, these results will aid to improve essential fatty acid content in seeds of tree peonies and other crops of agronomic interest.

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Primary Poster Presenter: Aruna Kilaru

Using Nanopore Sequencing to Understand Allelic Contributions to Aroma in Apple (0500-063)
Hall 2

Branched-chain esters derived from 2-methylbutanol and 2-methylbutanoate (2MB) are important contributors to apple aroma. These branched-chain compounds are products of isoleucine metabolism. A novel pathway, enabled by citramalate synthase (CMS), has recently been discovered to feed into the isoleucine synthetic pathway, bypassing regulation at threonine deaminase. CMS is a member of the 2-

isopropylmalate synthase family, but lacks a feedback regulatory region commonly found in these enzymes, and is thereby able to drive the formation of 2MB esters during apple fruit ripening. CMS action also explains observed increases in citramalic acid and isoleucine levels in apple fruit peel. Two alleles of MdCMS were found in 'Jonagold' fruit, generating active (MdCMS_1) and inactive (MdCMS_2) isoforms of the CMS protein. In order to further investigate how isoforms of CMS influence 2MB ester synthesis in apple, nanopore sequencing was used to haplotype the MdCMS locus of 24 apple varieties previously characterized as having widely divergent rates of 2MB ester synthesis. Expression levels of MdCMS were also ascertained. Four additional alleles of MdCMS were discovered that differed at four exonic SNPs and three intronic indels directly upstream of MdCMS. Initial results indicate that varieties homozygous for MdCMS_2 have extremely low ratios of 2MB esters to straight-chain esters. Apple varieties homozygous for alleles thought to produce active proteins, as well as those heterozygous for active and inactive proteins, possess similar 2MB:straight-chain ratios, suggesting a dominant-recessive pattern. The information gained will help breeders screen and predict aroma profiles from genomic DNA, saving the multiple years needed to otherwise to go from seed to fruit.

Co-author(s): Randy Beaudry

Primary Poster Presenter: Philip Engelgau

Using synthetic biology to investigate the subcellular architecture of the phylloquinone pathway (0500-005)
Hall 2

Phylloquinone (vitamin K1) is a vital electron carrier involved in photosynthesis in plants, green algae, and certain cyanobacteria. The phylloquinone pathway starts from the product of the shikimate pathway, chorismate, which is converted in plastids to o-succinylbenzoic acid (OSB). Subsequently, the succinyl side chain of OSB is activated by an OSB-CoA ligase, AAE14, and cyclized by naphthoate synthase to form 1,4-dihydroxy-2-naphthoyl-CoA (DHNA-CoA), which is hydrolyzed to 1,4-dihydroxynaphthoic acid (DHNA) by DHNA-CoA thioesterase. While the naphthoate synthase and DHNA-CoA thioesterase-catalyzed steps occur in peroxisomes, it remains an open question whether AAE14 acts in plastids, peroxisomes, or both compartments as it contains targeting signals for both organelles. To address this question, we are using a synthetic biology approach to investigate localization of AAE14 under control of its native promoter to evaluate the in planta localization in Arabidopsis. In parallel, we are functionally complementing a seedling-lethal aae14 Arabidopsis knockout with versions of AAE14 targeted to plastids, peroxisomes, or both organelles to determine where OSB-CoA ligase activity is required in the cell. By understanding the localization of AAE14, we will have better understanding of the subcellular architecture of the phylloquinone pathway and the necessity for transporter steps needed to mediate exchange of pathway intermediates.

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Primary Poster Presenter: Rachel McCoy

Wax Crystal-sparse Leaf 5 catalyzes alkanes to primary alcohols and is involved in rice leaf cuticul (0500-018)
Hall 2

Cuticular wax, composed of very-long-chain fatty acids and their derivatives, is crucial for plant development and tolerances to environmental stresses. Odd-numbered primary alcohols are components of plant cuticular wax, but their biosynthesis is unknown. We isolated a rice wax crystal-sparse leaf 5 (WSL5) gene using a map-based cloning strategy. WSL5 is predicted to encode a cytochrome P450 family member. Complementation of the mutant wsl5 with WSL5 genomic DNA rescued the cuticular wax-deficient phenotype, while WSL5 knock-out transgenic plants showed wsl5 phenotype, confirming the function of WSL5. The load of C23-C33 alkanes increased, while the C29 primary alcohol reduced markedly in wsl5 mutant and WSL5 knock-out transgenic plants. Overexpression of WSL5 increased the C29 primary alcohol and decreased alkanes in rice leaves. Heterologous expression of WSL5 increased the C29 primary alcohol and decreased alkanes, secondary alcohol and ketone in Arabidopsis stem wax, indicating WSL5 catalyzes alkanes to primary alcohols and competes with endogenous mid-chain alkane hydroxylase in alkane substrates. These results demonstrate that WSL5 catalyzes the terminal hydroxylation of alkanes yielding odd-numbered primary alcohols, and is involved in the formation of epidermal wax crystals on rice leaf.

Co-author(s): Du Zhang

Primary Poster Presenter: Le Qing Qu

Biochemistry: Photosynthesis and BioEnergy

Altering enzyme biogenesis properties of Tobacco chloroplasts to fold non-cognate Rubiscos (0500-052)
Hall 2

Recent advances in synthetic biology have shown the potential to introduce components of photosynthetic systems from bacteria into chloroplast towards enhancing carbon assimilation and plant growth (South et al. 2019). However, this potential is currently limited to proteins whose folding requirements are met in plants. Aigner and colleagues elegantly demonstrated the importance of protein folding machinery when developing a synthetic system in E. coli to express higher plant Rubisco, a process requiring six complementarity accessory proteins (Aigner et al 2017). Similarly, the folding requirements of some non-plant Rubiscos cannot be met, or are poorly met, in leaf chloroplasts. Making chloroplasts a more versatile

expression host could increase the range of Rubsicos able to be expressed, facilitating strategies to enhance photosynthesis. To do this we have developed a chloroplast transformed tobacco master line expressing the promiscuous protein folding chaperonin complex GroESL from E. coli. The selectable marker gene coding spectinomycin resistance has been excised through homologous recombination allowing for further plastome transformation. The plant's enhanced protein folding capacity is being tested by the introduction of different components of the cyanobacteria photosynthetic apparatus into tobacco chloroplasts. It is envisaged the GroESL protein folding machinery system will streamline the introduction of non-plant Rubiscos into the tobacco chloroplast, reducing the hurdle posed by variations in the folding requirements among proteins.

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Primary Poster Presenter: Sally Buck

Development of tools for optimal nutrient and irrigation management based on remote sensing of chlor (0500-053)
Hall 2

It is imperative to address the need in improved agriculture supply as the world population demand is expected to exceed agriculture supply by the year of 2050. There is a notable difference between crop yield predictions and final output of agriculture fields. This difference may be explained also by soil heterogeneity, varying topography, and the resulting variable nutrient uptake. Remote sensing techniques are implemented in agriculture to first corroborate yield maps attained by the farmers' equipment, and also explain the variability by assessing chemical and physical characteristics in crops. The premise of the technique is a detection of the sunlight reflected from the crop and contains an information on crop vitality, water content, pigments concentration and more. Advances in technology enable studying time dependent physiological activities embedded within the "reflected spectrum". One such activity is photosynthesis, where sunlight is absorbed by the crop in order to produce biomass. During photosynthesis, the apparatus emits fluorescence, which can then be discriminated from the "reflectance spectrum". There is a good agreement between fluorescence level and carbon dioxide assimilation rate when examining large areas such as happens by satellites. However, when increasing the spatial resolution to the level of single plants to subplots, the correlation between plant growth rate and its canopy fluorescence is lost. Therefore, this study aims to relate fluorescence signals to the physiological phenotype of the crop. The fluorescence signal convolves various profiles based on the scale analyzed, both spatially and temporally. Resolving and analyzing correctly the signal will enable its implementation in "smart products" that reduce the heterogeneity in the field, and increase crop yield, thereby assisting us to better feed the world, as our globe progresses towards a population of nine billiard inhabitants on 2050.

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Engineering CO2 neutral photorespiration in plants (0500-051)
Hall 2

The CO2-fixing enzyme RubisCO is restricted by its slow turnover rate and its oxygenase activity. Resulting photorespiration reduces plant carbon efficiency and is a major target to improve plant growth and agricultural yield. Maximizing plant carbon efficiency implies transforming photorespiration from a CO2 negative into a CO2 neutral process requiring bypassing the mitochondrial glycine decarboxylase (GDC), the major hub of photorespiratory CO2 loss. However, replacing the GDC requires an alternative provision of one carbon compounds due to its dual role in photorespiration and one carbon metabolism (C1). Our synthetic biology approach to engineer CO2 neutral photorespiration implies shifting cellular one carbon provision from the mitochondria to the cytosol. Cytosolic formate assimilation, based on tetrahydrofolate intermediates, will be used as the major route of C1-unit supply. Subsequent condensation of produced C1-units with photorespiratory derived glycine to make serine, transforms plant photorespiration into a CO2 neutral process by bypassing mitochondrial GDCInitial labelling experiments with exogenously supplied 13C-labelled formate indicate that in Arabidopsis the capacity for formate assimilation is limited and metabolic flux is directed towards methionine production. To redirect photorespiratory derived glycine to the cytosol we use the Arabidopsis shm1 mutant, deficient in mitochondrial serine hydroxymethyltransferase 1, as reporter line. We implemented a cytosolic four-step synthetic pathway for the efficient assimilation of formate via tetrahydrofolate intermediates into serine in our reporter line and identified current bottlenecks via 13C-formate feeding experiments. Further, mitochondrial formate dehydrogenase (FDH) was identified as a second contributor to photorespiratory CO2 loss. We eliminate this source of CO2 loss by targeted gene knockout using CRISPR/Cas9 system.

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Primary Poster Presenter: Marc-Sven Roell

Evaluation of rice truncated light harvesting antennae mutants generated using CRISPR/Cas9 (0500-049)
Hall 2

Light represents a finite resource that plants must compete for; one strategy to maximize light input involves the use of light harvesting antennae to capture more light in their upper canopies than can be used for photosynthesis, preventing light passage to competitors in the understory. To achieve a more uniform canopy light distribution and enhance whole plant energy conversion efficiency, we have used a

CRISPR/Cas9-mediated approach to engineer rice plants with truncated light harvesting antennae (TLA). Notably, mutations in two genes involved in antennae assembly, TLA3 and TLA4, are predicted to decrease chlorophyll content, without adversely affecting the activity of the photosystems or the electron-transport capacity of the thylakoids. Additionally, through our analysis, we have discovered a Poaceae-specific duplication of TLA4, which includes agronomically important staple foods such as rice, wheat, corn, millet, and sorghum. By studying the photosynthesis-related phenotypes of TLA3, TLA4, and its ortholog, which we have named TLA4L, we will determine if the grasses have established novel mechanisms for importing light harvesting antennae that can be leveraged for additional gains in quantum yield, especially in high-density crop monocultures.

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Primary Poster Presenter: daniel caddell

Genetic engineering of mutants with high biomass and lipid productivity.
(0500-042)
Hall 2

Engineering photosynthetic organisms is a long-standing goal of plant and algal biologists. Here we describe mutant photosynthetic organisms that have an attenuated CheY-like gene (CheY) encoding a two-component regulatory system with Regulator Receiver domain and myb-like DNA binding domain. The mutant organism with attenuated CheY-like gene exhibits a higher biomass productivity as a result of reduced chlorophyll content (primarily related to reduced light-harvesting antenna), increased rates of electron transport, and increased carbon fixation. These mutants are also characterized by ~20% increase in protein per TOC, which could yield an increase of 40-60% in areal protein productivity, when compounding increased percentage of protein with the increased biomass productivity. Under N- batch growth the mutants outperformed the wild type in both the biomass and lipid (FAME) accumulation (with up to 50% higher FAME production). To determine the effects of a CheY mutation in higher plants, we tested A. thaliana with mutations in CheY-like homologs. In the mutated organisms we observed a significant reduction in the amount of chlorophyll; more studies are needed to assess the effects of this mutation on biomass productivity. Overall, mutants with attenuated CheY provide a genetic means of increasing biomass, protein, and lipid content in photosynthetic organisms contributing towards the ongoing efforts towards more sustainable production of foods and chemicals.

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Primary Poster Presenter: Fedor Kuzminov

Identification of enzymes for genetic engineering of grasses with greater biomass accumulation (0500-048)
Hall 2

The major goal of this project is to increase the quality and quantity of grass biomass. Specifically, we aim to increase the amount of the easily digestible cell wall polysaccharide mixed-linkage glucan (MLG) in grass cell walls. To achieve this goal, we have identified a Brachypodium transcription factor BdTHX1 involved in MLG accumulation. BdTHX1 is co-expressed with and binds to a mixed-linkage glucan (MLG) biosynthesis gene BdCSLF6 and a GH16 member BdXTH8 encoding a grass-specific hetero-transglucanase with MLG:xyloglucan endotransglucosylase activity, suggesting a role in regulating the synthesis and restructuring of MLG. We found another Brachypodium THX transcription factor, BdTHX2, that shows similar expression patterns as BdTHX1. THX2 is highly co-expressed with BdXTH8 and both genes have higher expression in young vegetative tissues. To uncover more genes regulating MLG biosynthesis in expanding cells, we have performed ChIP-seq experiment using an anti-BdTHX2 antibody and elongating internode and elongating leaf. Examining those genes should lead to a better understanding of MLG deposition in young vegetative tissues and the mechanism of MLG's effect on plant development. With such information, it is possible to engineer crops like sorghum with the stem as a storage organ accumulating large amounts of MLG without affecting plant growth.

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Improving camelina seed oil content by integration of additive/synergistic yield traits (0500-044)
Hall 2

In most C3 crop plants, net photosynthesis is considered as a balance between the amount of CO2 fixed in carboxylation and the amount of CO2 released through the process of photorespiration and respiration. Photorespiration occurs when RubisCO oxygenates instead of carboxylates ribulose-1,5-bisphosphate (RUBP), leading to production of 3-phosphoglycerate (3-PGA) and a toxic molecule of 2-phosphoglycolate (2-PG). The toxic 2-PG is recycled through energy consuming steps involving peroxisome and mitochondria to 3-PGA that can re-enter Calvin-

Benson cycle, considerably reducing the efficiency of photosynthesis. In recent years, synthetic biology approaches have been utilized in several species to reduce photorespiration. In one approach, synthetic pathways to metabolize 2-PG within the chloroplasts (photorespiratory bypass; P-bypass) have been shown to the reduce photorespiration and increase photosynthetic performance. In another approach, components of algal Carbon Concentrating Mechanisms (CCM) have been introduced to increase the ratio of photosynthesis vs. photorespiration. We have combined these approaches by introducing a partial P-bypass pathway by expressing gene encoding for glycolate dehydrogenase (GDH) from bacteria in the CCM component, LIP36 (Low CO2 induced protein), a mitochondrial carrier protein from Chlamydomonas reinhardtii background in Camelina sativa. GDH oxidizes toxic glycolate to glyoxylate within chloroplast generating a competing pathway for plant photorespiratory cycle and LIP36 helps in maintaining redox homeostasis and increase in nutrient use efficiency. Both of these components individually results in higher assimilation of CO2 and increase in seed yield. Our preliminary results indicate a synergistic interaction between the two components. (Research is funded by DOE grant no. DOE-DE-SC0018269).

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Modification of pectin biosynthesis leads to higher plant growth in switchgrass, poplar and rice. (0500-043)
Hall 2

Plant biomass such as woody Populus and switchgrass perennials and the annual rice is composed of a complex polymeric matrix of lignin, cellulose, hemicellulose, pectin, and cell wall proteins. Plant cell walls account for the bulk of mature dry biomass. Cell walls have a significant role in plant development, morphology, cell growth and provide mechanical strength to plants and plant cells. Changes in cell wall composition lead to altered plant organ biophysical and mechanical properties and plant growth and development. Therefore, understanding the polymer interactions in cell walls and their structural complexity is required to engineer plants with enhanced functionality. Cellulose, hemicellulose, and lignin constitute the bulk of lignocellulosic biomass, and thus are the main targets of bio-feedstock and crop improvement efforts. However, increasing evidence indicates that the cell wall component pectin can also be an effective target for biomass feedstock improvement. The pectic polysaccharides are the most structurally complex of the plant cell wall glycans, consisting of the polysaccharides homogalacturonan (HG) and rhamnogalacturonans I and II. Here we show that reduced expression of the HG-biosynthetic α-1,4-galacturonosyltransferase GAUT4 in switchgrass, poplar and rice results in a significant increase in dry biomass yield in GAUT4-knockdown transgenic lines compared to their respective controls. Importantly, this trait was

maintained in a 3-year field-trial of switchgrass (Biswal et al. 2018, Nature Biotechnology. 36: 249-257). We provide evidence that reduced amounts of the pectic polymer(s) lead to loosened walls and hence increased growth in grass and woody plants. These data indicate that genetic manipulation of pectic polymer biosynthesis has great potential for increasing biomass yield and plant growth. ReferencesBiswal AK et al. (2018) Sugar release and growth of biofuel crops are.....pectin biosynthesis. Nature Biotechnology. 36: 249-257.

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Photosynthetic changes in Fe-deficient Chlamydomonas shifting from photoauto- to photohetero-trophy (0500-045)
Hall 2

Iron is essential for metabolic functions in photosynthetic organisms in the ocean and on land. Although iron is relatively abundant in the Earth's crust, it is typically not in a bioavailable form leaving organisms deficient in redox cofactors to drive photosynthesis and respiration. A typical sign of poor iron nutrition in plants is interveinal chlorosis. In previous work, we and others noted that iron-limited Chlamydomonas cells are chlorotic under photo-heterotrophic conditions where they provide iron preferentially to respiratory components, but not under photo-autotrophic conditions. Here, we are leveraging the power of a microbial system to monitor the dynamics of molecular changes that affect iron-limited photosynthesis in the presence of a carbon source. Specifically, we are using temporal transcriptomics and proteomics to monitor changes during the transition from photo-autotrophy to -heterotrophy.

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Primary Poster Presenter: Helen Liu

Photosynthetic response of C4 bioenergy sorghum lines to elevated ozone concentration (0500-054)
Hall 2

Atmospheric pollutants such as ozone (O3) increase oxidative stress in vegetation and threaten the stability of crop production. Current O3 pollution in the United States is estimated to decrease the yields of maize (Zea mays) up to 10%, however, bioenergy feedstocks, including sorghum (Sorghum bicolor), have not been studied for response to O3 stress. Using the unique capabilities of Free Air

Concentration Enrichment (FACE) technology, which provides elevated concentrations of O3 (100 nL L-1) in open-air plots at the field scale, we examined the photosynthetic response of 10 genotypes of sorghum to elevated O3. 10 genotypes of sorghum grown at FACE have similar photosynthetic performance at ambient condition and similar photosynthetic response to elevated O3. Elevated O3 concentration did not alter net CO2 assimilation rate (A), stomatal conductance (gs) or instantaneous water use efficiency (iWUE) and chlorophyll fluorescence across the 10 sorghum genotypes of sorghum. Elevated O3 concentration reduced the maximum carboxylation capacity of phosphoenolpyruvate (Vpmax) in PI452891, but did not affect the maximum CO2-saturated photosynthetic capacity (Vmax) in sorghum lines. There was a significant effect of O3 on slope but no difference in the intercept of the A-gs relationship between ambient and elevated O3 for sorghum. These results suggest that sorghum exhibits a greater O3 tolerance than maize, and provide important fundamental data for evaluating the yield stability of bioenergy feedstock crop.

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Primary Poster Presenter: Shuai Li

PoDRM2 FROM PURSALNE, ENHANCES STARCH ACCUMULATION IN ARABIDOPSIS THALIANA (0500-047)
Hall 2

Starch is one of the three major nutrients in human diets and is considered key resource for bio-ethanol production. Starch is produced during photosynthesis by plants as major energy storage. Increasing starch production will provide ample foods, not only for human consumption, but also for animal feedstock, as well as for potential bioenergy production when needed. Previously, we successfully cloned a homolog gene, DRM2, from Purslane and overexpressed PoDRM2 in Arabidopsis thaliana. Comparing the growth development between wild-type Columbia and homozygous PoDRM2 transgenic lines, we observed that the plant size of PoDRM2 transgenic lines were significantly larger than that of Columbia, suggesting a potential high efficiency in photosynthesis. To confirm this observation, starch synthesis/accumulation in leaves were investigated through iodine staining method. At the end of light period, PoDRM2-carrying Arabidopsis lines accumulated significantly more starch in its leaves than wild-type control. More strikingly, at the end of dark period, as predicted, wild-type control does not have starch accumulated, however, PoDRM2 transgenic lines accumulate starch as much as during light period. Furthermore, PoDRM2 transgenic line contains more chlorophyll than wild type control. These results indicate that PoDRM2 functions as a key player to regulate starch accumulation during photosynthesis. PoDRM2 encodes a methyltransferase. Genome-wide bisulfite sequencing identified more than 2,0500 genes with altered methylation status. 55 out of 61 genes on photosynthesis

pathway were changed with their methylation. Our results suggest that DNA methylation plays important role in plants regulating photosynthesis

Primary Poster Presenter: Shuxin Ren

Studying the role of SIGMA FACTORS in the phytochrome-mediated block of greening in Arabidopsis (0500-040)
Hall 2

The external light spectrum is a critical factor in the ability of plants to synthesize and accumulate chlorophyll. Previous studies reported an inability of wild-type seedlings to accumulate chlorophyll in response to far-red (FR) light exposure and subsequent growth in white light in the absence of sucrose, in a response known as the FR block of greening (BOG). The BOG response is known to be controlled by phytochrome A through repression of protochlorophyllide reductase (POR) genes by FR light coupled with irreversible plastid damage.Sigma factors are nuclear-encoded proteins that bind to the core plastid RNA polymerase and initiate transcription of genes in chloroplasts. Sigma factors are known to be regulated by phytochromes, with the expression of some sigma factors being previously reported to be reduced in phytochrome mutant lines, including phyA. In this study, we investigated the potential role of sigma factors in the phytochrome-mediated BOG response. Among all six Arabidopsis sigma factors mutant lines, our results indicated that only sig6 mutants exhibit accumulation of chlorophyll after FR treatment, similar to phyA mutants. At the molecular level, Sig6 appears to be involved in controlling protochlorophyllide accumulation by regulating the PORA gene in response to prior FR exposure.

Co-author(s): Beronda Montgomery

Primary Poster Presenter: Hussien Alameldin

The realized and potential maximum rates of electron transport in C3 photosynthesis (0500-041)
Hall 2

The model of C3 photosynthesis described by Farquhar et al. (1980) [A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149: 78–90] has become widely used for interpreting the gas-exchange and fluorescence characteristics of C3 leaves. Fitting this model to data can be used to estimate a number of parameters, including the maximum Rubisco activity (Vcmax, μmol CO2 m-2 s-1), and the maximum electron transport rate (Jmax, μmol e- m-2 s-1). The Vcmax and Jmax parameters are superficially similar in that the former provides insight into a leaf's capacity for CO2 assimilation, and the latter provides insight into a leaf's capacity for electron transport. However, they are fundamentally different in that Vcmax represents a leaf property that is fully resolved into its molecular details, whereas Jmax represents a vague, empirical concept. In this presentation, we will analyze Jmax with a new model of the light reactions that is

coupled to the original model of the dark reactions from Farquhar et al. (1980). The coupled model relates physiological indices of leaf metabolic activity (i.e., O2 and CO2 exchange, 650-850 nm fluorescence, 810-830 nm absorbance) to their underlying controls, both biochemical (i.e., densities and kinetics of Photosystem II, Photosystem I, Cytochrome b6f, and Rubisco) and environmental (i.e., absorbed irradiance, temperature, CO2 and O2). We will present model-based analyses of gas-exchange and fluorescence measurements that address three questions: What determines the maximum electron transport rate that is realized under saturating light and CO2, i.e., Jmax? How does Jmax compare to the potential maximum electron transport rate? What determines the potential maximum electron transport rate?

Co-author(s): Joseph Berry

Primary Poster Presenter: Jennifer Johnson

The structural basis of assembly of Rubisco into a phase-separated organelle (0500-046)
Hall 2

Photosynthesis in many organisms is limited by the slow catalytic rate of the CO2-fixing enzyme Rubisco. Oceanic microalgae make Rubisco run faster by packing it into a phase-separated organelle called the pyrenoid, where the CO2 concentration is much higher. We recently discovered that Rubisco's clustering in the pyrenoid of the model alga Chlamydomonas is mediated by the intrinsically disordered repeat protein EPYC1; however, the mechanism for this clustering has remained unknown. Here, we demonstrate the structural basis for the clustering of Rubisco by EPYC1. We identify ten evenly-spaced Rubisco binding regions on EPYC1. Single particle cryoelectron microscopy reveals that Rubisco has eight binding sites for EPYC1, one on each Rubisco small subunit. The Rubisco-binding regions of EPYC1 bind as extended polypeptides with a single alpha helix. The ten binding sites have sequence similarity, and extensive mutagenesis studies allow us to define the physicochemical properties that mediate Rubisco binding. The low affinity (Kd > 3 mM) of each binding site explains how the pyrenoid matrix can retain the properties of a liquid droplet despite the high valency of EPYC1 and Rubisco binding sites. Our discovery of the mechanism of formation of the matrix advances our structural and functional understanding of the pyrenoid, a phase-separated organelle that plays a biogeochemically fundamental role in the global carbon cycle.

Co-author(s): Sarah Port,
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Primary Poster Presenter: Shan He

Towards a holistic understanding of starch biosynthesis (0500-050)
Hall 2

Starch, the major storage carbohydrate in plants, is the key nutritional component of our staple crops and a feedstock for industry. It is composed of glucose polymers that form massive semi-crystalline granules. The precise structure and composition of starch determine its functionality and thus applications. However, how structure and composition are controlled at the level of the biosynthetic machinery it is not well understood. We previously demonstrated that Saccharomyces cerevisiae can be engineered to produce starch-like glucans using Arabidopsis genes, thus serving as a model for starch biosynthesis. That study systematically investigated the contribution of individual biosynthetic enzymes, but did not address the potential importance of expression levels or enzyme ratios. Such ratios may have a strong influence on starch structure given that the starch-biosynthetic enzymes act simultaneously and in an interdependent fashion on the same substrate.Here we employ a refined version of the yeast system, implementing also controllable variation in enzyme expression. Using promoters and terminators of varying strengths, we created yeast strains with a broad range of ratios between single enzyme isoforms. We show that these yeast strains differ both in respect to the amounts and structures of the glucans produced, allowing us to dissect the roles of enzyme ratios vs. enzyme specificities. In collaboration with the Ebenhöh lab in Düsseldorf, we are now using these data to inform a mathematical model of starch biosynthesis. Ultimately, such a model will help generate a systems-wide understanding of the synthesis of complex glucans, providing a basis for the targeted biotechnological improvement of crops.

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Primary Poster Presenter: Barbara Pfister

REORGANIZATION OF PHOTOSYSTEM II SUPERCOMPLEXES AND LIGHT-HARVESTING COMPLEX II IN PLANT THYLAKOID M (0500-090 (Screen 9))
Hall 2

It is well established that thylakoid protein phosphorylation adjusts photosynthetic performance to environmental changes: Balancing of light energy between PSI and PSII requires STN7 kinase to catalyze LHCII phosphorylation, triggering its redistribution from PSII to PSI. PSII core phosphorylation is triggered by high light-induced activation of STN8 and was postulated to control PSII supercomplex

disassembly, facilitating photodamaged PSII repair. Currently, it is hypothesized that reversible dephosphorylation by phosphatases (PPH1 and PBCP) is not regulated. However, how PSII supercomplex stability is determined by protein phosphorylation is unknown. Here, under dark and growth light conditions, the redistribution of protein complexes and PSII supercomplex stability in stacked and unstacked thylakoid domains were compared in Arabidopsis kinase double mutants stn7/8 (phosphorylation-deficient) and phosphatases double mutants pph1/pbcp (dephosphorylation-deficient). LHCII Movement out of grana under light was proved and quantified by two independent biochemical techniques besides using 77 K fluorescence spectroscopy and chl a/b ratio. Quantification of the different PSII assembly forms was performed using new established method combining Blue-Native polyacrylamide gel electrophoresis with D1 immune-dot-blotting. This data indicates that from dark to light, 21% of wild type dimeric PSII-LHCII holocomplex (C2S2M2) disassembles to smaller forms (monomeric and dimeric core). Unlike WT and stn7/8, changes in pph1/pbcp were not significant, proving that PSII/LHCII phosphorylation affects supercomplex disassembly in grana core. These data prove that protein phosphorylation induces partial disassembly of C2S2M2 holocomplexes accompanied by trimeric LHCII movement to unstacked regions, serving as light-harvester for PSI and that PSII-LHCII holocomplex stability is only mildly effected by subunit phosphorylation (Supported by the National Science Foundation [#MCB-1616982]).

Co-author(s): Helmut Kirchhoff

Primary E-Poster Presenter: Haniyeh Koochak

Biochemistry: Specialized Metabolites

Biosynthesis of Pyrethrins in Tanacetum cinerariifolium (0500-092 (Screen 6))
Hall 2

Pyrethrum (Tanacetum cinerariifolium) plants have been known since antiquity for the presence of a group of natural pesticides, called pyrethrins, in its flowers. The six types of pyrethrins produced in pyrethrum are all esters of a monoterpenoid acid (chrysanthemic acid or pyrethric acid) and a jasmonic acid-derived alcohol (jasmolone, pyrethrolone or cinerolone). Recently, we have begun to identify the enzyme responsible for the synthesis of the alcohols. By comparing the structure of these alcohols with that of JA, we hypothesized that jasmolone may be generated by hydroxylation of jasmone, a catabolite of JA, and that pyrethrolone could be derived from jasmolone by additional oxidation step. Through correlation analysis of transcriptomic data and metabolomics data of different pyrethrum tissues, eleven P450 candidate genes were selected. The candidate genes were transiently expressed in Nicotiana benthamiana leaves which were fed with jasmone, and the

tobacco tissues examined for the production of new alcohols. Using this approach, one introduced P450 gene was shown to encode an enzyme capable of hydroxylating jasmone to give jasmolone. This gene was accordingly named Jasmone Hydroxylase (TcJMH). Furthermore, by coexpressing TcJMH with the rest of the P450 candidates in the tobacco system, a second gene was found to encode an enzyme that converts jasmolone to pyrethrolone directly, and this gene was named Pyrethrolone Synthesis (TcPYS). The enzymatic activities of TcJMH and TcPYS were further verified in in vitro assays. Coexpressed of TcJMH and TcPYS with TcGLIP, the enzyme that forms the pyrethrin esters, in tobacco leaf fed with jasmone and chrysanthemic acid, led to the production the pyrethrin molecules jasmolin I (the ester of chrysanthemic acid with jasmolone) and pyrethrin I (the ester of chrysanthemic acid with pyretrolone), indicating the possibility of engineering the production of these human-safe natural pesticide in other plant species.

Co-author(s): Eran Pichersky

Primary E-Poster Presenter: Wei Li

Diverse Allyl-Glucosinolate catabolites independently influence root growth and development (0500-091 (Screen 1))
Hall 2

Plants utilize specialized chemicals to defend against biotic attackers. The generation of these defense chemicals is hypothesized to be costly for the plant and their production might limit growth or fitness in the absence of biotic attackers, or even in the presence of biotic attackers adapted to the compound. Hence, the plant must calibrate and fine-tune its defense metabolism to optimize the cost-to-benefit ratio for any given environmental response. Glucosinolates (GSLs) are sulfur-containing defense metabolites, produced in the Cruciferae family, including the model plant Arabidopsis thaliana. Previous work suggested that specific GSLs may function as signals to provide direct feedback regulation within the plant to calibrate defense and growth. This includes one of the most widespread GSL, Allyl-GSL that has been linked to resistance to numerous pests. Allyl-GSL treatment leads to alterations in plant biomass and endogenous metabolite content, with a suggested link between Allyl-GSL and auxin signaling. Using co-treatment with auxin and Allyl-GSL, we found that Allyl-GSL magnifies auxin signaling and responses. This auxin interaction is likely caused not by the Allyl-GSL but instead by three separate catabolic products coming from Allyl-GSL. Excitingly, each compound has a unique regulatory effect on plant development. These unique regulatory effects are reflected by different effects on the auxin signaling cascade or the progression of the cell cycle. The generation of these different catabolic products is mediated by specific enzymes that are controlled in response to diverse environmental stimuli. We propose that this allows the plant to measure the specific processes that are influencing defense metabolism. Thus allowing a specific response that is optimal to any given environment. These findings provide a better understanding of how plants integrate between growth and defense under changing biotic environments.

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Primary E-Poster Presenter: Ella Katz

Functional characterization of short chain dehydrogenases from Medicago truncatula (0500-093 (Screen 15))
Hall 2

Short chain dehydrogenases are a versatile class of enzymes found in all living organisms and referred to as the SDR superfamily. To date, more than 679,000 genes have been reported in this family. Due to their low substrate specificity, they are involved in intermediary metabolic functions, regulation of signaling, and the sensing of redox status to RNA processing. In a model legume plant, Medicago truncatula, our study using GWAS with metabolomics (mGWAS), and Pearson correlation analysis of microarray data revealed two short chain dehydrogenases MtSDR1 and MtSDR2 might be involved in the biosynthesis of triterpene saponins. RNA-seq expression analysis of the data obtained from the Ensembl Plants database shows that MtSDR1 is expressed in all the tissues with the maximum expression being present in pods, while MtSDR2 does not express well in any of the tissues. Phylogenetic analysis shows that MtSDR1 has a close relationship with a hypothetical chickpea short chain dehydrogenase, with ancestor branching from Solanaceae family. Invitro screening assays using MtSDR1 purified protein with methanolic extract (acid hydrolyzed and non-acid hydrolyzed) of M. truncatula aerial and root metabolites as substrate and with co-factors NAD(H) and NADP(H) showed that with different co-factors different products are formed. There was one unique product with m/z 441.34 [M-H]- was formed in the acid hydrolyzed methanolic extracts of both aerial and root tissues when NADH was used as the co-factor. Additionally, the substrate having m/z 439.32 [M-H]- was shown to be consumed in the reaction proving NADH was utilized during the reaction. In case of overexpressed and CRISPR-Cas9 knockout hairy root lines of MtSDR1, differential accumulation of metabolites was observed indicating its versatile function. In conclusion, our results indicate that MtSDR1 forms a novel product in invitro reaction, whereas in plants it has promiscuous functions in both roots and aerial tissues.

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Primary E-Poster Presenter: Santosh Kumar

Biochemical Characterization of Nuclear Apyrases in Arabidopsis thaliana

(0500-058)
Hall 2

Apyrases are nucleoside triphosphate-diphosphohydrolases (NTPDases) that regulate the concentration of nucleotides by removing the terminal phosphate from NTPs and NDPs. Two of the 7 apyrases in Arabidopsis, APY1 and APY2, are 87% identical in primary structure and play a crucial role in regulating auxin transport and plant growth. Here, to better understand how these apyrases exert their control over plant growth, we localized them in nuclei of etiolated seedlings, purified them from isolated nuclei, and biochemically characterized their enzymatic properties. Initial localization assays showed that APY1 and APY2 were highly expressed in nuclei purified from etiolated seedlings, just as a previously characterized pea apyrase was shown to be highly expressed in the nuclei of etiolated pea seedlings. After their extraction from nuclei of dark-grown Arabidopsis seedlings, APY1 and APY2 co-purified together as monomers on size exclusion chromatography and were shown to be more than 80% free of contamination from other proteins by SDS-PAGE analysis. The purified APY had the highest specific activity toward ATP and ADP substrates yet reported for any plant apyrase, but it could not hydrolyze AMP. It was recognized in immunoblots by antibodies highly specific for APY1 and APY2 and was inhibited by known APY inhibitors. Its Km, Vmax, and insensitivity to several phosphatase inhibitors were similar to those reported for untagged animal apyrases. Partial purification of native APY from the nuclei of apy1 (null for APY2) or apy2 (null for APY1) mutants revealed that both APY1 and APY2 had NTPDase activity, unlike prior reports on GFP-tagged versions of APY1, which had only NDPase and not NTPase activity. Current immunofluorescence and immunogold localization assays will reveal in what sub-nuclear locale(s) APY resides and thus help clarify its potential functions there.

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Primary Poster Presenter: Stanley J. Roux

CBDa synthase expression is correlated with cannabidiol (CBD) production in Cannabis sativa (hemp) f (0500-055)
Hall 2

Unfertilized female flowers of the dioecious Cannabis sativa plant are a rich source of cannabinoids, including tetrahydrocannabinoid (THC) and cannabidiol (CBD), which may have some medical applications. In fact, the FDA has recently approved a highly purified form of CBD as an efficacious pharmaceutical drug called Epidiolex® for reducing seizures in some epileptic conditions. No research has documented which cannabinoid biosynthesis pathway enzyme(s) contribute to rate-limitation of carbon flow to end-products (THC and CBD). The objective here was to monitor expression of genes encoding these enzymes in developing flowers of female hemp (< 0.3% THC) cannabis and examine if changes in expression of any of these genes is associated with increasing CBD. We used real-time (quantitative) qPCR to monitor expression of CBDa synthase, geranylpyrophosphate

(GPP):olivetolate geranyltransferase (GOT) synthase, olivetolic acid synthase, GPP synthase, and THCa synthase. High-performance liquid chromatography (HPLC) was used to monitor cannabinoid levels in developing flowers in three commercial strains of hemp. These strains include: 'Wife', 'Abacus', and (Otto 2 x BaOx) x (Colorado Cherry x BaOx). Hemp plants were grown in a greenhouse, and weekly samples were taken to assess gene expression and cannabinoid content. As the flowers matured, there was a strong upregulation in CBDa synthase expression. This was accompanied by a significant rise in CBD content. Expression of the gene (GOT synthase) encoding the enzyme that generates the first cannabinoid in the pathway cannabigerol (CBG) did not change during flower development. In addition, the high-level CBD producing strain, (Otto 2 x BaOx) x (Colorado Cherry x BaOx), had higher levels of CBDa synthase expression compared to the two other varieties that produced lower levels of CBD. Supported by Dr. David Levine research support fund and Connecticut Pharmaceutical Solutions LLC.

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Primary Poster Presenter: Peter Apicella

Characterization of fatty acid glycosides in tobacco using ester-exchange reaction method (0500-061)
Hall 2

For aroma formation in foods and tobacco, glycosides are important chemicals. Numerous studies have specifically examined alcohol glycosides and terpene glycosides, especially in flowers, tea, and wine. Generally, leaf tobacco materials include fatty acid glycosides that are degraded into aromatic fatty acids when heated or combusted. Fatty acids tend to have a low threshold value of smell. Therefore, it is important to accumulate information about the fatty acid glycoside composition in tobacco materials. Nevertheless, these glycosides have been analyzed much less frequently than major metabolites because of the necessary but complicated pretreatment procedures and the lack of a Gas Chromatography Mass Spectroscopy (GC/MS) database. This study examined new analytical methods that enable us to identify fatty acid glycosides much more easily by application of an ester-exchange reaction as a pretreatment procedure. After dried tobacco leaves were soaked in 20 mL of hexane, 5 mL of ethanol and 0.5 N sodium hydroxide aqueous solution were added. After shaking, organic solvent phase was collected and analyzed using GC/MS. Several lower fatty acid ethyl esters were identified as generated from glycosides by an ester-exchange reaction catalyzed by sodium hydroxide. The composition of identified esters corresponds to that of fatty acids combined with sugars that are originally present in leaf materials. Key points of this method are the following: suppression of ester hydrolysis in a nonpolar solvent; and continuous shifting of the ester-exchange reaction equilibrium until glycosides are fully consumed, which can be accomplished by the high solubility of generated free sugar in water. This method is amenable to the analytical apparatus because

considerable amounts of free fatty acids contained in tobacco leaves are neutralized and removed from the reaction field by the coexistence of alkaline. Aroma-related basic research can progress quickly using this effective method.

Primary Poster Presenter: Shun Yamauchi

Characterization of Medicago truncatula mutants defective in anthocyanin accumulation (0500-062)
Hall 2

Anthocyanins and proanthocyanidins (PAs) are flavonoid compounds produced by plants that confer various colors such as blue, red, and purple in fruits and vegetables. Anthocyanins provide tolerance to biotic and abiotic stresses in plants, and also some of these compounds are well-known for its antioxidant properties and therapeutic benefits to human health. Recently, anthocyanins are of significant interest because of their potential to be used as natural coloring agents and as therapeutic agents to improve neurodegenerative diseases such as Alzheimer's and Parkinson's. However, plants do not produce adequate quantities for the industrial and pharmaceutical applications. Hence, understanding the transcriptional regulation of accumulation of anthocyanin pigments in plants will enable us to improve crop plants with increased anthocyanin levels, and to metabolically engineer plants and microbes to produce anthocyanins in large quantities. To identify the novel transcriptional regulators of anthocyanin and PA accumulation, we are using a forward genetic approach in the model legume plant Medicago truncatula. M. truncatula wild-type leaves show reddish purple anthocyanin pigmentation whereas seeds are light brown in color due to the accumulation of PA. By screening M. truncatula Tnt1 retrotransposon mutant population, we identified three different mutants with the following phenotypes: (a) increased number of anthocyanin spots in leaves, (b) complete loss of anthocyanin pigmentation in vegetative organs and white color seeds, and (c) decreased anthocyanin pigmentation in vegetative organs and black color seeds. Among all the mutants, some of the mutant phenotypes are novel because there were no previously published reports on these phenotypes. Data on phenotypic characterization and efforts toward identifying the causative genes will be presented.

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Primary Poster Presenter: Vijaykumar Veerappan

DETERMINATION OF RHODANESE FROM THEOBROMA CACAO'S MESOCARP, SEEDS AND PODS (COCOA PLANT). (0500-064)
Hall 2

ABSTRACTThis study on the assessment of Rhodanese from Theobroma cacao's mesocarp, seeds and pods was aimed at determining rhodanese from these parts of the plant since its presence correlates with the presence of cyanide. Cyanide can be

detoxified by the enzyme to the less toxic thiocynate. Standard methods were used for preparation of reagents and enzyme assay. Protein concentration was determined by Bradford's method and the effect of metals and temperature on rhodanese activity was established. Protein and hence, rhodanese were found to be present in all three components (i.e. mesocarp, seeds and pod) examined. The activity of rhodanese was affected by the heavy metals tested (MnCl2, SnCl2, NaCl, KCl and HgCl2) while the optimum temperature of the enzyme was found to be between 45 and 60oC. It is therefore established that T. cacao's mesocarp, seeds and pods are not free of cyanide. Ipso facto, their consumption should be controlled.Keywords: Rhodanese, Theobroma cacao, Mesocarp, Seeds and Pods.

Primary Poster Presenter: Abiodun Ajiboye

Determining friend and foe: antibiotic efficacy of maize diterpenoids (0500-066)
Hall 2

Plants deploy specialized metabolites to communicate with other organisms and cope with environmental challenges. For instance, diterpenoid metabolites serve as key components of biotic and abiotic defenses in major crops such as rice and maize. Here, we report the discovery and functional characterization of a novel group of bioactive diterpenoids, termed dolabralexins, that occur perhaps uniquely in maize. Patterns of inducible dolabralexin accumulation in maize roots exposed to fungal pathogens or abiotic stress suggest broad relevant of dolabralexins in below-ground defenses. Furthermore, we show maize root diterpenes to selectively alter the root microbiome composition. Combining pathway engineering and bioassays of purified compounds, we demonstrate that dolabralexins exhibit potent and species-specific antibiotic activity against major Fusarium pathogens. These results and gene resources can provide new targets for breeding and engineering of crop resistance traits in maize and other crops, as well as the manufacture of useful biocides.

Co-author(s): Philipp Zerbe

Primary Poster Presenter: Katherine M. Murphy

Discovery and engineering of colchicine biosynthetic enzymes (0500-060)
Hall 2

Historically, plants have played a prominent role in human medicine. The medicinal effects of plants are due to bioactive small molecule natural products, many of which continue to serve as major sources of clinical pharmaceuticals. One such compound is the alkaloid colchicine, which is produced by plants from the Colchicum and Gloriosa genera and is used clinically for treating gout and other inflammatory diseases. Although previous studies have identified specific plant tissues associated with colchicine biosynthesis, along with the precursors from which this molecule is derived, the underlying biosynthetic genes have remained

unidentified. To facilitate colchicine biosynthetic pathway discovery, we have performed extensive metabolite profiling in Gloriosa superba and paired this to corresponding RNA-seq expression data, with the hypothesis that relative expression of biosynthetic genes should correlate to accumulation of colchicine alkaloids. By then using a combination of correlative expression analyses and enzymatic logic, we selected a testable number of candidate biosynthetic enzymes for functional screening via heterologous expression in tobacco. Through this methodology, we have discovered and characterized 7 novel enzymes that act to produce a late stage colchicine intermediate from a 1-phenethylisoquinoline substrate that is a known precursor to colchicine. Furthermore, by utilizing enzymes from plant primary metabolism, along with several involved in the biosynthesis of natural products from other plants, we have engineered a 16-enzyme pathway in tobacco that connects the newly discovered biosynthetic steps in colchicine biosynthesis to primary amino acid metabolism from the heterologous tobacco host, thus allowing for metabolic engineering of colchicine alkaloids. This result not only establishes a nearly complete metabolic route to colchicine, but also pushes the boundaries for the rate and magnitude of biosynthetic pathway discovery in plants.

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Primary Poster Presenter: Ryan Nett

Effect of Peptides on Alkaloids Occurrence in Hairy Roots (0500-072)
Hall 2

More and more signaling peptides have been discovered to participate in plant defense through stimulating jasmonate (JA) signaling pathway that activates defense genes and enhances the production of secondary metabolites. However, little is known pertaining to the role of peptides in hairy root cultures accumulating secondary metabolites. In this study, tomato hairy roots were first established. Afterwards, tobacco hairy roots were used as the study model to screen out peptides that could affect nicotine content. Based on the screening procedure established in tobacco hairy roots, a screening procedure for the tomato hairy root targeting its metabolite, tomatine, was subsequently developed. Through this process, we found nicotine content decreased by 20% in comparison with that in 0.1 μM MeJA after the addition of tobacco or tomato systemin. Tomatine content was elevated by 0.1 μM MeJA but decreased significantly after the addition of tomato systemin or flg22. These results demonstrate that in both tobacco and tomato hairy root cultures, the addition of specific peptides gave rise to a negative effect of 0.1 μM MeJA on accumulating secondary metabolites, suggesting an antagonistic effect between 0.1 μM MeJA and peptides.

Primary Poster Presenter: Yu Jie Lin

Hormone-induced alkaloid production in two varieties of the medicinal plant Catharanthus roseus (0500-071)

Hall 2

Many plant-derived secondary metabolites have chemical properties that give them therapeutic value for the treatment of cancers, hypertension, and other illnesses. In the medicinal plant Catharanthus roseus, the secondary metabolites of interest are the monoterpene indole alkaloids vinblastine and vincristine. These compounds are naturally produced at low levels in the most commonly used varieties of the plant, which makes the chemical extraction of the two alkaloids difficult and time consuming. Pharmaceutical scientists generally extract the intermediate compounds in the vinblastine and vincristine biosynthesis pathway, which are often more abundant, for in vitro synthesis of the final products, but this process can be cost prohibitive. Many scientists in this area of research use the plant signaling hormone methyl jasmonate to induce higher levels of the alkaloid products in the laboratory, but recent studies have found that ethylene also induces alkaloid production. While methyl jasmonate is too expensive for practical use in an agricultural production setting prior to biopharmaceutical extraction, ethephon (a commercially available ethylene derivative) is a viable and cost-effective option. With this in mind, we designed a study of the alkaloid induction patterns of these phytohormones in two varieties of C. roseus. Testing hormonal induction in the "Little Bright Eyes" variety has allowed us to compare to previous studies, while including "SunStorm Apricot" provides an opportunity for future investigation into the regulation of induction on a genome-wide scale. We have found that there are significant differences not only between the induction agent but also between the two varieties, which suggests that varietal selection is a crucial consideration for the study of metabolic pathways for medicinally important compounds.

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Primary Poster Presenter: Valerie Fraser

Inositol pyrophosphates: signaling at the interface between plant nutrition and defense (0500-073)
Hall 2

Diphospho-myo-inositol polyphosphates, also termed inositol pyrophosphates, are molecular messengers containing at least one high-energy phosphoanhydride bond and regulate a wide range of cellular processes in eukaryotes. So far, most of our knowledge about them comes from studies in yeast and animals, where they are described as signaling molecules that control many distinct cellular processes including phosphate homeostasis, DNA repair, telomere length maintenance, ribosome biogenesis, immune response and insulin signaling. Here we present our recent discoveries on the biosynthesis of inositol pyrophosphates in Arabidopsis and their role in nutrient and hormone perception as well as plant defense.

Co-author(s): Gabriel Schaaf

Primary Poster Presenter: Philipp Gaugler

Leveraging cross-species defense pathways to increase metabolic diversity and crop stress resilience (0500-069)
Hall 2

Maize (Zea mays) produces small-molecule defenses, termed specialized metabolites, to protect against pests and pathogens. To elucidate the origin of Zea-specific antimicrobial sesquiterpenoids, termed zealexins, we integrated transcriptomic co-expression analyses, forward genetics, CRISPR/Cas9-generated knockouts and heterologous gene expression in Nicotiana benthamiana to define 10 Zx biosynthetic pathway genes. The production of acidic zealexins is catalyzed by Zx5-7, a small family of highly promiscuous Cytochrome P450 CYP71Z enzymes encoded by a gene cluster on chromosome 5. Beyond zealexins, the Zx5-7 gene cluster further catalyzes the production of diverse acidic diterpenoids (kauralexins, dolabralexins) and sesquiterpenoids (costic acid, santalenoic acid). Sorghum (Sorghum bicolor) is a related cereal crop not yet known for antimicrobial terpenoids, instead relying on phenylpropanoid pathway defenses such as 3-deoxyanthocyanidins. The pathogen-elicited sorghum sesquiterpene synthase SbTPS1 produces α-zingiberene, β-bisabolene and β-sesquiphellandrene; however, simple non-volatile α-zingiberene acids are largely unknown in nature. To combine Zx and sorghum pathway enzymes for novel defense metabolite production possible through cross-genera enzymatic catalysis, we co-expressed Zx5-7 with SbTPS1 in N. benthamiana and observed production of a putative α-zingiberene acid. We anticipate that reactive specialized metabolites derived from novel associations may have useful growth inhibitory properties to combat pathogens and herbivores. Large-scale purifications, NMR-based structural elucidation and bioassays are currently underway prior to transgenic delivery of SbTPS1 into maize. Our work seeks to combine unique strengths of defense-related pathways present in different yet related crop genera to yield novel enzyme combinations and new specialized metabolites that can challenge rapidly evolving pest and pathogen pressures.

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Primary Poster Presenter: Evan Saldivar

Light regulation of vinca-alkaloid vindoline biosynthesis in Cathranthus roseus seedlings (0500-070)
Hall 2

Catharanthus roseus (Madagascar periwinkle) produces more than a hundred of terpenoid indole alkaloids (TIAs) including the anticancer drugs, vincristine and vinblastine, which are derived from the coupling of vindoline and catharanthine. Light affects vindoline biosynthesis in young C. roseus seedlings. A seven-step enzymatic process sequentially converts tabersonine to vindoline; however, the molecular regulatory mechanism controlling expression of the genes encoding these enzymes is not elucidated. We identified a Leu-Leu-Met (LLM)-domain GATA

transcription factor, CrGATA1, that regulates light-induced vindoline biosynthesis in C. roseus. CrGATA1 and the vindoline pathway genes, T16H2, T3O, T3R, D4H, and DAT, are predominantly expressed in areal parts of C. roseus seedlings and significantly induced by light. In addition, CrGATA1 activated the promoters of five vindoline pathway genes in plant cells. Two GATC-motifs in the D4H promoter are critical for the CrGATA1-mediated transactivation. Transient overexpression or virus-induced gene silencing (VIGS) of CrGATA1 in C. roseus seedlings altered vindoline pathway gene expression and vindoline accumulation. In addition, we showed that a C. roseus Phytochrome Interacting Factor (CrPIF1) is a repressor of CrGATA1 and vindoline biosynthesis. Transient overexpression or VIGS of CrPIF1 in C. roseus seedlings altered the expression CrGATA1 and vindoline pathway gene, as well as vindoline accumulation. CrPIF1 repressed activities of the CrGATA1 and DAT promoters by binding to the G/E-box/PBE elements. Our findings reveal a regulatory module involving PIF-GATA that controls light-mediated biosynthesis of specialized metabolites.

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Primary Poster Presenter: Yongliang Liu

Medicinal Genomics: Exploring the diversity of iridoid compounds in blueberry for human health benef (0500-056)
Hall 2

Blueberry (Vaccinium corymbosum) is an economically important fruit crop that is native to North America. Fresh market production of blueberries in the United States was valued at $5.68 billion in 2015 and was planted over 36,349 hectares. In addition to its commercial value, blueberries are prized for their positive health benefits, containing high levels of antioxidants, which has been linked to a decreased risk of cancer and heart disease. Iridoids are another class of known pharmacologically important compounds that have recently been found in blueberries. Iridoids are present in over 15 plant families and are potent natural products with a wide range of biological activities in humans including, anticancer, antibacterial and anti-inflammatory. No work however, has been able to detect monotropein, an iridoid glycoside compound, in any North American blueberry species (V. corybosum, V. angustifolium, V. virgatum), the most commonly used germplasm for cultivated blueberry. To address this research limitation I have collected over 80 berry and leaf samples from multiple species and commercial varieties of blueberry to survey for monotropein production. The glycoside iridoid monotropein was successfully identified in a subset of cultivars in the diversity panel, as well as all wild blueberry species in this panel, indicating iridoid production can be targeted through breeding efforts that incorporate wild germplasm. Currently, both metabolite and transcriptomic data are being leveraged to identify key iridoid biosynthetic pathway genes in blueberry. In addition to

providing molecular markers to breed for higher iridoid content, knowing how iridoids are synthesized will enable much improved access to these compounds for future clinical research.

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Primary Poster Presenter: Courtney Leisner

Proanthocyanidin subunit composition determined by functionally diverged dioxygenases (0500-059)
Hall 2

Proanthocyanidins (PAs) are primarily composed of the flavan-3-ol subunits (-)-epicatechin and/or (+)-catechin, but the basis for their different starter and extension unit compositions remains unclear. Genetic and biochemical analyses show that in the model legume Medicago truncatula, two 2-oxoglutarate-dependent dioxygenases, anthocyanidin synthase (ANS) and its homolog leucoanthocyanidin dioxygenase (LDOX), are involved in parallel pathways to generate, respectively, the (-)-epicatechin extension and starter units of PAs, with (+)-catechin being an intermediate in the formation of the (-)-epicatechin starter unit. The presence /absence of the LDOX pathway accounts for natural differences in PA compositions across species, and engineering loss of function of ANS or LDOX provides a means for obtaining PAs with different compositions and degrees of polymerization for use in food and feed.

Co-author(s): Xiaolan Rao,
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Primary Poster Presenter: Ji Hyung Jun

Regulation of glandular secretory trichome formation in Artemisia annua (0500-094 (Screen 1))
Hall 2

Trichomes are hair-like structures that exist on the epidermis aerial part of many plants and are classified into two general categories: glandular and nonglandular. Glandular trichomes are characterized by their capacities to synthesize and secrete large quantities of secondary metabolites, which make them as the cell factories for metabolic engineering. It is of great industrial value to regulate the density of glandular trichomes. However, the regulatory network for glandular trichome development remains largely unclear. Artemisia annua possesses two kinds of trichomes: glandular secretory trichomes (GSTs) in which artemisinin is biosynthesized and stored, and the T-shape (nonglandular) trichomes (TSTs). Some transcription factors have been identified to promote GSTs' development in A. annua (Shi et al., 2018; Yan et al., 2017, 2018). However, none of them specially regulates GST initiation without influencing TST formation or cuticle development,

indicating specific regulator of GST initiation has not been identified. In this study, we report on the identification of a powerful glandular trichome-specific WRKY transcription factor, which positively regulates glandular trichome initiation in A. annua. Ectopic expression of its mint homologous genes in A. annua also significantly induced GST formation, indicating that GST formation in A. annua and other plant species with trichomes such as mint may share the similar molecular mechanism. This allows us to take the full advantage of this WRKY transcription factor by genetic engineering to optimize trichome density and improve the production of specialized metabolites in many important aromatic and medicinal plant species, which has clearly industrial value.

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Primary E-Poster Presenter: Kexuan Tang

Biochemistry: Transport

Specific inhibition of potassium influx but not efflux via the AKT1-KC1 complex by cesium in Arabido (0500-095 (Screen 13))
Hall 2

Cesium shares similar physicochemical properties with the macronutrient potassium and is known to compete with potassium inside and outside of plants and inhibit plant growth at high concentrations. However, the detailed molecular mechanisms of how cesium inhibits potassium accumulation in plants are not understood. Here we show that mutation on a member of the major potassium channel AKT1-KC1 complex renders Arabidopsis hypersensitive to cesium. Electrophysiological analysis demonstrated that cesium, but not sodium, rubidium or ammonium, specifically inhibited potassium influx but not efflux through the AKT1-KC1 complex. In addition, a lack of KC1 further led to an inability of Arabidopsis to accumulate potassium in the plant body due to uncontrollable potassium leakage through the homomeric AKT1 channel that occurs in the absence of KC1, leading to a vast loss of potassium. These data indicate that reduced potassium accumulation due to competition in AKT1 and other potassium channels by cesium plays a major role in plant growth retardation. Therefore, in this study, we provided the actual evidence at the plant level to what has long been believed; potassium channels, predominantly AKT1, are blocked by cesium.

Primary E-Poster Presenter: Ryoung Shin

ALA4 and ALA5 are lipid flippases that are critical for vegetative growth in

Arabidopsis (0500-077)
Hall 2

Aminophospholipid ATPases (ALAs) are lipid flippases involved in the uptake and translocation of specific lipids across membrane bilayers. Arabidopsis thaliana contains 12 ALAs that sort into five phylogenetic clusters, including five in cluster 2 (ALA8, 9, 10, 11, and 12) and four in cluster 3 (ALA4, 5, 6, and 7). Here we show that double mutants lacking ALA4 and 5 (cluster 3) are severely dwarfed, characterized by reduced growth in rosettes (6.5-fold), roots (4.3-fold), bolts (4.5-fold), and hypocotyls (2-fold). Plant size reductions correlated with reductions in cell size, suggesting that ala4/5 mutants are dwarfed in part due to cellular expansion defects. Dwarfism was also associated with perturbations in the content of both glycerolipids and sphingolipids, most notably a ~2-fold increase in glucosylceramides (GlcCers) which could potentially inhibit growth. Uptake assays in yeast suggested that ALA5 was capable of transporting specific lipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS), as well as the sphingolipid sphingomyelin (SM). However, this assay detected no transport for GlcCers, suggesting GlcCer increases in ala4/5 mutants likely arise from indirect pathways. In comparison to other ALAs, the PC > SM > PE > PS transport profile for ALA5 was very similar to that of ALA10 (cluster 2), with the most notable exception being that ALA10 can transport lysophosphatidylcholine. Interestingly, a suppressor mutant screen on mutagenized ala4/5 seedlings was used to identify three dominant suppressor mutants that had near wildtype rosette growth, all of which caused similar disruptions in a putative regulatory domain of a cluster 2 ALA. These results suggest that the biochemical activity of ALA4/5 from cluster 3 is of critical importance for plant and cell growth, and that this distinct activity originates from a putative regulatory domain that differentially controls the activity of cluster 2 and cluster 3 ALAs.

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Primary Poster Presenter: James Davis

Domesticated cotton (Gossypium spp.) phloem loads from the apoplast using a single member of its nin (0500-078)
Hall 2

Cotton (Gossypium spp.) domestication converted a perennial bush into a determinate annual crop with a high sink demand that exceeds source capacity. Agronomists describe "cutout" as when growth of new leaves and branches cease because available carbohydrates are dedicated to maturing existing fruits. Understanding how cotton mobilizes photoassimilate out of source leaves is important to unraveling source-sink relationships and may provide insight into processes contributing to yield. Gossypium ssp. have 'moderate' numbers of

plasmodesmata (PD) in minor vein phloem (Gamalei's Type 1–2a) and closely related Tilia spp. (Basswood; both genera are Malvaceae) have 'abundant' PD in minor vein phloem (Type 1). Based on these frequencies, we conducted experiments to determine if cotton uses passive phloem loading through PD rather than active loading through the apoplast. Esculin, a fluorescent Suc analog, did not accumulate in cotton veins but [14C]-Suc did, as determined by autoradiography. Nine sucrose transporter (SUT) genes were identified per diploid genome; only GhSUT1-L2, showed appreciable expression in mature leaves. Each SUT was functionally tested in planta by virus-induced gene silencing (VIGS). GhSUT1-L2 VIGS resulted in chlorosis, reduced rates of photosynthesis, and accumulation of starch and soluble sugars in mature leaves. In heterologous systems, only GhSUT1-L2 cDNA stimulated esculin and [14C]-Suc uptake into yeast, and only the GhSUT1-L2 promoter caused uidA (GUS) reporter gene expression in the collection phloem of Arabidopsis thaliana. Collectively, these results argue that apoplastic phloem loading mediated by GhSUT1-L2 is the dominant mode of phloem loading in cotton.

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Primary Poster Presenter: John Evers

GOT1B is required for localization of storage protein RNA and for export of proglutelin from ER (0500-076)
Hall 2

The rice seed storage proteins, glutelin, prolamine, and α-globulin, are deposited in two types of intracellular compartments in the endosperm cell. Prolamines are deposited as intracisternal granules within the protein body endoplasmic reticulum (PB-ER) to form protein body (PB)-I, whereas glutelins and α-globulins are exported from the ER to the protein storage vacuole (PSV) to form PB-II. The glutelin precursor2 (glup2) mutant lines, which accumulates abnormally high amounts of proglutelin show missense mutations in the Golgi transport 1 (GOT1B) gene. Microscopic examination of glup2 endosperm indicated the presence of proglutelin-α-globulin-containing intracisternal granules surrounded by prolamine-containing small granules within the ER lumen. Immunocytochemical studies showed that GLUP2/GOT1B protein was distributed on the PB-ER, cisternal ER (cis-ER), Golgi-derived dense vesicles, and the PSV. These observations together with the abnormal accumulation of proglutelin and α-globulins as intracisternal granules suggest that GLUP2/GOT1B participates in the transport of the proglutelin and α-globulin from the ER. As assessed by in situ RT-PCR analysis of developing endosperm sections, prolamine and α-globulin RNAs were found to be mis-targeted from their usual sites on the PB-ER to the cis-ER, the normal sites of proglutelin synthesis. These results indicate that GLUP2/GOT1B is required for localization of prolamine and α-globulin RNAs to the PB-ER in addition to their role in transport of the coded proteins from ER. Two other mutant lines, Glup1 and glup7, display abnormally high amounts of proglutelin (Ueda et al., 2010) and mutant PBs similar

to that seen in glup2 mutant, suggesting that the responsible genes of Glup1 and glup7 mutants are also involved in the transport of proglutelin from ER.

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Primary Poster Presenter: Masako Kushima (Fukuda)

New modulators for intracellular trafficking of PIN-FORMEDs (PINs) (0500-075)
Hall 2

The subcellular polarity of PIN auxin efflux carriers is important to form local auxin gradients and to mediate plant growth and organogenesis. Several protein kinases/phosphatases have been known to modulate intracellular PINs trafficking. To expand the scope of PIN polarity regulation, we took advantage of the proteomic tool to find the modulators that directly interact with PINs and affect their trafficking and plant development. First, we used the bacteria-expressed central hydrophilic loops (HL) of 3 different PINs as baits to pull out their interacting proteins (PIPs) from whole Arabidopsis seedling proteins. Next, PIPs, differentially displayed on SDS-PAGE, were analyzed by mass spectrometry to identify the proteins and genes. These putative PIPs include protein kinases, GTPase, protein-interacting protein, etc. These PIP candidates interacted with PINs-HL in yeast 2-hybrid and in vitro pull-down assays. Intracellular PIN trafficking and plant development were considerably changed in the loss-of-function PIP mutant backgrounds. This approach reveals novel modulators that regulate intracellular PIN trafficking and auxin-mediated plant development.

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Primary Poster Presenter: KwangHo Maeng

Regulation of CDC48/p97 Dependent Plant Growth by the Phytohormone Gibberellin (0500-079)
Hall 2

The phytohormone Gibberellin (GA) regulates various aspects of plant growth and development, including seed germination, stem elongation, flower induction and fruit set through the destabilization of the growth repressor, DELLA. CDC48/p97 is a highly-conserved homohexameric AAA-ATPase molecular chaperone that uses the energy of ATP hydrolysis to unfold and/or extract client proteins from membranes, protein complexes and other cellular structure. The Plant ubiquitin regulatory X (UBX)-containing protein, PUX1, negatively regulates CDC48/p97 by promoting the disassembly of the active homohexameric complex into its inactive monomers. Preliminary data indicate that the Gibberellin receptor, GID1 (GA-INSENSITIVE DWARF1), interacts with two forms of PUX1, a 38kD full-length and a 34 kD putative truncated form suggesting a previously undefined molecular mechanism by

which GA controls plant growth and development.In vitro characterization of the interaction have shown that the binding of PUX1 to GID1 is GA-independent and mediated through a region of PUX1 containing the UBX domain. Additionally, PUX1/GID1/CDC48 was showed to form a ternary complex that was also GA-independent. Furthermore, in vivo studies of fluorescently tag PUX1 in plants have shown that PUX1 is also localize in the nucleus. Whereas GA biosynthesis mutant suffer from dwarfism, lines with elevated levels of GA show increased growth due increase cell division and elongation. Similar to lines that have enhance GA-signaling, pux1 loss-of-function mutants exhibit accelerated growth compared to wild type and behave like GA-hypersensitive mutants when treated with Paclobutrazol, a GA biosynthesis inhibitor. Together our findings suggests a new mechanism by which GA-signaling promotes plant growth and development in part by inhibiting PUX1 function, thereby maintaining levels of active CDC48/p97 necessary for proper cell division and expansion.

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Primary Poster Presenter: Jessica Heath

Structural Basis of Selectivity in Type I and Type II Pores of Plant Nodulin-26 Intrinsic Proteins (0500-074)
Hall 2

The evolution of land plants led to an amplification and diversification of the aquaporin superfamily of membrane channels. Among the subfamilies of plant specific aquaporin-like changes are the nodulin-26 intrinsic proteins (NIPs) which are multifunctional transporters of water, ammonia, glycerol and metalloid nutrients that participate in a number of important osmoregulatory and metabolic functions. In addition to transporting molecules essential to plant growth, NIPs also transport harmful metalloid hydroxides, such as Arsenite [As(III)]. NIPs share the canonical hourglass fold of the aquaporin family, but possess substitutions within the aromatic arginine (ar/R) selectivity filter within the channel pore. The nine proteins of the NIP subfamily in the model plant Arabidopsis thaliana can be subdivided into two ar/R subgroups: the NIP subgroup I, which form an aquaglyceroporins that are permeated by glycerol, water and ammonia, and the NIP subgroup II, which form metalloid transporters for boric acid which lack aquaporin activity and are essentially "water tight". In spite of the amino acid differences in the Ar/R region, all of the NIP subfamilies are permeable to As[OH]3. In this work we investigate the role of the distinct ar/R structures in determining substrate specificity for NIP I and NIP II proteins, and propose a model through which a single amino acid substitution results in the selectivity for water or metalloid nutrients such as boric acid through the ar/R selectivity filter. (Supported by NSF grant MCB-1121465).

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Primary Poster Presenter: Zachary Beamer

Biotic Interactions: Plant-Animal

Structural modification of the central metaxylem in nematode-infected roots: parasitic strategy or p (1000-105 (Screen 3))
Hall 2

Cyst nematodes are soil-dwelling parasites that substantially reduce yields of many crops. They establish feeding sites deep within the root vasculature tissue and divert nutrients from the host plant to serve their own needs. To better understand this host-parasite relationship, wheat roots were inoculated with cereal cyst nematodes (CCN, Heterodera avenae) and infected root tissue was examined using confocal microscopy. To support this, we developed methods to obtain high-quality three-dimensional images of thick (up to 150 µm) sections of root tissue. This provided unprecedentedly clear views of the feeding sites and surrounding tissues. Surprisingly, segments of the central metaxylem (cMX) vessels near the feeding sites looked very different from the expected narrow hollow tubes. In the atypical cMX segments, individual elements were short and plump rather than long, narrow and cylindrical. We determined that during a period of 15 days in which cMX vessel elements would normally elongate and then mature to form a hollow tube, cMX vessel elements near CCN infection sites do not elongate. Instead they grow radially, becoming plump. Their outer walls undergo secondary thickening and not all walls between elements degrade. It is still not clear whether this anatomical change benefits the parasite or is part of the host's defense (or both). Our findings raise new questions about how these developmental changes are induced and how the host plant survives the blockage of what should be a major conduit for transporting water and nutrients from root to shoot.

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Primary E-Poster Presenter: Kara Levin

Discovery of an immune receptor for a Herbivore-Associated Molecular Pattern (HAMP) to combat chewin (1000-106 (Screen 8))
Hall 2

Plants recognize molecular patterns from a diverse array of attacking agents and mount dynamic inducible defense responses. Specific receptors are known to detect molecular patterns from bacteria, fungi, oomycetes, parasitic plants and nematodes; however, receptors specifically enabling the detection of chewing

herbivores have remained elusive. To bridge this void, we utilized a set of ATP synthase-derived elicitors found in caterpillar oral secretions, termed inceptin-related peptides, to characterize a receptor for chewing herbivores. Inceptin-induced response variation across diverse cowpea (Vigna unguiculata) germplasm was leveraged for both QTL mapping and a Genome Wide Association Study (GWAS) to identify a narrow list of leucine-rich repeat receptor-like kinases (RLKs) and proteins (RLPs) as parsimonious candidates. A single RLP, termed Inceptin Receptor (INR), was sufficient to impart inceptin-elicited responses to Nicotiana benthamiana. Wild-type tobacco (N. tabacum) plants do not respond to inceptin; however, stable transgenic lines expressing INR gain inceptin-induced defenses and reduce the growth of beet armyworm (Spodoptera exigua) caterpillars. Phylogenetic analyses place INR in a restricted number of legume genomes that likewise display demonstrable inceptin-induced responses. Our results demonstrate that a novel receptor for herbivory can be used to enhance immunity to caterpillars in distant and inceptin-insensitive plant species. Insights surrounding INR will help establish common principles of herbivore-associated receptor function and inform discovery pipelines for new lineage-specific receptors that recognize novel invasion patterns.

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Primary E-Poster Presenter: Adam Steinbrenner

The soybean cyst nematode employs diverse strategies for robust host defense suppression (1000-107 (Screen 1))
Hall 2

Heterodera glycines, the soybean cyst nematode, is a sedentary plant parasite that is the economically most damaging soybean pest. Once sedentary inside the root, infective nematodes induce root cells to re-differentiate into a different cell type to form a feeding site (syncytium). The nematodes develop into adulthood while exclusively feeding from their syncytia, which requires that these feeding cells remain viable throughout the nematode's life. Plants, on other hand, induce defense responses potentially including programmed cell death (PCD) at the sites of infection. Thus, successful SCN parasitism relies on effectively suppressing plant defense responses. Similar to other pathogens, H. glycines delivers effector proteins during infection. Effector proteins are synthesized in esophageal gland cells and secreted into the plant tissue through a hollow mouth spear, the stylet. Functional characterization studies of effectors show that once inside the host cells, these proteins interact with plant components and modulate cellular pathways in favor of parasitism. We have identified multiple effectors that play specific roles in host defense suppression. Our research shows that H. glycines effectors suppress plant defenses using different strategies including interacting with and re-targeting or modifying of host proteins with critical roles in host defenses. This presentation will portray functional characterization studies of defense-suppressing effectors and will discuss diverse strategies to achieve nematode virulence.

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Primary E-Poster Presenter: Parijat Juvale

A putative resistance gene RESISTANT TO MYZUS PERSICAE (RMP) is required for the plant defense again (1000-108 (Screen 11))
Hall 2

Resistance (R) genes have principal functions in plant defense against pathogens and pests. The RESISTANCE AGAINST POWDERY MILDEW8 (RPW8) locus in Arabidopsis thaliana accession Moscow-0 (Ms-0) contains two non-canonical R genes RPW8.1 and RPW8.2 that confer resistance against powdery mildew disease caused by Golovinomyces spp. In the accession Columbia (Col-0), which is susceptible to powdery mildew, this locus contains the RESISTANT TO MYZUS PERSICAE (RMP) instead of RPW8.1 and RPW8.2. We find that RMP contributes to basal resistance against green peach aphid (GPA; Myzus persicae Sülzer), which is an important pest of a wide variety of plants from over 50 families. In no-choice assay, which monitors the combined effects of antixenosis and antibiosis, the GPA colonization was greater on the rmp mutant than on the wild type (WT). Aphid fecundity was significantly higher on the rmp mutant than on the WT plants. Artificial diet assays demonstrated that phloem sap-enriched petiole exudates collected from the rmp mutant accumulate lower levels of an antibiosis activity against the GPA, which is correlated with the increased fecundity of GPA on the rmp mutant compared to the WT plants. When given a choice GPA preferred the rmp mutant than the WT plants. Similarly, dispersal assays indicate that in comparison to the WT plants, emigration of GPA from rmp mutant was reduced, thus indicating that the insect prefers to stay on rmp plants compared to the WT plant. RMP is required for turning on premature senescence and cell-death in response to extracts derived from the GPA. We hypothesize that RMP is required for response to the aphid-derived elicitors of plant defense. Future efforts are directed towards understanding the mechanism underlying RMP’s function in defense against the GPA.

Co-author(s): Jyoti Shah

Primary E-Poster Presenter: Moon Twayana

A cowpea aphid salivary enzyme with dual roles in altering host immunity and physiology (1000-003)
Hall 2

One of the biggest threats to cowpea, an important crop to drought-ridden areas of the world, is the cowpea aphid, Aphis craccivora. The cowpea aphid feeds by

inserting its stylets into the host tissues and navigating to the phloem element where it acquires plant sap. During this navigation to the phloem, aphids deposit saliva, into cells and the apoplast, containing effector proteins that alter plant immune responses and physiology for the benefit of the aphid. Here we report the salivary profile of the cowpea aphid native to California. Over 76% of the proteins identified in the A. craccivora saliva, were not previously reported from this aphid species. One of the enzymes found in this saliva, not previously identified in the saliva of any aphid species, is diacetyl/L-xylulose reductase (DAX). DAX is a member of the short-chain dehydrogenases/reductases. Using an orthologous expression system, A. craccivora DAX (AcDAX) was expressed and purified. AcDAX found to both oxidize xylitol to xylulose, via the reduction of NADP+ to NADPH, and to reduce methylglyoxal, via the oxidation of NADPH to NADP+. The enzyme was found to have a Km of 0.46 mM and kcat of 1.45 s-1 in converting xylitol to xylulose, comparable to its human homolog (DCXR) (Km = 0.11 mM, kcat = 1.7 s-1), and to have a Km of 1.57 mM and kcat of 0.24 s-1 in the reduction of methylglyoxal. The conversion of xylitol to xylulose, an intermediate in the non-oxidative phase of the pentose phosphate pathway, by this aphid salivary enzyme could be an indicator of an alternate energy source for the aphid. While the reduction of methylglyoxal by DAX is a way for the aphid to reduce plant cytotoxicity and alter plant immune signaling.

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Primary Poster Presenter: Jacob MacWilliams

Aphid resistance in diploid and allotetraploid soybeans: Two genomes are better than one (1000-012)
Hall 2

Enhanced resistance to pests and pathogens, resulting from the additive effects of two sets of defensive genes, may provide a selection for polyploidy, which has arisen frequently in the course of plant evolution. The allotetraploid perennial soybean Glycine dolichocarpa has resistance to both Aphis glycines (soybean aphid) and Acyrthosiphon pisum (pea aphid), whereas its diploid progenitors, Glycine tomentella D3 and Glycine syndetika, show resistance to only A. glycines or A. pisum, respectively. Transcriptomic and metabolomic assays demonstrated species-specific variation in the responses of perennial soybeans to A. glycines and A. pisum infestation. Resistance to A. pisum feeding was associated with isoflavone accumulation, whereas resistance to A. glycines increased with flavone content. This observation was recapitulated in artificial diet assays, where isoflavones had a greater negative effect on A. pisum and flavones had a greater negative effect on A. glycines. Correlative analysis of gene expression and aphid resistance in the three perennial soybean species identified likely resistance (R) genes. The functions of two cysteine-rich receptor-like protein kinases were confirmed through overexpression and expression silencing. Together, the observed additive effects of flavonoids and R genes in aphid resistance support the hypothesis that

allotetraploidy in perennial soybeans provides an evolutionary advantage through the combination of two plant defense systems.

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Primary Poster Presenter: Georg Jander

Assessing the Effect of High-Quality Synthesized Silver Nanoparticles (AgNPs) on Plant Health and In (1000-011)
Hall 2

The limited studies that have been conducted on plants exposed to silver nanoparticles suggest that toxicity is species-related and dose-dependent. Silver nanoparticles are small (10-9 or 1.0 nm) clusters of elemental silver and are commonly found as anti-microbial agents in products such as bandages, clothing, or tubing. As production of silver nanoparticles for industrial purposes increases, so does the risk of leaching and environmental contamination. The long-term effects of exposure on crops and plants are unknown. In previous studies, we have successfully synthesized and characterized AgNPs for research purposes using TEM. Initial studies on the effect of AgNPs on Brassica rapa growth and herbivory suggested a detrimental effect on plant health, but no significant differences were seen between treatment or control plants' germination, herbivory or insect pupation rates after herbivory. As of today, there are no published studies documenting the effect of silver nanoparticle treatment on plant resistance to herbivores. We exposed AgNP-treated Arabidopsis thaliana plants to 48-hour herbivory trials and assessed plant damage and insect performance. We used digital image analysis and customized scripts in Mathematica to analyze our data. Initial results demonstrate obvious qualitative growth and pigmentation effects from AgNP treatments in doses as low as 50ppm. We present a non-destructive, cost-effective technique for measuring a variety of plant phenotypes including resistance to insect herbivory, pigmentation, and growth. Final results on the effect of AgNPs on plant herbivory will also be presented.

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Primary Poster Presenter: Erin Rehrig

Deciphering the metabolic code: acylsugar structural effects on insect herbivory (1000-006)
Hall 2

Plants interact with their environment using a myriad of lineage-specific specialized metabolites. Some specialized metabolites, such as acylsugars produced in tomato and the broader Solanaceae family, convey resistance against herbivores and offer humans environmentally safe pesticides. Acylsugars show striking inter- and intra-

specific chemical diversity, due to quick evolution and great variation in the enzymes on the biosynthetic pathway. Literature suggests potential adaptation and selection on these metabolites in response to herbivore pressure. However, critically assessing the anti-herbivory activity of an array of acylsugars is often hindered by variation in other traits in species of interest. To overcome this hurdle, I am utilizing CRISPR/Cas9 and knowledge of pathway enzymes to alter or remove the acylsugar biosynthetic pathway in isogenic backgrounds of Solanum lycopersicum and its wild relative, Solanum pennellii, for herbivory experiments. Knockout lines of the first three acylsugar acyltransferase (ASAT) genes in both S. pennellii LA0716 and S. lycopersicum cv. M82 backgrounds accumulate no detectable acylsugars. I observed loss of tetra-acylated acylsucroses in slasat4 plants, while the spasff1 mutants fail to convert acylsucroses to acylglucoses. All these lines show normal growth and trichome morphology that allows the separation of acylsugar-mediated chemical defenses from trichome structural-mediated physical defenses. These plants also provide an ideal system for comparing effects of acylation patterns on anti-herbivory activity. Results suggest that the performance of the specialist, Manduca sexta, is hindered by presence of acylsugars, while acylsucroses have a larger negative effect on insect growth performance than acylglucoses. Moreover, these mutant plants present a molecular engineering platform for further creating desired acylsugar chemotypes by utilizing knowledge gained from variant ASATs.

Co-author(s): Rob Last

Primary Poster Presenter: Yann-Ru Lou

Developing tools for improving agriculture traits of the superfood grain-Eragrostis tef (1000-005)
Hall 2

Tef (Eragrostis tef) is a native cereal plant critical to food security in the Horn of Africa. The tef grains are rich in minerals and protein, they are gluten-free and safe for diabetics, blood-sugar management, weight control, and colon health. Therefore, in the last two decades, tef has been gaining in popularity as a lifestyle food all over the world. Tef production is mostly associated with low yields resulting from lodging, weeds, and pests. To improve tef agriculture traits, such as pest resistance, it is necessary to elucidate its biosynthesis in the molecular levels, both gene expression and biosynthesis of chemical defense metabolites. The main goal of this research is to identify the effect of pest on tef leaves and to characterize the biosynthetic pathway of the chemical defense metabolites against phloem-feeding insects. To address this, we first characterized the insect species that fed on tef plants in field conditions. In the field survey, we identified a diverse insect species, including leaf chewers and phloem feeders. Secondly, we developed a Foxtail mosaic virus (FoMV) vector for virus-induced gene silencing (VIGS) in tef. The tef plants showed a severe bleaching phenotype using a reporter gene, phytoene desaturase (PDS), after three weeks from inoculation. Next, we will perform a high throughput transcriptomic (RNA-seq) and metabolomic (GC-MS) analysis to expose the genes and metabolites involved in the chemical defense pathway. To show the

gene function, we will reduce the gene expression with the FoMV-VIGS system we developed and confirm their activity using GC-MS. Overall, this will allow us to expose the plant chemical defense in tef and use this knowledge to improve pest resistance and increase yield.

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Primary Poster Presenter: Vered Tzin

Elucidating a Nymph-Based Whitefly Resistance Mechanism in Alfalfa
(1000-010)
Hall 2

Whiteflies are polyphagous, obligate phloem-feeders that cause feeding damage, vector plant viruses and cause sooty mold infections due to their honeydew secretions. Host plant resistance is the most effective means of whitefly control, as whiteflies develop insecticide resistance rapidly and have few natural predators. Here, we report a Bemisia tabaci MEAM1 (Middle Eastern Asia Minor 1)-resistance mechanism in alfalfa (Medicago sativa). Whitefly-resistant (R) alfalfa inhibit MEAM1 first-instar nymphs from developing to their later instars. High-throughput R assays were used to phenotype 83 unique alfalfa genotypes derived from a germplasm populations developed from MEAM1-resistant parents. A spectrum of phenotypes were observed and a highly susceptible (S) and three R genotypes were characterized further. Infestations of R and S alfalfa with two other B. tabaci species (MED1, Mediterranean1, and NW1, New World-1) showed that while MEAM1 nymph development is delayed on R plants, MED1 develops at the same rate on R and S genotypes and NW1 do not develop past early instar stages. The three whitefly species also displayed different egg oviposition behaviors. While MEAM1 and NW1 females laid similar numbers of eggs on R and S plants, MED1 laid more eggs on selected R genotypes compared to S. Choice experiments were also performed with R or S plants over a 72-hour interval and differences in the whitefly species were revealed. MEAM1 and MED1 preferred S plants over the three R genotypes. Surprisingly, NW1 preferred R genotypes. Collectively these data indicate that alfalfa’s R mechanism is complex and likely species-specific. Genotype-by-sequencing and high-resolution melting analyses of plants from R and S populations identified two major candidate resistance loci on Chromosomes 3 and 8. These loci are devoid of canonical NLR genes and at the center of each of these loci are related transcription factors with probably roles in defense.

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Primary Poster Presenter: Patrick Thomas

Exploiting the natural variation of the wild emmer wheat to identify new insect defense mechanisms (1000-004)
Hall 2

Wheat is a staple crop that suffers from severe economic yield losses worldwide caused by insect herbivory feeding. Cereal aphids are one of the major wheat pests, causing direct and indirect damage that reduce the overall plant biomass. To increase yield, there is a continuous demand to improve resistance by identifying chemical defense and physical defenses and their biosynthesis pathways. While cultivated wheat was bred for high yield and may have lost traits associated with resistance to herbivory, the wild wheat progenitor is untapped sources for a wider range of resistance mechanisms. Our main goal is to expose the defense mechanisms of the wild emmer wheat. In this project, we screen a panel of 200 accessions of tetraploid wild emmer wheat seedlings and quantify their response to Rhopalosiphum padi aphid infestation. We measure the metabolic diversity of the major chemical defense compounds in wheat named benzoxazinoids as well as primary metabolites using LC-MS and GC-MS, respectively. In addition, we assess the genetic variations of physical defense mechanisms by counting trichrome density on the leaves. The preliminary results suggest a large variation in the levels of the benzoxazinoids. Also, the analysis of trichrome density has a negative correlation with aphid reproduction and salicylic acids inducing aphid resistance. For the next step, we will perform genome-wide association studies (GWAS) to identify the loci and genes involved in these pathways. The knowledge will be gained from the high-throughput analysis will be used for pre-breeding and marker-assisted selection to improve wheat resistance to aphids.

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Improvements in corn protection against rootworm insects with diversity of insecticidal molecules (1000-001)
Hall 2

Western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte) is one of the major insect pests of corn in the United States. WCR larvae feeds on corn roots and are causing significant economic losses when unprotected. Exposure to insecticidal protein expressed in plants trigger development of resistance in insects to these proteins over time decreasing the potency of Biotech traits. Discovering novel insecticidal molecules representing new mechanism of actions (MOA) will enable sustainable insect control and complement the current biotech traits which are based on the expression of the Bacillus thuringiensis Cry3Bb and Cry34/35

proteins. The dsRNA was shown to have insecticidal activity when derived from essential rootworm genes and will be utilized in next generation product. Comparison of mechanism of action of insecticidal protein and dsRNA indicate utilization of very diverse processes, membrane pore formation and RNAi pathway induction, respectively which suggest extended durability of future corn varieties containing both MOAs. In addition, new proteins with good activity on WCR have been discovered. These new proteins have sufficient sequence and structural diversity compared with Cry3Bb to provide new MOAs for the control of WCR. Diet bioassay data with these new proteins indicate that these proteins can control both populations of fully susceptible WCR and those that shows tolerance to Cry3Bb. Transgenic corns expressing these new proteins have demonstrated superior root protection in greenhouse tests and field trials. These new active molecules provide improved tools for the effective control of WCR populations in corn crops.

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Primary Poster Presenter: stanislaw Flasinski

Oxylipins affect photosynthesis and energy- related transcriptome and proteome in a marine diatom (1000-009)
Hall 2

During herbivory, various marine diatoms release polyunsaturated aldehydes (PUAs) into the environment which affect neighboring unwounded cells. In the diatom Phaeodactylum tricornutum, cells exposed to sublethal concentrations of the PUA 2E, 4E/Z-Decadienal (DD) maintained photosynthetic efficiency and developed increased survival to lethal concentrations of DD within a several hour time frame. However, the molecular mechanisms for these responses and the roles of photosynthesis- and energy- related pathways for supporting cell survival are poorly understood. This study reports on sublethal DD-induced transcriptomes and proteomes at 3 and 6 h. RNA-Seq analysis revealed 7320 and 4631 significantly differentially expressed protein coding genes at 3 h and 6 h, respectively (Adjusted P value <0.05). Based on LC-MS/MS analysis, 71 proteins at 3 h and 48 proteins at 6 h were significantly differentially expressed ($P < 0.05$, ≥ 1.5 fold). Among the differentially expressed genes at 3 h, strong down-regulations were evident for genes involved in photosynthetic light harvesting, chlorophyll biosynthesis, glycolysis and TCA cycle. At 6 h, the majority of transcripts in these categories exhibited a range from modestly down-regulated to slightly up-regulated. Overall, DD-induced early response showed transient suppression of transcripts involved in photosynthetic light harvesting, chlorophyll biosynthesis and energy pathways. In contrast, the corresponding proteins exhibited modest to slight up- or down-regulations or no detectable changes for both time points. Thus, during the early exposure to DD, photosynthesis- and energy- related proteins remained predominantly stable despite extensive differential expression of their encoding transcripts. The early DD stress regulations observed in the P. tricornutum

transcriptomes and proteomes may contribute to understanding physiological bases for the ecological success of diatoms and aid in future microalgae agriculture.

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Primary Poster Presenter: Mona Mehdy

Oxylipins affect photosynthesis and energy- related transcriptome and proteome in a marine diatom (1000-009)
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Singlet Oxygen for Defense Against Aphids (1000-007)
Hall 2

A luciferase-based reporter gene that is selectively stimulated by singlet oxygen (1O2) was used to compare levels of this reactive oxygen species (ROS) in

Arabidopsis thaliana leaves in response to infestation by the green peach aphid, Myzus persicae. Results indicated that 1O2 levels were significantly higher in infested plants compared to uninifested controls 24h after infestation. In addition, the conditional flu mutant in Arabidopsis, which accumulates excess 1O2 when it is transferred from light to dark and back to light again (L:D:L shift), was used to test the effects of 1O2 on green peach aphid infestations. When flu was grown in continuous light and did not accumulate excess 1O2, aphid population growth was comparable on flu and wild-type (WT, Col-0) plants. In contrast, when the plants were exposed to a L:D:L shift, flu accumulated higher 1O2 than WT plants, and supported significantly fewer aphids. These results suggest that 1O2 may contribute to plant defenses against aphids.

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Primary Poster Presenter: Hillary Fischer

The vacuolar amino acid transporter AtAVT1A functions in both nematode infection and senescence (1000-008)
Hall 2

Root-knot nematodes (Meloidogyne spp.) are destructive plant pathogens which are responsible for crop losses in the billions each year. The stage 2 juvenile penetrates the host root slightly behind the tip and then migrates up the stele until it establishes its feeding site, comprised of giant cells, which are redifferentiated root cells with transfer cell-like properties. These giant cells act as a sink in the host root and divert nutrients and water to the nematode. Previous research identified the putative vacuolar amino acid transporter gene AtAVT1A as being up-regulated in the root during nematode infection. To determine if AtAVT1A played a role in nematode parasitism, we tested two T-DNA insertion lines for nematode infestation. In both AtAVT1A knockout lines the number of reproductive adult female nematodes was significantly lower than that of the Columbia wild type. In order to characterize the role of AtAVT1A during the course of normal plant development, a promoter:GUS fusion construct was created and subsequently used to produce transgenic plants. The AtAVT1Apro:GUS plants displayed GUS staining in the hydathodes, pedicels, siliques, the vasculature of cotyledons and in the vascular tissue of lateral root junctions with the primary root. We also observed strong GUS staining in the leaves during senescence. To monitor the expression of AtAVT1A during senescence, a time course was established in which the seventh leaf was removed and RNA isolated between days 23 and 42. Gene expression was measured using qPCR, which showed minimal expression at days 23 and 28, followed strong induction from days 32-42 as senescence occurred. Root-knot nematode infection and senescence both involve the remobilization and redirection of nutrients in the plant, including amino acids. AtAVT1A seems to have been hijacked by root-knot nematodes from its normal role in plant development to help supply the giant cells with amino acids.

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Primary Poster Presenter: Heather Marella

Volatile indole prime seeds for long-term anti-herbivore defense (1000-002)
Hall 2

Herbivore-induced plant volatiles (HIPVs) play a crucial role in facilitating plant interactions above and belowground including priming plants for future stress. Seeds may lay dormant for years in the soil and are likely exposed to an array of HIPVs, which we hypothesized will affect the growth, development, and defense profiles when the seeds grow into mature plants. Despite the ecological relevance, the long-term effects of seed exposure to HIPVs on growth and defenses of the future plant is unknown. Here we investigated the effect of A. thaliana and M. truncatula seed exposure to five HIPVs (cis-3-hexenol, cis-3-hexenyl acetate, trans-2-hexenal, β-caryophyllene and indole) on the on growth and anti-herbivore defense of resulting plants. Our result demonstrates that seed exposure to indole reduced the performance of chewing herbivore beet armyworm (Spodoptera exigua) and phloem-feeding pea aphid (Acyrthosiphon pisum). Induction of defense genes was largely unaffected by seed exposure to indole in either plant species. In addition, M. truncatula plants derived from cis-3-hexenol and cis-3-hexenyl acetate exposed seeds grew faster, produced larger leaves and taller plants while HIPV'S exposure to seeds didn't affect the growth of A. thaliana plants. Our results demonstrate the role of seeds in plant volatile mediated interactions which is potentially a novel ecological mechanism of plant-to-plant communication.

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Primary Poster Presenter: Abhinav Kumar Maurya

Biotic Interactions: Plant-Microbe

A post-translationally-modified RNA-binding protein negatively regulates immune responses through al (1000-109 (Screen 12))
Hall 2

Quantitative phosphoproteomics has emerged as a valuable tool for identifying components of signaling pathways that are not readily distinguishable from transcriptional or genetic studies. To identify novel candidate regulators of defense signaling in maize and Arabidopsis, we profiled rapid changes in the phosphoproteome of both species following treatment with Plant Elicitor Peptides (Peps), conserved signals regulating innate immunity in higher plants. We observed

an SR-class RNA-Recognition Motif (RRM)-containing protein, designated Immunoregulatory RRM (IRR), to be dephosphorylated in both maize and Arabidopsis within minutes of treatment. Both insertional knockouts of the IRR-encoding gene in Arabidopsis and VIGS-mediated silencing of the IRR gene in maize resulted in a Pep-hypersensitive phenotype with strongly upregulated defense responses compared to wild type or empty vector control lines. SR-class RRM proteins are associated with alternative splicing of pre-mRNA, and correspondingly, IRR was found to physically interact with a CC1-like splicing factor. RNA-Seq revealed differences in alternative splicing in irr knockout plants compared to wild type. Retained-intron events in transcripts encoding defense signaling proteins were observed, resulting in frame-shifts that would lead to truncated proteins. RIP-PCR demonstrated that IRR physically interacts with these alternatively-spliced transcripts in a phosphorylation-dependent manner. Current studies are focused on the in vivo function of truncated proteins caused by IRR-mediated alternative splicing and on global identification of IRR-interacting transcripts through RIP-Seq.

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Primary E-Poster Presenter: Keini Dressano

The sprout inhibitor 1,4-dimethylnaphthalenes induces changes in gene expression in potato and reduc (1000-110 (Screen 15))
Hall 2

The compound 1,4-dimethylnaphthalene (DMN) is used to control sprouting of stored potato tubers. Using RNA-seq gene expression profiling was established for potato tubers (Solanum tubersum cv La Chipper) treated with DMN post-harvest. DMN was applied at three different times during storage; just after harvest during endo-dormant, midwinter after endo-dormancy was broken, and early spring when sprouting was prevented via exposure to cold storage temperatures. Changes in gene expression were lowest during endo-dormancy while midwinter and spring treatments exhibited a greater and more diverse expression response. Across all treatments a number of transcripts associated with stress response and pathogen exposure were induced by DMN. To determine if the reduction in fungal diseases seen by growers and processors after DMN exposure was solely a function of potato gene expression a series of fungal culture were treated with DMN. Alternaria alternate, Aspergillus niger, Fusarium oxysporum, and Pithium ultimum exhibited reduced growth when treated with DMN at levels used to control potato sprouting in commercial storages. Thus, we conclude that reduction of pathogens in storage by DMN may be a result of both induction of genes in potato tubers associated with pathogen response as well as a fungistatic activity of DMN.

Primary E-Poster Presenter: Michael Campbell

Breaking the Disease Triangle by the Circadian Clock (1000-112 (Screen 13))
Hall 2

The outbreak of a disease is determined by interactions between the host, its environment and the pathogen. This "disease triangle" model has been used to predict epidemics in humans as well as in agricultural plants. Because plants are sessile organisms, every aspect of the plant physiology, including immunity against pathogens, is influenced by the environmental conditions, such as light, temperature, and humidity. Moreover, in the absence specialized immune cells, plant defense occurs in coordination with plant growth. In my talk, I will discuss the intricate interconnections between the circadian clock and plant immune mechanisms. I will then show how circadian clock integrates environmental signals in timing as well as gating immune responses to protect against infection while avoiding conflicts with growth-related activities.

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Primary E-Poster Presenter: Xinnian Dong

Identification and functional characterization of putative effector proteins of Plasmodiophora brass (1000-111 (Screen 10))
Hall 2

In silico, 120 putative Plasmodiophora brassicae secretory protein effector (PE) coding clones were identified from a cDNA library generated from 35 day old canola clubroot galls. Expression patterns, over 0 to 28 days post infection (dpi) of A. thaliana Col-0 with P. brassicae pathotype 3, have been established for 75. Fourteen of these 75 were not expressed in resting spores of P. brassicae but were expressed at later stages of infection/disease progression, suggesting that these genes may be associated with pathogenicity, primary and/or secondary infection and disease progression. Secondary infection is an important indicator of susceptibility versus resistance to clubroot disease.Programmed Cell Death (PCD) inhibition tests have been carried out on a subset of PEs, to identify an inhibitory response when co-infiltrated with PCD inducers. A small number of PEs showed an inhibitory effect on PCD triggered by PiNPP1 (P. infestans necrosis causing inducer protein); PbHyOxyΔsp (Δsp - without signal peptide) showed the highest level of inhibition. Unexpectedly, PbSTK1Δsp was found to induce necrosis. Stable Arabidopsis transgenic lines of XFP-tagged-PbPEs have been generated, for subcellular localization of the Pb PEs in a host plant. Diversified localizations of PEs throughout the plant cell include, nucleus, cytoplasm, endoplasmic reticulum, vesicles, punctate structures, mitochondria, peroxisomes and plasmodesmata. Two PEs, PbeIF2aΔsp and PbUbox2Δsp, show a plasmodesmata (PD)-specific localization. Visualization of these PEs in PDLP1 (Plasmodesmata localized protein 1) and PDLP5 (Plasmodesmata localized protein 5) Arabidopsis lines is underway.

PD-specific localization of Pb PEs suggest a possible role in movement of spores and/or secretory proteins through PD to facilitate disease spread and progression.

Primary E-Poster Presenter: Peta Bonham-Smith

A generalist pathogen view of plant resistance evolution (1000-113 (Screen 1))
Hall 2

Molecular models of how resistance mechanisms evolve in host-pathogen interactions are predominantly based on co-evolutionary arms races associated with specialist pathogens. However, it is not clear how applicable and extendable this model is to pathogens with other lifestyles like generalist pathogens with the ability to infect highly divergent hosts. To test how a generalist pathogen deals with plant resistance evolution, we infected 98 diverse strains of the generalist necrotroph fungus Botrytis cinerea on 90 genotypes representing eight Eudicot species (tomato, sunflower, lettuce, chicories, Brassica, soybean and Arabidopsis). For the crop species, six wild and six domestic lines were tested. For Arabidopsis, wild type, phytoalexin, JA and SA signaling mutants were included. We show that Botrytis interaction with the Eudicot do not follow existing models. In contrast to expectations, Eudicot-Botrytis interactions had little association to the evolutionary distances between plant species. For example, two Chicory sister species separated such that one was more closely linked to soybean and the other to Brassica. Arabidopsis mutants behaved as a single cluster suggesting that the patterns being identified are not destroyed by abolishing major defense pathways. Additionally, domestication, believed to have dramatic effects on resistance, only slightly impacted this pathosystem. Finally, GWAS revealed that Botrytis virulence and host specificity are distinct polygenic traits. This suggests that rather than a co-evolutionary arms race focused on one or a few major virulence loci, evolution of the Eudicot/Botrytis interaction relies on genome-wide standing genetic variation that differs even between closely related species.

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Primary E-Poster Presenter: Celine Caseys

Low temperature enhances plant immunity through salicylic acid pathway and alters the intersection b (1000-115 (Screen 9))
Hall 2

Temperature has a large impact on plant immune responses. Earlier studies identified intracellular immune receptor NLR genes and salicylic acid (SA) as targets

of high temperature inhibition of disease resistance. Here we report that low temperature enhances both apoplastic and stomatal defenses to bacterial pathogen Pseudomonas syringae pv. tomato DC3000. This enhancement is dependent on SA signaling and is accompanied by upregulation of multiple SA biosynthesis and signaling genes at lower temperature. Although the SA signaling is antagonized by jasmonic acid and ethylene in a temperature-independent manner, the inhibition by ethylene is through the SA biosynthesis gene SID2/ICS1 at normal temperature but not at lower temperature. Rather, SA biosynthesis regulation genes SID1/EDS5 and ICS1, in addition to SID2, are likely repressed by ethylene at lower temperature. Thus, low temperature enhances SA pathway to promote immunity and at the same time uses ethylene to represses multiple SA regulators to achieve a fine-tuned immune responses.

Primary E-Poster Presenter: Zhan Li

Tomato seed-associated bacteria confer protection of seedlings against Pseudomonas syringae (1000-114 (Screen 5))
Hall 2

The plant microbiome is known to benefit host health in numerous ways, including providing protection against pathogens. Here, we provide evidence that tomato seed-associated microbiota play an important role in early seedling health. To test the importance of seed epiphytic communities for seedling susceptibility to the common bacterial pathogen, Pseudomonas syringae pv tomato (Pst), we transplanted naturally occurring seed epiphytic microbial communities back onto seeds prior to germination and compared disease susceptibility to those that were not re-inoculated after surface sterilization. We found that the epiphytic microbiome can protect seedlings against Pst establishment and disease, and we further show that this protective effect is not due to a plant genotype by microbiome interaction. Using 16S amplicon sequencing, we found that these microbiomes were dominated by species of Pantoea, and we took a culturing approach to show that these isolates, including both P. agglomerans and P. dispersa, are sufficient to protect against Pst. By varying concentration of first the pathogen and then the protective symbiont, we then examined the dose-response of protection and found that applying a higher concentration of protective inoculum to the seeds does not correlate to a lessened Pst load but does reduce disease burden. Instead, the most protective dose of Pantoea in terms of Pst growth corresponds to the original density at which protective bacteria were found on seeds. Overall, our findings contribute to a broader understanding of the importance of vertically transmitted plant-associated microbes, with implications for the design and efficacy of biocontrol agents.

Contribution of LER genes to variations of temperature sensitivity in immunity in Arabidopsis natura (1000-120 (Screen 13))
Hall 2

Plant immune responses induced upon pathogen infection are modulated by many abiotic signals including temperature. How temperature modulates immune responses is not fully understood. Here, we analyzed resistance phenotypes to bacterial pathogen Pst DC3000 at 16C, 22C and 28C in 100 Arabidopsis natural accessions. Using the genome wide association study, we identified one gene cluster associated with temperature modulated plant immunity. This gene cluster contains three homologous genes named as LER1 (Low temperature Enhanced Resistance 1), LER2 and LER3. The LER homologs in animals are involved in glutathione redox cycle but the LER functions in plants have not been reported. To investigate the role of each of these Arabidopsis LER genes, we generated multiple mutant combinations of LERs in Col-0 background using CRISPR-Cas9 technology. The ler2 single mutant did not exhibit disease phenotype at 22C, but had a strong autoimmune phenotype at 16C while the ler1 ler3 double mutant showed autoimmunity at both 16C and 22C. Further analysis indicates that extensive natural polymorphisms on LER genes affect their RNA expression and thus the immune response in the natural accessions. We are now investigating how LER genes influence glutathione redox cycle to differentially modulate plant immunity at different temperatures. Our findings thus reveal LERs as a key modifier of temperature modulated immunity and will provide new insights on plant innate immunity regulation by glutathione redox status.

Co-author(s): Jian Hua

Primary E-Poster Presenter: Zhixue Wang

Functional analysis of "transcription factor-miRNA-NLR gene" network balancing the plant resistance (1000-118 (Screen 5))
Hall 2

Transcription factors and miRNAs play important roles in gene regulation at the transcription and post-transcription levels respectively, which form complex regulatory networks with the target genes. The regulation networks are studied a lot in animal, but are rarely reported in plants. NLRs are the most important R (resistance) genes in plants, and play critical roles during ETI defense pathway. miRNAs can target NLR genes and regulate plants resistance. Our preliminary data showed that nta-miR6019/6020 can target and cleave N gene, and suppress the plant resistance to TMV; meanwhile, the expression of miR6019/6020, and its regulation to N gene are regulated by plant growth. However, the regulation mechanism of plant growth to NLR-silencers is still unclear. Next, we aim to characterize the molecular mechanisms underlying the regulation of NLR genes and plant immunity by NLR-silencers, and investigate the growth-associated transcription factors (TFs) upstream of NLR-silencers, which will provide new components for the construction of regulation network of "TFs-miRNAs-NLR genes". This study will facilitate further understandings of the regulation mechanisms of small RNAs in plant innate immune, and provide useful clues to balance the growth and defense of crops in agricultural production.

Primary E-Poster Presenter: Yingtian Deng

HOS15 and HDA9 negatively regulate immunity through histone deacetylation on intracellular immune re (1000-119 (Screen 6))
Hall 2

Growth-defense balance is crucial for plant survival and fitness. Plant intracellular immune receptor Nod-Like Receptor (NLR) genes are precisely modulated to ensure timely and effective immune responses. Epigenetic modification has emerged to be an important player in plant immunity. However, the mechanism underlying this regulation for growth-defense balance is poorly understood. Here, we uncovered HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 15 (HOS15) and HISTONE DEACETYLASE 9 (HDA9) as two negative regulators of plant immunity. We found that loss-of-function HOS15 and HDA9 confer enhanced resistance to pathogen infection. Further analysis showed that HOS15 and HDA9 directly bind to many NLR genes and repress their expression. The repression is likely through reducing H3K9ac level, as seen in one of the NLR genes, SUPPRESSOR OF npr1-1, CONSTITUTIVE 1 (SNC1). In addition, HOS15 represses the basal expression of NLRs, and HDA9 specifically functions during the infection stage to prevent overstimulation of NLR genes. Together, this study uncovers a previously uncharacterized histone deacetylase complex in plant immunity and highlights the importance of histone modifying enzymes acting together with their cofactors to fine-tune the defense responses.

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Primary E-Poster Presenter: Leiyun Yang

Comparative genomics provide a rapid detection of Fusarium oxysporum f. sp. conglutinans (1000-122 (Screen 14))
Hall 2

Fusarium oxysporum f. sp. conglutinans (Foc) is the causal agent of Fusarium wilt disease of Brassica oleracea. A rapid, accurate, and reliable method to detect and identify plant pathogens is vitally important to integrated disease management. In this study, using a comparative genome analysis among Fusarium oxysporum (Fo), we developed a Foc-specific primer set (Focs-1/Focs-2) and established a multiplex-PCR assay. In the assay, the Focs-1/Focs-2 and universal primers for Fusarium species (W106R/F106S) could be used to detect Foc isolates in a single PCR reaction. With the optimized PCR parameters, the multiplex-PCR assay showed a high specificity for detecting Foc and was very sensitive to detect as little as 100 pg of pure Foc genomic DNA or 1000 spores in 1 g of twice-autoclaved soil. We also demonstrated that Foc isolates were easily detected from infected plant tissues, as well as from natural field soils, using the multiplex-PCR assay. To our knowledge, this is a first report on detection Fo by comparative genomic method.

Primary E-Poster Presenter: Yuhong Yang

Elucidating the Role of the Putative RNA-Binding Protein SUP8 in Regulating Pathogen Defense and Flo (1000-121 (Screen 9))
Hall 2

Plants are frequently challenged by pathogens and pests. Defense responses of plants are often intricately connected with growth and development to maximize the use of limited resources. Successful control of plant diseases requires a thorough understanding of defense mechanisms and factors affecting development of plants. We identified an Arabidopsis mutant sup8 in a genetic screen for suppressors of acd6-1, a small mutant whose defense level is grossly in an inverse correlation with its size. SUP8 encodes a putative protein with KH repeats that was previously demonstrated a role in flowering time regulation. Consistent this known role of SUP8, two sup8 alleles showed delayed flowering. In response to Pseudomonas syringae, sup8 mutants exhibited increased bacterial growth and diminished accumulation of salicylic acid, a key defense signaling molecule. The sup8 mutants also showed reduced response to flg22, a defense elicitor derived from the conserved region of P. syringae flagellin proteins, for reactive oxygen species (ROS) production, callose deposition at the cell wall, and seedling root growth inhibition. In addition, sup8 plants exhibited enhanced disease resistance to the necrotrophic fungal pathogen Botrytis cinerea. Interestingly, treatment with methyl viologen, an inducer of superoxide production, also yielded a compromised response in sup8 mutants. Our data implicate a role of SUP8 in mediating crosstalk between pathogen defense and development, possibly through regulating ROS.

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Primary E-Poster Presenter: Hua Lu

3D Electron and Confocal Microscopy of Extracellular Vesicle Production in Plants (1000-048)
Hall 2

Electron microscopy studies indicate that extracellular vesicles (EVs) may play a central role in the communication between plants and microbes, both in leaves and in roots. The Innes laboratory has developed methods for the purification of EVs from the leaf apoplast and has shown that these vesicles contain biotic and abiotic stress response proteins (Rutter and Innes, 2017), as well as diverse small RNAs (Baldrich et al., 2019). I am investigating two fundamental questions concerning plant EVs: How many types of EVs exist, and how are they produced? To answer these questions, I am using Medicago truncatula as the host plant and its fungal pathogen Colletotrichum destructivum as the pathogen. I am currently using Serial Block Face Scanning Electron Microscopy (SBF-SEM) to create a 3- dimensional image of infection sites, which will enable me to assess the origin of EVs during

infection. To assess the diversity of EVs, I am using Golden Gate cloning to tag four different vesicle cargo proteins with different fluorescent proteins in a single T-DNA construct. EVs will be purified from transgenic Arabidopsis plants and examined by confocal microscopy to determine how often the fluorescent signals overlap. These analyses will help us determine the heterogeneity of EVs. If different classes exist, I will then investigate whether there are independent mechanisms of EV production.Baldrich, P., Rutter, B.D., Zand Karimi, H., Podicheti, R., Meyers, B.C., and Innes, R.W. (2019). Plant Extracellular Vesicles Contain Diverse Small RNA Species and Are Enriched in 10 to 17 Nucleotide "Tiny" RNAs. The Plant Cell, Vol. 31: 315–324Rutter BD, Innes RW (2017) Extracellular Vesicles Isolated from the Leaf Apoplast Carry Stress-Response Proteins. Plant Physiol 173: 728-741

Primary Poster Presenter: Suchismita Ghosh

A Novel Approach to Develop Broad-Spectrum Resistance to Plant Parasitic Nematodes (1000-047)
Hall 2

Plants and their parasitic nematodes are in a continuous co-evolutionary struggle for dominance. Consequently, plants have evolved strategies to perceive invading parasites and pathogens, by receptors that recognize conserved molecular patterns of the invaders, to induce pattern-triggered immunity. This perception in both tomato (Solanum lycopersicum) and Arabidopsis (Arabidopsis thaliana) requires the well-known co-receptor BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1/SOMATIC EMRYOGENESIS RECEPTOR KINASE 3, (BAK1/SERK3). By RNA-seq analysis of Arabidopsis bak1-5 mutant and wild-type Col-0 roots, infected with the root-knot nematode Meloidogyne incognita, a lectin receptor-like kinase (LecRK; AtG-LecRK-VIII.8) was identified to be upregulated in BAK1-dependent manner. Interestingly, null mutants of AtG-LecRK-VIII.8 displayed enhanced resistance to M. incognita infection. AtG-LecRK-VIII.8 or ENHANCED RESISTANCE TO NEMATODES1 (ERN1) belongs to G-type LecRKs (G-LecRK) encoding the typical G-LecRK domains, that includes the S-domain, and an atypical EGF domain. The ern1-1 and ern1-2 mutants did not show altered plant or root growth phenotypes compared to the wild-type. The enhanced resistance of ern1-1 and ern1-2 mutants was manifested by faster and higher levels of ROS burst and increased transcript accumulation of immune-related genes. Complementation of the ern1-1 mutant, with ERN1 behind the native or 35S promoters, reversed the enhanced resistance phenotype. Our results demonstrated that ERN1 is a negative regulator of nematode-triggered immunity. Considering that homologs of ERN1 exist in numerous crop species, it could be targeted for engineering resistance to root-knot nematodes.

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1:30 PM - 3:00 PM

A plant SnRK kinase is targeted by a bacterial effector to promote virulence (1000-101)

A critical component controlling bacterial virulence is the delivery of pathogen effectors into plant cells during infection. Pathogen effectors alter host metabolism and immunity for pathogen benefit. Multiple effectors are phosphorylated by host kinases, and this posttranslational modification is important for their activity. In this work, we sought to identify host kinases involved in effector phosphorylation. We found that bacterial effectors are enriched in motifs for phosphorylation by CDPK and SnRK kinases. Targeted yeast-two hybrid was used to identify bacterial effectors capable of interacting with CDPK and SnRK kinases. The conserved Pseudomonas effector AvrPtoB acts as an E3 ubiquitin ligase and is required for bacterial virulence. We identified a member of the SnRK kinase family that was able to phosphorylate and associate with AvrPtoB in planta. Mass spectrometry was used to quantify AvrPtoB phosphorylation, with the snrk knockout mutant exhibiting reduced phosphorylation compared to wild-type Arabidopsis. In addition, this SnRK member was required for AvrPtoB virulence and affected AvrPtoB-mediated degradation of host targets. These data identify a conserved kinase family that can be targeted by pathogen effectors to promote disease.

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Primary Poster Presenter: Lei Lei

A poplar receptor-like kinase mediates the symbiotic interaction between plant and mycorrhizal fungi (1000-026)
Hall 2

The soil-borne microbe/fungi can establish a mutualistic relationship with plant roots, to provide a large variety of nutrients to host plants in exchange of photosynthesized sugars. However, the molecular signal mediating the establishment of that relationship remains unclear. Our previous genetic mapping and resequencing data identified a receptor-like kinase coding gene PtRLK1 deletion event in Populus deltoides associated with a lack of root colonization by ectomycorrhizal fungi -Laccaria bicolor. And further study showed the overexpression of PtRLK1 could introduce the L. bicolor colonization in the P.t. x P.d. hybrids roots. Here, through introducing PtRLK1 into P. deltoides and P. tremula x alba 717-1B4, we are able to show that the PtRLK1 can help to establish the symbiotic relationship between non-host poplar plants and L. bicolor. In addition, our qPCR results showed the defense-related genes were down-regulated in the inoculated root sample of transgenic P. deltoides but up-regulated in that of transgenic P. tremula x alba 717-1B4 line. Accordingly, we observed the L. bicolor slightly penetrated into the root cells of transgenic P. deltoides but fully filled every root cells including the vasculature of the transgenic P. tremula x alba 717-1B4,

suggesting a sophisticated switch mechanism may present in the plant to regulate the beneficial or pathogenic responses to the mycorrhizal fungi inoculation. Furthermore, we also overexpressed PtRLK1 into perennial grass-switchgrass and annual crop grass-rice, to investigate if this receptor-like kinase also can introduce the mutualistic mycorrhizal colonization into the roots of non-host grass species for benefiting biomass and crop yield. To date, we have inoculated the root of transgenic non-host plants with L. bicolor, and a total of 57 inoculated root samples have been collected. All RNA, metabolite, protein, and phosphor-protein have been extracted from these root samples and are ready for -omic analyses.

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Primary Poster Presenter: Zhenzhen Qiao

A potent high-resolution DNA variant filtering improves in silico detection accuracy (1000-088)
Hall 2

The accurate detection of DNA mutations is a very critical step for gene function discovery studies. Current next-generation sequencing (NGS) technologies have rapidly accelerated the pace of discoveries in molecular genetics, but are characterized by an inherently higher sequencing error rates, and the concomitant adverse effects of false-positive in silico mutation detections. Various algorithmic techniques had been proposed for filtering false-positives. Two undesirable consequences of standard hard-filtering techniques are false-negative predictions, where bona fide mutations are erroneously discarded, and also the potential for low-concordance of predicted mutations among the leading DNA mutation detection algorithms. We describe a general high-resolution computational method for uncovering false-negative induced DNA mutations in NGS sequencing data for mutagenized populations. Regardless of the variant-calling algorithm utilized, the method uses a binning approach by assigning predicted mutations with the same variant-call quality-score in the same category bin, and then empirically determining the quality-score threshold at which the exactitude of the predicted mutations in the NGS sequencing data mirrors the experimentally described DNA mutation spectrum of the mutation inducing agent. To evaluate the proposed technique, we applied the method to a previously described EMS-mutagenized sorghum population. Using the SAMtools variant-calling algorithm and a variant quality-score threshold of 12, almost 96% (3,141,908) of the 3,274,606 SNPs were GC□AT mutations. Alternatively, using the GATK algorithm and a quality-score threshold of 28, 93% (3,211,794) of the 3,435,789 SNPs were GC□AT mutations. This preliminary result represents an 87% (1,521,203 likely false-negatives) increase over the previously predicted 1,753,403 EMS-induced SNPs. The result also shows a high 94% (3,075,884 SNPs) concordance between the SAMtools and GATK variant-calling algorithms.

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A UBC13-Interacting E3 Ligase Acts with the FLS2-BAK1-BIK1 Complex to Regulate Plant Immunity (1000-090)
Hall 2

The tomato UBC13-type ubiquitin-conjugating enzyme (E2) Fni3 (Fen-interacting protein 3) was previously shown to regulate plant immunity through K63-linked ubiquitination. However, the underlying molecular basis for the regulation remains to be elucidated. In this study, we found a tomato RING type ubiquitin ligase (E3), SlFti1 (Solanum lycopersicum Fni3-interacting protein1) and its homolog SlFti1B working with Fni3 to catalyze K63-linked ubiquitination. The Arabidopsis closest homologs, AtFti1 and AtFti1B were also found acting with UBC13 to catalyze K63 ubiquitination. Of the AtFti1 and AtFti1B, only AtFti1 associated with members of the FLS2-BAK1-BIK1 receptor complex. The interaction of AtFti1 with FLS2 and BAK1 was enhanced upon challenging with the immunogenic epitope of bacterial flagellin, flg22. By contrast, the interaction of AtFti1 with BIK1 was diminished upon flg22 treatment. Both BAK1 and BIK1 phosphorylated AtFti1 in vitro and the phosphorylation by BIK1 on the Ser395 of AtFti1 inhibited its E3 activity. Overexpression of AtFti1 (OEX) in Arabidopsis resulted in an elevated level of FLS2 and inhibition of flg22- and pathogen-induced degradation of FLS2. Consistently, AtFti1 OEX lines showed elevated sensitivity to flg22 in the seedling growth assay and increased resistance to Pseudomonas syringae pv tomato (Pst) after flg22 treatment. These results suggest that the E3 activity of AtFti1 is inhibited by BIK1 phosphorylation in immunity-non-activated cells. Upon flg22 treatment which initiates FLS2-mediated immune signaling, the inhibition is relieved due to the reduced association of AtFti1 with BIK1. The released AtFti1 then functions as a positive regulator that stabilizes FLS2 by inhibiting its degradation. Our findings add a new layer of regulation to the pathogen-associated molecular pattern (PAMP)-induced plant immunity.

Primary Poster Presenter: Yi Zhang

A Unique Mutation Emphasizes the Dynamic Relationship between Calcium and ROS in Immune Signaling. (1000-064)
Hall 2

Dynamic changes of cytosolic calcium concentration in plant cells play an important role for the plant innate immune response. Plants also use changes in intracellular and extracellular Reactive Oxygen Species (ROS) to communicate perception of environmental cues as well along with electrical, hydraulic and nitrogen oxide

signaling events. In order to translate these signals to downstream responses, the fundamental mechanisms of these signals need to be elucidated. Here, we explore the dynamics of calcium and ROS signals for plant immune responses highlighting a particularly interesting yet unidentified mutant silent knight 1 (silk1), which uniquely produces no calcium response to any stimuli tested. Interestingly, the mutant is significantly susceptible to both biotrophic and necrotrophic pathogens. Using pharmacological calcium and ROS inhibitors and generators, and the silk1 mutant, we explore how these signals may initiate or regulate each other by which plants defend themselves against microbial pathogens. We will present data representing the importance of calcium and ROS regulation during immune responses and how each particular immune stimuli may require calcium and ROS signals in plants.

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Primary Poster Presenter: Matthew Marcec

Analysis of competition between Rhizobium leguminosarum and Rhizobium rhizogenes within legume nodul (1000-031)
Hall 2

Rhizobia bacteria provide leguminous plants useable nitrogen through their capability to fix atmospheric nitrogen (N2) into ammonia (NH3). Specifically, rhizobia fix nitrogen in nodules, specific root structures, and receive carbohydrates from the plant in a reciprocally beneficial symbiosis. Nitrogen fixation is energetically costly to rhizobia as it uses resources that could be used towards growth and reproduction. Therefore, natural selection favors 'cheaters'; rhizobia living in nodules that fix less nitrogen. To maintain this symbiosis, plants must favor rhizobia that fix the most nitrogen and/or use sanctions to penalize 'cheaters'. I am investigating this relationship by isolating rhizobia and observing their interactions between each other and Pisum sativum (pea). Five bacterial strains were isolated from nodules of Lupinus sp. and P. sativum. 16S rDNA analysis identified four of the strains as Rhizobium leguminosarum and the other as R. rhizogenes. Nodulation assays showed that R. rhizogenes and one of the R. leguminosarum strains do not nodulate pea. Successful nodulating strains resulted in healthy green plants with pink nodules, however co-inoculation with nodulating and non-nodulating strains resulted in white nodules and yellow leaf coloration signifying stress. Furthermore, biofilm production was found to differ among strains of R. leguminosarum. All strains were resistant to ampicillin, and green fluorescent protein (GFP) and mCherry fluorescent markers were introduced into the bacteria. This fluorescence will be used to identify bacteria in co-inoculated pea nodules to observe occupancy patterns of the bacteria within the nodules of the pea plant. This study will increase our understanding of the interactions between agriculturally applied bacterial bio-fertilizers and naturally occurring soil rhizobia.

Co-author(s): Ashwarya Sharma

Primary Poster Presenter: Michelle Lum

Analysis of fungicide resistance of Botrytis cinerea causing Ginseng gray mold in Korea (1000-042)
Hall 2

The gray mold fungus Botrytis cinerea causes losses of commercially important fruits, vegetables and ornamentals worldwide. In addition to being one of the most acute problems impeding chemical control of fungal diseases, the evolution of fungicide resistance is an emblematic case of local adaptation to spatially heterogeneous and temporally variable selection pressures. Fungicide treatments are effective for disease control, but bear the risk of resistance development. B. cinerea was isolated from ginseng fields of Korea in 2018, and the resistance of these isolates against four fungicides (fludioxonil , fenhexamid , polyoxin B , boscalid) was examined. Among 874 isolates collected in Eumseong, Jeungpyong, Chungju, Goesan, the percentages of resistance against fludioxonil (FlyR), fenhexamid (FenR), polyoxin B (PolR), boscalid (BosR) fungicides were 3.2%, 34.3%, 99.4%, 90.4%, respectively. Most of all isolates was resistance to polyoxin B/boscalid and sensitive fludioxonil (PolR+BosR+FlyS). Also, of the total strains, 2.4% were found to be resistant to all four fungicides. These fungicide resistant isolate will increase the risk of gray mold rot and hamper the effectiveness of current strategies for fungicide resistance management. Phylogenetic analysis using combined sequences (RPB2, HSP60, and G3PDH genes) clearly showed that all isolate of B. cinerea were different from Botrytis spp. As a result, it was also confirmed that ginseng isolate was closely related to apple, pepper, cucumber, and tomato isolates that were distantly related to strawberry isolate with each other in nucleotide level.

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Primary Poster Presenter: Seung-Ho Lee

Antimicrobial Properties of Salvinia molesta on Common Bacteria (1000-022)
Hall 2

Very little has been documented about the positive characteristics of the invasive fern Salvinia molesta. The goal of this research was to determine if an extract created from mature S. molesta plants has antibacterial properties. Extract was tested on the following bacterial species: Escherichia coli, Salmonella typhimurium, Staphylococcus epidermidis, Bacillus megaterium, Enterobacteria cloacae, Serratia marcescens, Proteus vulgaris, and Pseudomonas aeruginosa. Each species was exposed to small doses of S. molesta extract, and absorbance was measured after exposure, which can be interpreted as population density. B. megaterium, P. vulgaris, E. coli, E. cloacae, and S. epidermidis all showed significant inhibition after being exposed to the extract. For E. coli, the response was dose dependent. The Kirby-Bauer test also was conducted on the following bacteria: E. coli, S.

typhimurium, S. epidermidis, B. megaterium, S. marcescens, Staphylococcus aureus, Alcaligenes faecalis, P. vulgaris, Streptococcus pyogenes, P. aeruginosa, and Klebsiella pneumoniae. Small paper discs were saturated with extract and placed on a petri dish that was inoculated with bacteria. Inhibition circles on the paper discs were present for the following bacteria: E. coli, S. typhimurium, E. cloacae, S. aureus, P. aeruginosa, S. marcescens, A. faecalis, and S. epidermidis. Some of the inhibition rings were faint but still visible. In conclusion, S. molesta extract has the capability of controlling bacterial species. Additional tests should be conducted to determine the extent of inhibition generated by S. molesta extract on these common bacterial strains.

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Primary Poster Presenter: Amy Erickson

Arabidopsis TCP8 promotes brassinosteroid signaling at an interface of defense and development (1000-072)
Hall 2

Plants are regularly challenged by microbial pathogens in native and agricultural environments, translating to enormous economic loss annually. A complex innate immune system allows plants to recognize pathogen-specific molecular signatures, triggering a system-wide defense response known as PAMP-triggered immunity (PTI). A second layer of defense is triggered in response to the activity of specialized effector proteins which target host components to suppress PTI and otherwise promote pathogen fitness. The strong physiological response and cell death associated with host detection of effectors is known as effector-triggered immunity (ETI). An active immune response, however, is metabolically expensive and comes at the cost of plant growth and development; accordingly, optimization of the balance between defense and development is critical to plant fitness. The TCP transcription factor family consists of well-characterized transcriptional regulators of plant development and morphogenesis. Three closely-related Class I TCPs have been confirmed to promote ETI, and our recently published work identified complementary roles in the regulation of EFR-dependent PTI. Here, we present the direct activation of key components of the growth-related brassinosteroid signaling pathway by TCP8, as well as potential mechanisms for its regulation. We propose a model by which environmental context may dictate subnuclear localization and promoter occupancy of TCP8 and other TCP TFs between PTI and BR signaling-responsive genes, ultimately prioritizing either defense or development.

Co-author(s): Walter Gassmann

Primary Poster Presenter: Benjamin Spears

Auxin plays multiple roles during Pseudomonas syringae pathogenesis

(1000-061)
Hall 2

Pseudomonas syringae pv. tomato strain DC3000 (PtoDC3000) is a model for studying bacterial pathogenesis in plants. Recent observations show that PtoDC3000 uses many strategies to manipulate auxin physiology in Arabidopsis to promote pathogenesis. For instance, PtoDC3000 can synthesize the auxin indole-3-acetic acid (IAA), and it can also manipulate host auxin signaling via secreted virulence factors. Auxin inhibits host defense responses mediated by the plant hormone salicylic acid (SA), and previously we showed that auxin synthesis by the pathogen, mediated through the AldA enzyme, promotes pathogen growth by suppressing SA-mediated defenses. However, other findings suggest that auxin promotes PtoDC3000 growth in planta via a mechanism independent of suppression of SA-mediated defenses. Thus, auxin plays multiple roles during pathogenesis. To test if host auxin signaling is important during pathogenesis, we took advantage of transgenic plants carrying an inducible dominant negative allele in the AXR2 gene, GR-axr2-1, that impairs auxin signaling. We observed a significant repression of auxin responsive genes (IAA-19 & GH3.3) in GR-axr2-1 plants, and bacterial growth was reduced in these plants compared to the wild type. These findings indicate that host auxin perception is required for normal susceptibility to PtoDC3000. To investigate if auxin also has a direct effect on PtoDC3000, we tested the effect of IAA on expression of virulence genes in culture, and observed that IAA down regulates genes involved in Type III secretion, but induces expression of other genes likely to play a role at later stages of infection. We quantified expression of these genes in plant tissue, including in an Arabidopsis mutant with elevated auxin levels, and observed the same pattern. Our results suggest that, in addition to suppressing host defenses, IAA acts as a microbial signaling molecule to coordinate expression of virulence genes involved at various stages of pathogenesis

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Primary Poster Presenter: ARNAUD THIERRY DJAMI TCHATCHOU

Biosynthesis and secretion of the microbial sulfated peptide RaxX and binding to the rice XA21 immun (1000-033)
Hall 2

Despite the importance of immune receptors in biology of plants and animals, the precise biochemical mechanisms leading to the biosynthesis and secretion of the peptide activators of these immune receptors remains unexplored. Here we describe the biosynthesis, processing, and secretion of a peptide activator that binds a rice immune receptor. Our studies also reveal that this peptide activator is the first member of an important class of peptides present in both eukaryotic and

prokaryotic species. These results provide a major advance in our knowledge of the biology of peptide ligands.

Co-author(s): Pamela Ronald

Primary Poster Presenter: Dee Dee Luu

Carbon cycling and metabolic coupling in the root – rhizosphere - microbiome continuum (1000-094)
Hall 2

Understanding cell interactions within and between organisms is key to determining and controlling the flow of materials and energy through the environment. This in turn requires measuring the production and assimilation of substrates across multiple organisms in situ, which is currently a grand challenge. Such interactions involving plants and rhizospheric microbiomes are thought to affect the health and the overall performance of the plants, health of the soil, and the belowground carbon budget. However the communicating signals and the underlying molecular mechanisms that orchestrate the carbon flow from plants to microbes to soil is poorly understood. We aim to understand the metabolic coupling between plant roots and the rhizospheric microbiome using a simplified Brachypodium-Pseudomonas system. We are specifically targeting the allocation of photosynthate (assimilated CO2 as sugars) between source (net sugar-producing) and sink (net sugar-consuming) tissues, and the partitioning of newly formed photosynthate into different compounds as a function of the presence or absence of the bacteria. We have used a novel approach by coupling stable isotope probing with multi-omic analysis techniques to understand the molecular changes taking place in plant microbe soil interactome. Plants were grown with and without bacteria and probed with isotopically labelled CO2 for two diurnal cycles and harvested for subsequent analysis. A gradual decrease in the isotopically labelled carbon was visible as the newly synthesized photosynthate was allocated to different plant organs. The isotopic signal decreased as it moved from the roots to the rhizosphere to loose soil then was undisguisable from the baseline levels in the bulk soil. Significant changes in the leaf, root and soil metabolite profiles were observed when plants were exposed to bacteria compared to those that were not. By coupling multiple high resolution mass spectrometry based techniques, we are currently investig

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Primary Poster Presenter: Pubudu Handakumbura

Characterization of a novel chicken foot nodule mutant in the model legume plant Medicago truncatula (1000-065)
Hall 2

Legume plants such as soybeans and pea interact with the soil bacteria rhizobia and form unique organs called nodules to capture and convert inert atmospheric nitrogen (N2) into usable form by symbiotic nitrogen fixation (SNF). In a forward genetic screen to discover novel genes that are involved in SNF process, Dr. Veerappan identified chicken feet-like nodules (cfn) mutant by screening Tnt1 mutant population in the model legume plant Medicago truncatula (barrel medic). cfn mutant was named after its characteristic clustered nodule-like structures on roots similar to a chicken foot. cfn mutant plants show defective root architecture, decreased nodule numbers, reddish-purple shoot (N2 deficiency symptom) and white nodules indicating deficiency in SNF. In cfn mutant, nodule-like structures acquire root-like identity and occasionally root transforms into nodule-like organs. Comparison of cfn mutant phenotype with previously characterized M. truncatula root architecture and nodule organ identity defect mutants nodule root (noot), compact root architecture1 (cra1) and cra2 indicates that cfn is a novel mutant. Segregation analysis of cfn mutant phenotype using R2 (Regeneration 2) population shows that cfn phenotype is controlled by a single, recessive mutation. cfn mutant plants are lethal, failed to reproduce and needs to be maintained in heterozygotes. Towards finding the causative mutation underlying cfn mutant phenotype, I mined the Medicago Tnt1 mutants database and performed whole genome sequencing. Identification of the causative mutation in cfn mutant will provide novel mechanisms that control root architecture, nodule organ identity and SNF.

Primary Poster Presenter: Roshani Budhathoki

Characterization of a novel defense signaling peptide in Medicago sativa (1000-091)
Hall 2

As a first line of defense to fend off pathogen attack, plants deploy a robust innate immune response coordinated by numerous phytohormones, including peptides. To identify potential regulators of immune responses in Medicago sativa (alfalfa), we employed activity-led purification using a suspension-cultured cell-based assay to detect induced extracellular alkalinization, a conserved early event associated with defense signaling. A 12 amino-acid peptide from foliar alfalfa tissues with no sequence similarities to previously-identified peptide signals was found to be a potent activator of extracellular alkalinization, and confirmed through testing of synthetic analogues. Termed alfalfin, the peptide triggers extracellular alkalinization when applied at nanomolar concentrations, an effect that is potentiated by pretreatment with methyl-salicylate. Application of alfalfin to trifoliate leaves from juvenile plants triggers ethylene emission and reactive oxygen species production in a dose-dependent manner. As compared to treatments with jasmonic acid, alfalfin does not stimulate accumulation of trypsin inhibitors in foliar tissues. However, crude extracts from alfalfin-supplied plants have greatly increased Phenylalanine

Ammonia Lyase (PAL) enzyme activity compared to water-supplied plants. As PAL is the first committed step in the production of phenylpropanoid metabolites produced in response to biotic and abiotic stressors, we are currently examining metabolomic changes triggered by alfalfin treatment. Together these data indicate that alfalfin elicits early defense responses and may be directly or indirectly involved in salicylic acid signaling, as well as defense-related production of phenylpropanoids. Presently, we are pairing metabolomic profiling with investigation of global transcriptional changes in response to the Alfalfin through RNA-seq to better understand the breadth of immune responses regulated by this new peptide signal.

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Primary Poster Presenter: Marlo Hall

Characterization of a putative PAMP-induced secreted peptide in the HR-resistance of potato to PVY (1000-075)
Hall 2

Potato (Solanum tuberosum) is the fourth most cultivated food crop in the world after rice, wheat, and corn. Potato Virus Y (PVY) (genus Potyvirus) is a devastating pathogen that can reduce yield or lead to a complete loss of a marketable product. In recent years, new recombinant strains of PVY such as PVYN-Wi and PVYNTN have been replacing the once-dominant strain, PVYO, in major US potato production areas. While strain-specific hypersensitive response (HR) genes are commonly found in commercial potato varieties and provide useful means to manage the virus, little is known about the resistance mechanism mediated by these genes, and how some virus strains break this mechanism. To better understand HR, we previously analyzed changes in gene expression in the variety Premier Russet (PR) upon inoculation with PVYO. PVYO and PVYN-wilga, but not other PVY strains, trigger HR in PR. These transcriptomics analyses showed that the gene PGSC0003DMG400014879 was the most highly differentially expressed. We hypothesized that it plays a major role in HR. This prompted us to further characterize its function in the resistance response to PVY. It is annotated as an ABC transporter in the potato genomics database SpudDB. However, by using various bioinformatic tools, we were able to show that the encoded protein is homologous to the Arabidopsis PAMP-Induced secreted Peptide (PIP) family. We named the potato gene StPIP. The StPIP prepropeptide has a putative transit peptide for extracellular secretion, and its propeptide contains two highly conserved regions that may be processed into two mature peptides. Transgenic PR lines overexpressing StPIP under the control of the CaMV35S promoter did not show major differences in the response to PVYO or PVYN-Wi. However, when inoculated with PVYNTN, StPIP-overexpressing plants showed earlier onset of and more severe symptoms than the control, suggesting that StPIP facilitates the spread and accumulation of PVYNTN.

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Primary Poster Presenter: Max Combest

Chickpea wild relatives: resource for improving biological nitrogen fixation. (1000-099)
Hall 2

Nitrogen fixation efficiency is a desirable trait for legume breeding, but is highly variable in farmer's fields. Here we evaluate the symbiotic performance phenotypes and transcriptomic landscape of domesticated and wild host relatives. We focused on the genetic diversity of chickpea (Cicer arietinum) wild relatives: C. echinospermun (Ce), and C. reticulatum (Cr); as well as the microbial diversity of nitrogen fixers Mesorhizobium mediterraneum (Mm), and M. ciceri (Mc). We ask whether geographical co-occurrence of host and microbes represent the most effective symbiosis outcomes for nitrogen fixation, and found that cultivated chickpea responds similarly to diverse M. ciceri and M. mediterraneum strains from the native range. Contrastingly, chickpea wild relatives have differential biomass performance depending on Mesorhizobium species. Ce biomass was twofold larger with its natural occurring symbiont Mc, also nodulation was higher. While the converse interaction with Mm was deficient on nodulation and biomass conversion. Under inefficient symbiosis with Mc, Cr allocate more biomass/nodule, nodule number is variable per genotype, and does not reflect performance. We provide evidence of functional co-evolutionary associations among wild Cicer spp. and Mesorhizobium spp. We are further investigating representative host-rhizobia pairs of cultivated chickpea and wild relatives, which exhibit contrasting efficient (co-evolved) and inefficient (heterologous) outcomes described above. Sequencing (RNAseq) of 110 samples corresponding to leaf, roots and nodules, resulted in aprox. ~2500 millions of reads currently being analyzed to deduce the host transcriptomic landscape, differential gene expression among metabolic and regulatory genes associated with nitrogen fixation efficiency in Cicer spp. The symbiotic adaptations represented in wild systems, can inform the efforts to mobilize nitrogen fixation efficient traits to cultivated genotypes.

Co-author(s): Douglas Cook

Primary Poster Presenter: Laura Perilla-Henao

Comparative analysis of endophytic bacterial community of in vitro and field conditions by pure-cult (1000-052)
Hall 2

Plant endophytes are non-pathogenic and plant-colonizing bacteria to affect upon plant health positively as growth promoter. In this study, we carried out endophytic community analysis by pure-culture methods and Next Generation Sequencing (NGS)-based whole metagenomic sequencing from rice representative cultivars of Milyang 23 (indica) and Gihobyeo (japonica). Endophytic community by a pure-culture method in rice was conducted and identified as total 840 endophytic bacterial isolates. Proteobacteria (63.45%) showed most dominant phylum

compared with other phyla. Comparison analysis of endophytic isolates through alpha- and beta-diversity were carried out by considering as rice cultivars, plant anatomy, and environmental conditions. Afterwards, whole metagenomic analysis of bacterial endophytes was conducted by using 300 bp paired-end products of Illumina's MiSeq platform. Putative bacterial reads extracted from rice resequencing data were mapped to the bacterial genome database (NCBI) and were classified with each taxonomic level. Proteobacteria (59.59%) showed the dominancy on rice endophytes. Alphaproteobacteria (61.44%) of Proteobacteria was dominant in class level showing differences from taxonomic distribution of pure-culture methods in class level. This research suggested the novel approaches of endophytic community analysis by cross-validation of pure-culture methods and NGS-based analysis using rice resequencing data.

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Primary Poster Presenter: Joon-hui Chung

Conserved Biochemical Defenses Underpin Host Responses to Oomycete Infection in an Early Divergent L (1000-068)
Hall 2

The expansion of plants onto land necessitated the evolution of robust defence strategies to protect against a wide array of microbial invaders. While host responses to microbial colonization are extensively explored in evolutionary young land plant lineages like angiosperms, we know relatively little about plant-pathogen interactions in earlier diverging land plants thought to better represent the ancestral state. Here, we define the transcriptional and proteomic response of the early divergent liverwort Marchantia polymorpha to infection with the oomycete pathogen Phytophthora palmivora. We uncover a robust molecular response to oomycete colonization in Marchantia that consists of conserved land plant gene families. Direct macro evolutionary comparisons of host infection responses in Marchantia and the model angiosperm Nicotiana benthamiana further reveal a shared set of orthologous microbe-responsive genes that include members of the phenylpropanoid metabolic pathway. In addition, we identify a role for the Marchantia R2R3-MYB transcription factor MpMyb14 in activating phenylpropanoid (flavonoid) biosynthesis during oomycete infection. Ectopic induction of MpMyb14 led to the accumulation of anthocyanin-like pigments and dramatically enhanced liverwort resistance to P. palmivora infection. Collectively, our results demonstrate that the Marchantia response to oomycete infection displays evolutionarily conserved features indicative of an ancestral pathogen deterrence strategy centred on phenylpropanoid-mediated biochemical defences.

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Convergent gene loss in aquatic plants reveals novel plant immunity and drought responses components (1000-069)
Hall 2

Plants colonization of land from water altered selection on numerous traits. A high dependence on microbes was crucial during this transition. Since, there have been several migrations within monocots and dicots to an aquatic environment however, little is known about the effect of such a change on plant-microbe interactions. We annotated the immune receptor family, Nucleotide Binding Leucine Rich Repeat proteins (NLRs), across 19 flowering plants genomes and identified four aquatic species with low numbers of NLR-encoding genes relative to other plants . The loss of NLRs in these aquatic species is not random with no remaining TIR-1 and RPW8 NLRs. Most importantly all three members of the EDS1, PAD4 and SAG101 signalling complex are absent in all four species. These observations indicate a convergent loss of this signalling pathway among the four species representing two independent transitions to water. We identified 44 additional genes lost only in aquatic species, which we named AngioSperm Terrestrial-Retained, Aquatic-Lost (ASTRAL). We hypothesised that ASTRAL genes may be involved in defense response similarly to the lost NLRs and EDS1 complex. Fifteen ASTRAL genes are differentially expressed upon pathogen infection. Whilst 11 ASTRAL genes were differentially expressed upon drought. The ASTRAL gene set provides information on pathways required for adaptation to land and novel candidates for roles in plant immunity functions previously obscured by genetic redundancy.

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Cyclin-dependent like kinases in the formation of the host membrane compartment in arbuscular mycorr (1000-081)
Hall 2

Intracellular interactions between plant cells and biotrophic microorganisms, either symbiotic or pathogenic, always involves the formation of a specialized host membrane compartment which houses the microorganism. This membrane compartment functions as the interface between the cytoplasm of the host cell and microorganism and regulates, for example, the exchange of mineral nutrients. In

the symbiotic interaction of plants with arbuscular mycorrhizal (AM) fungi, the intracellular membrane compartment in the root cortical cells consists of the plant periarbuscular membrane (PAM) which surrounds a highly branched fungal hypha called an arbuscule. Currently, the intracellular signal transduction pathways involved in the development of this compartment are largely unknown. Here we report on cyclin-dependent like kinases (CKLs), a class of kinases whose function in plants has not been described previously. A Medicago truncatula ckl1 and ckl2 double mutant and a mutant of the single symbiotic CKL gene of Brachypodium distachyon are unable to support arbuscule development. MtCKL1 and MtCKL2 are expressed specifically in root zones colonized by the AM fungus. The two proteins show overlapping but slightly different sub-cellular locations; MtCKL1 is located mainly in nucleoplasm, cytoplasm and rarely on the PAM, whereas, MtCKL2 is located primarily on the PAM and the plasma membrane. MtCKL1 and MtCKL2 interact with cyclins and mutation of the putative cyclin-binding domain indicates that the interactions with cyclins are required for protein function. The hypotheses that the CKLs function in endoreduplication or in polar exocytosis were tested and rejected. Currently, we hypothesize that symbiotic CKLs regulate the formation of symbiotic membrane compartment via signal transduction from a putative receptor complex on the PAM, either via phosphorylation of a new substrate or by dissociation from the PAM and re-location to the nucleus where they may regulate transcription.

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Cytokinin-Mediated Processes Promote Heat-Induced Disease Susceptibility of Arabidopsis (1000-014)
Hall 2

Under increased temperatures, such as those predicted as a result of global climate change, plant defense responses are attenuated leading to a process known as heat-induced disease susceptibility (HIS). The plant growth hormone cytokinin is known to regulate responses to both biotic and abiotic pressures. To address the role of cytokinin in HIS of Arabidopsis to Pseudomonas syringae pv. tomato DC03000 (Pst), wild-type plants and a cytokinin receptor mutant (ahk2,3 mutated on ARABIDOPSIS HISTIDINE KINASE 2 and 3) were exposed to Pst at two different temperatures, normal (22°C) and high (28°C). Pst populations were measured to assess pathogen fitness and host susceptibility. Stomatal conductance, fluorescent microscopy, and gene expression were measured to evaluate how cytokinin signaling impacts HIS. Results show that ahk2,3 plants are less susceptible at 28°C, with Pst populations plateauing 36 hours post inoculation. Moreover, pathogen-induced stomatal closure and expression of defense-marker genes were impaired under heat stress. However, a synthetic cytokinin reporter showed that high temperature increased cytokinin signaling. These results suggest that apart from its role in defense under normal temperature conditions, under high temperature cytokinin promotes physiological

conditions that contribute to pathogen proliferation, and highlight the value of cytokinin-based approaches to understand plant susceptibility and improve crop protection under increased temperatures.

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Cytoplasm-nucleus partitioning of Sw-5b is required to dictate host immunity against Tospovirus (1000-083)
Hall 2

Plant and animal intracellular nucleotide binding-leucine-rich repeat (NLR) immune receptors play critical roles in mediating host immunity against pathogen invasions. Many plant NLRs carry non-canonical integrated domains. However, the functions of most non-canonical integrated domains remained largely unknown. Tomato Sw-5b NLR that confers a resistance to tospovirus carries a non-canonical N-terminal Solanaceae domain (SD). In this study, we determined that the SD of Sw-5b functioned as a critical intracellular translocation modulator, allowing Sw-5b immune receptor to translocate from cytoplasm to nucleus to trigger the immunity against tospovirus invasion. The forced cytoplasmic localization of Sw-5b could only induce cell death but not the immunity to tospovirus infection, whereas the forced nuclear localization of the receptor triggered the host systemic immunity. The coil-coil (CC) domain of Sw-5b alone was not sufficient to transport the CC-NB-ARC-LRR into nucleus, and transgenic Nicotiana benthamiana plants expressing the Sw-5b CC-NB-ARC-LRR was unable to confer the immunity to tospovirus. Strikingly, the non-canonical SD domain of Sw-5b was capable of translocating Sw-5b into nucleus to induce the immunity. Furthermore, the translocation of Sw-5b into nucleus depended on both importins □□and□□. Silencing both importin □□and□□□expression completely□inhibited Sw-5b trafficking into nucleus, and thus disrupted both local and systemic immunity against tospovirus. Taken together, the non-canonical domain of Sw-5b NLR played critical roles in translocating the receptor from cytoplasm to nucleus to induce host local and systemic immunity against tospovirus invasion.

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Different plant responses of susceptible and tolerant citrus varieties to HLB (1000-019)
Hall 2

Candidatus Liberibacter spp. is the presumed causal agent of citrus Huanglongbing (HLB). Candidatus Liberibacter asiaticus (Las), the prevailing HLB pathogen, is transmitted by phloem-feeding citrus psyllids, Diaphorina citri. HLB is currently considered the most devastating citrus disease in the world. HLB symptoms include yellow shoots, leaf blotchy mottle, lopsided fruit with color inversion, and seed abortion. Moreover, starch accumulation and phloem damage affecting source-sink flow of photoassimilates, are also observed in the infected citrus plants. Even though this disease affects all citrus species, some varieties show less severe symptoms and much slower decline than others. The tolerance mechanism remains largely unknown. In the present research, we investigated gene expression, starch, callose, and ROS quantification induced by Las and Pseudomonas syringae pv. tabaci Flg22. The vegetative material chosen for the comparative experiments were a HLB tolerant cultivar (LB8-9 Sugar Belle®) and a very susceptible sweet orange cultivar (Valencia). Our results showed differential gene expression, callose deposition patterns, and ROS induction responses between these two cultivars. Our results aim to shed light on citrus innate tolerance mechanism against HLB.

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Discovery of novel RNA-based fungicides to control Fusarium head blight of cereals (1000-020)
Hall 2

The most devastating disease of cereals, Fusarium head blight (FHB), is caused by Fusarium graminearum (Fg). Management of FHB disease in cereals is largely based on basal resistance of commercial varieties and chemical control. Upon availability of the genomes of wheat and Fg new tools are being exploited to be integrated into existing control strategies for FHB. Transcriptome analysis of a range of wheat varieties expressing different levels of resistance to Fg helped us to uncover the Fusarium genes that are likely to play a role in early FHB infection of wheat kernels. A subset of early expressed genes involved in FHB kernel infection were selected for RNAi-mediated silencing to reduce pathogen growth using Spray-induced gene silencing (SIGS). SIGS is a non-GMO based technology which relies on the sequence specificity in dsRNA molecules to transcriptionally silence target genes. The use of dsRNA molecules to silence genes that support pathogen growth and mycotoxin production might offer a viable tool in integrated pest management (IPM) strategies to control FHB epidemics. Our research identified novel Fg gene targets that can be used as future next-generation fungicides.

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Distinct RGS1 Signalosome Architectures on the Plasma Membrane Define Two Signaling Pathways (1000-016)
Hall 2

The non-canonical 7-TM AtRGS1 is a regulator of G signaling in Arabidopsis. Endocytosis of AtRGS1 releases the heterotrimeric G protein complex and permits nucleotide exchange in Gα resulting in the self-activated Gα-GTP and downstream signaling events. Cell surface receptors perceive D-glucose and flg22 and induce AtRGS1 endocytosis through requisite phosphorylation of di-serine residues on the C-terminus. We show a signaling system where AtRGS1 endocytosis has two origins: Clathrin-mediated- (CME) and Sterol-dependent-endocytosis (SDE), and both origins are ligand specific. The WNK8 kinase and FLS2/BAK1 RLK cluster are necessary for AtRGS1 phosphorylation of the same requisite di-serines, but function specifically in response to D-glucose and flg22 respectively. Additionally, two distinct populations of AtRGS1 are present and associate with the Clathrin Light Chain (CME) and Flotilin1 (SDE) endocytosis proteins in a signal dependent manner. Individual components of the heterotrimeric G protein complex are also required, but for flg22 induced AtRGS1 endocytosis, not D-glucose induced AtRGS1 endocytosis. We show a system where a single 7-TM protein that regulates G signaling responds to two signals with two separate origins of endocytosis where the origins of endocytosis have distinct downstream signaling pathways.

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Effect of alive Neochloris oleoabundans microalga as fertilizer is several commercial plants (1000-095)
Hall 2

Algae have been used has fertilizers since long time ago, then they were substituted mainly by inorganic fertilizers. However, since some years the market, pushed by consumers preferences; is looking for more organic products and organic agriculture is being rocketed with this purpose According to the Forschungsinstitut für biologischen Landbau (Research Institute of Organic Agriculture), Mexico is among the three countries having more organic farmers, and these demands more organic supplies for this kind of agriculture. Currently there are several commercial additives and products derived from algae in the market, but they are mainly marine algae extracts and some with other added compounds. In order to help the organic production in the country, we started a project in collaboration with the Compañía Manufacturera de Artefactos Eléctricos, S.A. de C.V., to check the

potential of microalgae as biofertilizers. Several consortia were assayed but along the time, the composition of the consortia was modified even under controlled culture conditions, making difficult to keep a standard quality and the observed effects in the assayed cultivars were not clear nor stable. We decided then to try just with one strain and we selected Neochloris oleoabundans because of the facility of culture and handling. Different concentrations of N. oleoabundans cultures were assayed with commercial plants like strawberry, cucumber, and tomato among others in comparison with inorganic fertilizers and other commercial algae extracts and different plant traits were measured. Different storage conditions were also approached to end with a liquid presentation of alive N. oleoabundans as the most promising product and the results of these assays as well as some data about putative phytohormones released from this presentation will be shown in this work.

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Effect of microbial inocula and soil composition on root & shoot growth & soil microbiology in corn (1000-097)
Hall 2

The Center for Root and Rhizobiome Innovation (CRRI) was established at the University of Nebraska with the mission of improving plant health and yield through a synergy with soil microbiology. The U.S. already produces the bulk of the world's corn, but yield gains solely through directed breeding and improved cultural practices are unlikely to meet the coming demand. The United Nations projects world population to surpass 9.8 billion by 2050 and 11.2 billion by 2100. It is with these challenges in mind that new approaches involving an increased focus on the soil are being pursued by the CRRI. In this study, we outline our efforts in Aim 2 of the project isolating and culturing chemotactic bacteria that are subsequently used as inocula for corn plants grown in either sterile Turface, sand, or potting soil. Plants were sampled at the VE, V3, and V5 stages of development. Root and shoot fresh and dry weights were collected as well as data on root branching and architecture at each time point. The root adherent substrate was also used for DNA isolation to assay the persistence of the inocula in culture through next generation sequencing. We present here our preliminary results from our ongoing study. This work is funded by grant OIA-155741: RII Track 1-Center for Root and Rhizome Innovation from the National Science Foundation (NE EPSCoR) and by a grant from the NU Foundation.

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Effective Agrobacterium-mediated transformation methods for gene function analyses in ice plant (Mes (1000-018)
Hall 2

Ice plant (Mesembryanthemum crystallinum L.) can grow in salinity and drought environments. It has been used as a model plant to study salt-tolerant mechanisms. However, the molecular studies are hampered by the lack of efficient transformation and regeneration procedures in ice plant. Three types of ice plant tissues, cultured cells, tissue-culture-grown and pot-grown seedlings, were used to develop efficient Agrobacterium-mediated transformation protocols. The 5-day-old cultured ice plant cells co-incubated with the Agrobacterium tumefaciens concentration of 2.5×109 cells mL-1 for 48 hours showed the highest transient transformation efficiency. Additionally, the intact or cut root tip seedlings were infected with the A. tumefaciens strain EHA105 harboring the pBISN1 binary plasmid with a GUS (β-glucuronidase) reporter gene. The 3-day-old cut root tip ice plant seedlings yielded the best transient transformation rate of 90% with GUS staining distributed in cotyledon and hypocotyl. The transient transformation assay results of ice plant cells and seedlings demonstrated that the concentrations of Agrobacteria, the durations of co-incubation time, and the growth stages of plant tissues were three important factors affecting the transformation efficiencies. Furthermore, the pot-grown ice plant seedlings were injected with the Agrobacterium rhizogenes strains containing the pCAMBIA1303 binary plasmid with a GUS reporter gene to establish transformed roots. Both GUS staining and immunoblot results demonstrated that lateral roots of hydroponically grown ice plant expressing GUS proteins seven weeks after infections, suggesting the ice plants were successfully transformed and generating transformed roots. The developed Agrobacterium–mediated transformation protocols will be helpful for exploration of the molecular mechanism of salt tolerance in ice plant.

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Elucidating the molecular influence of soil microbes on the maize root transcriptome (1000-053)
Hall 2

The association between soil microbes and plant roots is present in all natural and agricultural environments. Microbes can be beneficial, pathogenic, or neutral to the host plant development and adaptation to abiotic or biotic stresses. Recent progress in investigating the functions and changes in microbial communities in diverse environments at genomic levels has been made using high-resolution next-generation sequencing (NGS) technologies. The aim of this study was to determine

how maize root transcripts and small RNAs change in response to a controlled inoculation of known microbes over a defined time course. In this study, the maize inbred line B73 was inoculated with a cocktail of ten microbes. At each time point after inoculation DNA and RNA were isolated from roots. The V4 region of the 16S rRNA gene was amplified from the DNA and sequenced with the Illumina MiSeq platform. The sequencing results indicated that most of the microbes successfully colonized maize roots. The colonization was dynamic over time and varied with the specific microbe. We also performed small RNA sequencing and mRNA RNA-Seq on root samples at 11 time points after inoculation. The transcriptome and small RNA analyses revealed epigenetic and transcriptional changes in roots due to the microbial treatment. These results will be presented. The long-term goal of this research is to use these data to develop predictive models for plant responses to microbial, which will ultimately provide novel insights into how microbes alter root function through characterization of the changes that occur in the root transcriptome.

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Engineering of a new plant virus-based vector system for gene function studies in pepper (1000-070)
Hall 2

While pepper (Capsicum annuum L.) is a highly recalcitrant species for genetic transformation studies, plant virus-based vectors can provide alternative and powerful tools for transient regulation and functional analysis of genes of interest in pepper. In this study, we established an effective virus-based vector system applicable for gain- and loss-of-function studies in pepper using broad bean wilt virus 2 (BBWV2). BBWV2 was engineered as a dual-gene expression vector for simultaneous expression of two recombinant proteins in pepper cells. In addition, enhanced and stable expression of recombinant proteins from the BBWV2-based dual vector was established via co-expression of a heterologous viral suppressor of RNA silencing. We also developed BBWV2-based virus-induced gene silencing (VIGS) vector. Successful silencing of the phytoene desaturase (PDS) gene using the BBWV2-based VIGS vector was accomplished in various pepper cultivars. Additionally, we optimized the BBWV2-based VIGS system in pepper by testing the efficiency of PDS gene silencing under different conditions. This BBWV2-based vector system represented a convenient approach for rapid and simple analysis of gene functions in pepper.

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Expanding jasmonate complexity: a novel 9,13-duel specific allene oxide cyclase (AOC) connects death (1000-074)
Hall 2

Cyclopentanone oxylipins, termed jasmonates, orchestrate diverse roles in reproduction and stress protection. Jasmonates are derived from the dioxygenation of linolenic acid (18:3) precursors by lipoxygenases (LOX) acting with regiospecificity at carbon 13 (13-LOX) followed by the sequential action of 13-allene oxide synthases (13-AOS) and 13-allene oxide cyclases (13-AOC) to form 12-oxo-phytodienoic acid (12-OPDA). In maize a series of linoleic (18:2) and 18:3 derived 9-LOX cyclopente(a)nones, termed death acids, can co-occur as positional isomers of the jasmonate pathway and display broad transcriptional activities. To understand how death acids, such as 10-oxo-11-phytodienoic acid (10-OPDA) and 10-oxo-11-phytoenoic acid (10-OPEA), are enzymatically formed, we employed metabolite-based Genome-Wide Association Studies and linkage analyses in biparental populations to uncover a significant shared locus. The candidate gene was part of a large family of enzymes associated with stress protection. Agrobacterium-mediated heterologous co-expression assays in Nicotiana benthamiana confirmed that combinations of a 9-LOX (ZmLOX5), a 9,13 duel-specific AOS (ZmAOS1) and the 9-AOC candidate produced substrate specific production of both 10-OPEA and 10-OPDA. In maize ZmLOX8 plays a documented role in jasmonate biosynthesis yet functions as a 9,13 duel specific LOX. Surprisingly, combinations of 18:3 with ZmLOX8, ZmAOS1 and the novel 9-AOC resulted in a mixture of 9 and 13-LOX cyclopentenones namely 10-OPDA and 12-OPDA. Thus within even a single pathway series the parallel existence 9,13-duel specific LOX, AOS and AOC enzymes forces a reconciliation of complex biosynthetic interactions between jasmonates and death acids during oxidative stress and pathogen attack. Importantly we now define the first death acid pathway node and a path forward for the critical examination of an expanded family of jasmonate-like signals in crop stress protection.

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Exploring the repertoire of citrus immune perception (1000-017)
Hall 2

The Rutaceae family, comprised of 160 genera, includes ornamental species and economically important fruit trees such as citrus. Citrus production worldwide has been significantly affected by the bacterial disease, Huanglongbing (HLB). All commercial citrus varieties are disease susceptible to HLB. However, Australian wild citrus relatives, which are sexually compatible with cultivated citrus, display resistance to a variety of diseases, including HLB. Plants depend on surface localized receptors known as pattern recognition receptors (PRRs) to perceive

conserved microbe associated molecular patterns (MAMPs) as non-self. MAMPs represent a variety of materials including proteins, lipids, or other components essential for pathogen growth and survival. After MAMP perception, reactive oxygen species (ROS) are rapidly induced and activate defenses. ROS serves as an antimicrobial as well as a secondary immune signal to prevent pathogen spread. To assess pathogen perception in the Rutaceae family, including citrus and wild relatives, we utilized a microplate assay to quantify ROS production after MAMP treatment. A total of 90 genotypes including 31 genera were analyzed. The ability to perceive bacterial flagellin, cold shock protein and chitin was analyzed. Our results suggest that susceptible sweet orange varieties, Washington Navel and Valencia orange, were least responsive to tested MAMPs. In contrast, wild citrus relatives exhibited segregation in MAMP perception and response magnitude. Putative Rutaceae PRRs for tested MAMPs were identified based on homology to known immune receptors. Progress on the characterization of these PRRs will be reported. An understanding of MAMP perception in cultivated citrus can guide PRR transfer from model plants or citrus relatives for broad-spectrum disease resistance.

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Finding the weak spots: investigating how bacterial secreted proteins induce disease susceptibility (1000-025)
Hall 2

Plants interact with a wide range of microbes. This exposure requires complex metabolic circuitry to maintain plant health and productivity. Bacterial pathogens that invade the apoplast or vasculature manipulate plant metabolism to favor their growth and colonization by secreting enzymes that release nutrients and cause disease. This study characterized the roles of two highly conserved type II secreted proteins: LesA (lipase/esterase) and ArgG (argininosuccinate synthase). LesA is a cell wall degrading lipase/esterase associated with bacterial virulence, and ArgG is involved in arginine metabolism and potentially associated with nitric oxide (NO) production and oxidative stress response. Mutations were made in these two genes in the gamma-proteobacterial pathogens - Xylella fastidiosa (Xf) and Xanthomonas arboricola pv juglandis (Xaj) – to investigate their role in disease development in their respective plant hosts - grapevines and walnuts. The enzymatic activity of these two proteins modulates plant defense response and alters cell wall by impairing antioxidant reactions and modulating phenolic metabolism to enhance plant susceptibility and disease development. Proteomic analysis was used to highlight the network of proteins present in the plant sap that correlate with these specific responses in infected grapevines by using data-independent acquisition (DIA). In walnut shoots, the modulation of polyphenol oxidase expression (JrPPO1 and JrPPO2 genes) enhanced disease susceptibility triggered by Xaj secreted proteins visualized by melanin deposition in symptomatic tissues in vitro. These

results provide valuable insight into the role of the plant immune response to pathogen secreted proteins in the plant apoplastic and vascular space.

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Fungal infection modifies the attraction of Brassica napus for herbivores (1000-037)
Hall 2

Plants are in their natural environment exposed to numerous biotic attackers. Thus concurrent incidence of pathogens and pests on the same plant and even the tissue occurs quite often. The interaction plant – pathogen –pest represents a dynamic system dependent on timing of infection and infestation, which influences plant susceptibility or resistance. Our results demonstrate that a fungal pathogen Leptosphaeria maculans, which is an infectious agent of a serious blackleg disease of oilseed rape (Brassica napus), modifies attraction for the subsequent herbivore infestation in dependence on insect trophic type. A choice-test revealed that while the leaves infected by L. maculans become more attractive for the chewing insect diamondback moth (Plutella xylostella) the sucking insect, a cabbage aphid (Brevicoryne brassicae), strongly preferred leaves of uninfected plants. Mechanisms underlying plant – pathogen –pest interaction were investigated on base of transcription of defence genes, hormonal analysis and changes in a glucosinolate profile. The results indicate the antagonism between salicylic and jasmonic signalling pathways. Both herbivores gave a strong preference to true leaves over cotyledons.The work was supported from European Regional Development Fund-Project " Centre for Experimental Plant Biology " (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

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Global analysis of translational regulation and metabolic dynamics during plant ETI (1000-027)
Hall 2

Plants are sessile organisms that have evolved multi-layered defense mechanisms including transcriptional and translational reprogramming to counter pathogen infection. Transcriptional reprogramming in plant immunity has been extensively studied during the last decades, but the impact of translational regulatory mechanisms are largely unknown. To understand genome-wide global translational

regulatory mechanisms during the immune response, we performed global translatome analysis during effector-triggered immunity (ETI) to the bacterial pathogen Pseudomonas syringae pv. maculicola ES4326 carrying the effector AvrRpt2. We used ribosome footprinting, which is the deep sequencing of ribosome protected mRNA fragments. Interestingly, translational regulation during ETI is very different from our previous translational analysis on pattern triggered immunity (PTI), with induction of genes in metabolic pathways, especially aromatic amino acid metabolism. We further identified novel immune translational regulators that are specifically involved in several metabolic pathways. Together, our study provides novel molecular mechanism for global translational reprogramming during ETI, connecting metabolic dynamics and translational regulation in plants.

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1:30 PM - 3:00 PM

GmBIR1 is a negative regulator of defense responses in soybean (1000-084)

Hall 2

Receptor-like kinases (RLKs) recognize Pathogen-associated Molecular Patterns (PAMPs) and trigger defense responses. The roles of the RLKs in defense responses in model plant Arabidopsis have been well established. However, the functional importance of the RLKs in soybean remains largely uninvestigated. In a high throughput screening using virus-induced gene silencing (VIGS) mediated by Bean pod mottle virus (BPMV), a RLK that played a negative role in regulating defense responses in soybean was identified. Because the identified RLK shares the highest homology with Arabidopsis BAK1-interacting Receptor-like Kinase 1 (BIR1), we thus designated it as GmBIR1. The GmBIR1-silenced plants displayed a severe stunted stature. In addition, massive cell death was observed on the leaves of the GmBIR1-silenced plants, suggesting a constitutive activated defense responses. Consistent with its defense-related phenotype, both salicylic acid (SA) and hydrogen peroxide (H2O2) were over-accumulated in the GmBIR1-silenced plants. As expected, GmBIR1-silenced plants exhibited significantly enhanced resistance to both Pseudomonas syringae pv. glycinea (Psg) and Soybean mosaic virus (SMV), two different types of pathogens, compared with the vector control plants. Taken together, our results indicated that the GmBIR1 plays a negative role in regulating defense responses in soybean.

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Green Tea (Camellia sinensis) Extract as a Seed Treatment to Control Bacterial Fruit Blotch Disease (1000-043)
Hall 2

Bacterial fruit blotch (BFB) of cucurbits is a seedborne and seed transmitted disease caused by the phytopathogenic gram-negative bacteria, Acidovorax citrulli. There are no effective disease management strategies to prevent BFB other than to quarantine or destroy contaminated seeds and plants. Green tea, which is an accessible, affordable, and widely accepted natural compound has been reported to have antimicrobial effects on gram negative bacteria. In this study, we have evaluated the antagonistic effects of both green tea and black tea. Both green and black tea are from the plant Camellia sinensis but are processed differently. Our results indicated that green tea, unlike black tea, when added to in vitro growth medium inhibited growth of certain bacterial concentrations of A. citrulli. We have tested four bacterial concentrations of A. citrulli; 10^(8), 10^(7), 10^(6), 10^(5) cells/mL. Green and black tea were tested after standardization to OD390=1.0 by spectrophotometry and diluted to 20%, 10% and 5% and added to Luria broth medium with and without the selective antibiotics. Ten percent green tea increased the effectiveness of antibiotics against 10^(7) cells/mL A. citrulli growth on LB medium. Black tea showed no antagonistic effects. Green tea contains polyphenols called catechins that have antimicrobial activity that is lacking in black tea. We are investigating the roles of several green tea compounds such as catechin, epicatechin, epigallocatechin, and epicatechin gallate. A goal is to develop green tea extract as an organic seed treatment to control BFB disease.

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Primary Poster Presenter: Merve Kiremit

Harnessing nature: The tri-trophic interactions of endophytic Metarhizium in Maize (1000-038)
Hall 2

Recent studies have revealed that many insect-pathogenic fungi, including Metarhizium (Hypocreales: Clavicipitaceae), are also endophytes that can benefit their host plant through plant disease antagonism, plant growth promotion and insect growth suppression. Our research focuses on the molecular and physiological aspects of plant-Metarhizium-insect interactions. We isolated Metarhizium from agricultural soil and created single spore cultures. We inoculated surface sterilized seeds of maize (Zea mays L.) with the conidiospores of M. robertsii J.F. Bisch., Rehner & Humber 2009 to establish endophytic colonization of plants. We evaluated V4 maize for endophytic colonization of leaf and root, plant height, chlorophyll

content, above-ground biomass, relative growth rate of second instar black cutworm, Agrotis ipsilon (Huffnagel, 1766) (Lepidoptera: Noctuidae). We also studied the defense gene expression in maize in response to endophytic colonization.We recovered M. robertsii from 91.06 ± 4.05 % (n=116) of maize plants grown from treated seed and more frequently in root sections (49.66 ± 2.33 %) compared with leaf sections (33.33 ± 2.43 %) of endophytically colonized plants. Height (P = 0.03; F2,227 = 3.73) and above-ground biomass (P = 0.002; F2,211 = 6.37) of endophytically colonized plants was significantly greater in compared to control plants. Chlorophyll content did not differ (P = 0.35; F2,227 = 1.05) among treated and control plants. In insect feeding bioassays, the relative growth rate of 2nd instar black cutworm was lower (P = 0.01; F2,211 = 4.66) when fed on maize leaves from endophytic plants compared to control plants. Plant defense genes involved in jasmonic acid and salicylic acid pathways were upregulated.Endophytic colonization of maize plants by M. robertsii had growth promotive effects on maize plants, growth suppressive effects on black cutworm larvae, and altered the gene expression pattern of key defense genes in maize.

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Primary Poster Presenter: Imtiaz Ahmad

Hormone signaling, growth, and defense response - Arabidopsis ndr1-1 takes center stage (1000-073)
Hall 2

Hormone signaling in plants is intricately involved in the cellular communication that coordinates growth, development, and defense responses. Diversion of resources supporting plant growth and development can occur during stress responses through hormonal imbalances and cross-talk. We analyzed the growth and development and defense-related hormone signaling in Arabidopsis thaliana mutants in response to the soil borne pathogen Verticillium spp. We discovered the interplay between defense and gibberellic acid (GA) hormone signaling that regulates growth and flowering time. Infection by two Verticillium species enhanced the early flowering phenotype of the Col-0 (WT) in a GA-dependent manner. Preliminary results indicate that Verticillium infection led to significant reduction in the levels of jasmonic Acid (JA) in the Col-0 (WT), while the levels of bioactive GA4 were significantly increased. These changes in GA levels were accompanied by shifts in expression levels of genes involved in the GA signaling pathway. We provide evidence that the widely characterized defense mutant ndr1-1 in A. thaliana displays early flowering and accelerated growth and significantly elevated GA levels compared with those of the wild type Col-0, and activates a branch of defense controlling CC-NBS-LRR genes (RPM1, RPS2 and RPS5) in a salicylic acid (SA)-dependent manner. In further support of this, ndr1-1 mutants have enhanced susceptibility and accelerated flowering in response to infection by another soil borne fungal pathogen, Fusarium oxysporum. NDR1 involvement in pathogen-

mediated changes in flowering time and growth response represents an unexplored avenue for Salicylic Acid SA- GA cross-talk in plants upon activation of defense.

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Identification of Genes that are Differentially Expressed in Nicotiana glutinosa Defense Responses A (1000-051)
Hall 2

Plants defend themselves against infectious pathogens through inducible defenses operating at the molecular level through immune receptors that are activated by pathogen elicitors. Two inducible responses to pathogens are termed extreme resistance (ER), in which the pathogen is eliminated without cell death, and the hypersensitive response (HR), which limits the pathogen to the infected area by local programmed cell death. Specific Nicotiana glutinosa accessions display either ER or HR depending on the member of the Polerovirus genus infecting the plant. N. glutinosa accession TW59 exhibits HR when infected with turnip yellows virus (TuYV) as well as potato leaf roll virus (PLRV), while accession TW61 exhibits HR only when infected by PLRV and exhibits ER when infected by TuYV. This variation allows us to compare the mechanisms through which ER and HR are executed. To study these outcomes at the transcript level, leaves of TW61 were agro-infiltrated with TuYV and PLRV infectious clones alongside leaves agro-infiltrated with the empty pBIN61 vector as a control. RNA was extracted to analyze the gene expression changes in response to each virus through Next Generation RNA-sequencing. We hypothesized that there would be specific changes in gene expression associated with ER, versus resistance accompanied by HR, as well as an overlap in differential gene expression for these two responses. We identified 484 significantly differentially expressed genes from the TW61 transcriptome and selected 15 as candidates based on high relative fold-change, and different patterns of expression. The differential expression of these candidate genes was further quantified through reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Our analysis has identified genes with potential roles in both ER and HR, or with unique functions in one defense pathway. These findings could contribute to a better understanding of these agriculturally relevant plant defense responses.

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Primary Poster Presenter: Allyson Weir

Identification of Host Target Proteins of a Pathogen Effector in Lettuce by Mass Spectrometry (1000-028)
Hall 2

Downy mildews are oomycete-caused diseases on several important agricultural crops including grapes, crucifers, and lettuce. During infection, pathogens release proteins called "effectors" which help to defeat the plant immune system. RXLR3 is an effector protein secreted by Bremia lactucae, the downy mildew pathogen of lettuce, that is known to suppress host small RNA-mediated RNA interference. However, the underlying mechanism remains unknown. We hypothesized that RXLR3 captures siRNAs and sequesters them in peroxisomes to inhibit their further functioning. Since RXLR3 does not contain a peroxisome-targeting signal, we searched for possible host target proteins using immunoprecipitation (IP) and mass spectrometry (MS). Candidate target proteins were purified from protein extracts of lettuce overexpressing RXLR3. Several different antibodies and protein constructs were used during IP to control for nonspecific binding. The eluates were then sent for MS, whose results were using Significance Analysis of INTeractome (SAINT). We generated a list of candidate proteins with the highest SAINT scores and are following up with further screening and protein binding assay. A greater understanding of RXLR3's function will provide new insights into the importance of RNA interference in the plant immune system and might lead to novel treatment of downy mildew in lettuce and other plants.

Primary Poster Presenter: Chi Zhang

Identifying conserved elicitors of immunity in Candidatus Liberibacter sp. (1000-040)
Hall 2

Citrus Huanglongbing (HLB), caused by phloem-limited Candidatus Liberibacter bacteria, is a destructive disease threatening the worldwide citrus industry. The mechanisms of pathogenesis are poorly understood and no efficient strategy is available to control HLB. Here, we use a comparative genomics screen to identify elicitor candidates from Candidatus Liberibacter. The core genome of multiple Candidatus Liberibacter pathogens was extracted, and these core genes were analyzed for their selection patterns. We developed a high-throughput microtiter plate-based screening assay to efficiently screen candidate elicitor peptides in citrus species for their ability to induce reactive oxygen species (ROS) production, a common immune response in plants. We found that one candidate peptide could trigger production of ROS and induce defense gene (GST1 and WRKY22) expression in HLB tolerant citrus genotypes. Our findings will help to better understand the pathogenesis of HLB and eventually develop sustainable management strategies against this destructive pathogen.

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Identifying suppressor of sunn-1 hypernodulating phenotype using MutMap and bulk segregant analysis (1000-063)
Hall 2

Legumes, capable of forming nitrogen fixing nodules in symbiosis with rhizobia, control the number of nodules using a systemic signaling pathway referred as Autoregulation of Nodulation (AON). In Medicago truncatula, rhizobial infection triggers local signaling events resulting in MtCLE12 and MtCLE13 induction in the nodule meristem initiating AON. CLEs are translocated to shoot where they bind to a receptor complex containing the leucine-rich repeat receptor-like kinase SUNN, followed by subsequent signal transduction to roots resulting in termination of new nodule formation. Mutation of SUNN results in a 5-10 fold increase in nodule number. We undertook a forward genetic screen using EMS mutagenized seeds of sunn-1, harboring an amino acid change in the kinase domain, to identify suppressor of sunn-1 (sos) lines. The weak allele was used for the potential of revealing novel genes whose protein product directly or indirectly interacts with SUNN to affect its function, or to identify a pathway component, or complementary protein in parallel pathway that alleviates the need for active SUNN protein in the pathway. We present phenotypic analysis of one of the suppressor lines, sos16 - a root acting suppressor as verified by grafting experiments, and a mapping strategy for identifying the cause of suppression by using a combination of MutMap and bulk segregant analysis. SNP data obtained from whole genome sequencing of 11 pooled backcrossed sos16;sunn-1 lines was used to identify the region of suppression, determined to be linked to sunn-1. Further confirmation of linkage comes from genetic markers data from a mapping cross with the A20 ecotype and from segregation data of F2's in a cross with A17 wild type. We are testing SNP markers in bulk segregants to identify the cause of suppression. This work is supported by NSF IOS ##14444 & #1733470.

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Immune receptor function is regulated by multiple intramolecular and intermolecular interactions (1000-039)
Hall 2

Pseudomonas syringae causes disease in many plant species, using the type III secretion system to inject type III secreted effector (T3SE) proteins into plant cells. T3SEs primarily function to suppress plant immunity. Plants can evolve NOD-like receptor (NLR) proteins that directly or indirectly recognize T3SEs. HopZ1a is a member of the YopJ superfamily of T3SEs found in animal- and plant-infecting

bacteria, and is an acetyltransferase. HopZ1a acetylates the pseudokinase ZED1, which triggers recognition by the ZAR1 NLR. ZAR1 has emerged as a key signaling hub in plant immunity, as it is also required for the recognition of unrelated effectors from P. syringae (HopF2a) and Xanthomonas campestris (AvrAC), as well as a related effector from X. perforans (XopJ4). Regulation of NLR signaling is critical to prevent autoactivation of immunity in the absence of the pathogen. We previously established an Agrobacterium-based transient assay system in which we demonstrated that ZAR1-mediated recognition is conserved from the Brassicaceae to the Solanaceae. Here, we carried out structural modeling of ZAR1 along with molecular and functional assays, to demonstrate that ZAR1 function is regulated by multiple intramolecular and intermolecular interactions. This work identifies molecular determinants of ZAR1 immune receptor function and activation.

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Increased fruit quantity and quality of tomato (Solanum lycopersicum) in response to Azomite® volcan (1000-093)
Hall 2

In previous experiments, Berg and Koskella (Current Biology 28: 2487, 2018) have shown that phyllosphere (above ground) microbiota of tomato provide protection against plant pathogens in nutrient- and dose-dependent manner. We have investigated whether these phyllosphere microbes from field-grown tomato leaves improve nutrient assimilation and increase tomato yield in greenhouse-grown tomatoes, ultimately providing a benefit to the tomato industry. Foliar application of microbiota from field-grown tomatoes on to leaves of greenhouse-grown tomatoes alone resulted in increased tomato production and tomato weight. We have also extended the experiments to measure the effects both of phyllosphere microbiota and Azomite® volcanic ash fertilizer (AZOMITE soil products, LLC). Two formulations of Azomite® (Granulated and Ultrafine) were tested. The Granulated formulation applied at start of sowing and planting promotes vegetative growth, early flowering and fruiting (tomato quantity), whereas the Ultrafine product application once every week on top soil increases tomato weight and fruit pigmentation (tomato quality). Combination of these treatments with the natural tomato leaf phyllosphere microbiota produced the best results. We are currently carrying out 16S rRNA gene sequencing to determine microbiome dynamics between soil, rhizosphere, root and phyllosphere, and Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) analyses to quantify the flux of nutrient elements between soil and tomato plants. These results will be used to draw any functionally significant correlations between the phyllosphere microbiota (through whole genome sequencing), and plant assimilation of available nutrients from soils

treated with or without Azomite® volcanic ash fertilizer, potentially providing novel methodologies to increase yield in agricultural context.

Influence of geographical location and host genotype on pecan seedling microbiome composition (1000-100)
Hall 2

Carya illinoinensis (pecan) is native to North America, ranging from Illinois, U.S.A. to Oaxaca, Mexico. Commercial production of pecan has increased and it is now grown in diverse areas around the world. The host genetics and the plant microbiome may play a role in the ability of pecan to adapt to new environments. As such, it is fundamental to elucidate the influence of geographical location and genetics on the microbial populations of pecan. Recent technological innovations used to assess microbiomes have made it possible for us to gain insight into the microbial communities associated with pecan. In a previous study, we explored microbial diversity in two pecan genotypes that were micropropagated that had and had not undergone antibiotic treatments. Microbiome analysis revealed that antibiotic treatments led to a shift in microbiome composition and an overall decrease in microbial diversity. The control samples of each genotype revealed a unique microbial composition. To further investigate factors affecting the microbiome, we sought to determine the microbial composition of a controlled cross using a cultivar and a native genotype (Lakota × 87Mx3.211), from two different geographical locations, Georgia and Texas. Seeds were planted in a soilless potting mix and grown in a quarantine facility. Total DNA was extracted from ten seedlings, and sequenced using next generation sequencing technology on each sample to determine the bacterial and fungal compositions. Data was analyzed using the Qiagen CLC Microbial Genomics Module. These analyses allow determination of whether microbiome differences are due to genotype and/or geographical location. After subtracting chloroplast, mitochondria, and other abundant bacterial sequences, initial 16S results indicate that all samples have many of the same classes of bacteria, but with some differences that may be based on parentage and/or geographical origin.

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Primary Poster Presenter: Kimberly Cervantes

Inositol pyrophosphates and their role in P-homeostasis of arbuscular mycorrhizal fungi (1000-035)
Hall 2

Arbuscular mycorrhizal (AM) fungi form symbiotic interactions with the roots of approximately 80% of plant species in natural and agricultural systems. In this

association, the obligate biotrophic fungus is supplied with fixed carbon by the plant and in return the fungus improves the nutrient uptake of the plant roots, especially in soils with high clay content or with an alkaline pH. One of the main nutrients transferred to the host plant is inorganic phosphorus (Pi). Phosphorus (P), an essential element, often limits crop growth as only a minor fraction of soil P is plant available. Because worldwide P-deposits that can be exploited to produce P fertilizer are limited and because P is a strong pollutant in open water bodies, there is a high interest to increase crop P-efficiency among others by taking advantage of AM fungi. Only limited information on the molecular mechanism of the symbiosis-specific Pi-metabolism in mycorrhiza is available, due to the difficulty of genetic manipulation of AM fungi. Thus, alternative strategies have to be applied to get more insight into this process.Here we report on the identification of mycorrhizal enzymes that are involved in the biosynthesis of molecular messengers, the inositol pyrophosphates, which were recently shown to play important signaling roles in Pi-homeostasis in yeast and plants.

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Primary Poster Presenter: Verena Pries

Insights Behind the Molecular Mechanism of Cytokinin-Induced Priming
(1000-077)
Hall 2

Priming is the indirect enhancement of the immune response of plants to pathogens. Compared to unprimed plants, the immune response from primed plants, upon pathogen attack, is much stronger. Recent research in Arabidopsis thaliana has shown that the plant hormone cytokinin has a priming effect against biotrophic pathogens, a phenomenon we call cytokinin-induced priming. Our research demonstrates, that like other priming agents, priming with cytokinin induces a low level of defense gene expression but after a cytokinin-primed plant is challenged by a pathogen, defense gene expression is more robust. The molecular mechanisms behind priming remains largely unknown, although recent studies have indicated that chromatin modifications may play a role. Our research on chromatin mapping using Assay for Transposase-Accessible Chromatin using sequencing (ATAC-Seq) indicates that priming by cytokinin involves differential accessibility to various regions in the genome. Further, we show that priming by cytokinin does little to alter the plant metabolome initially. However, after the plant experiences pathogen challenge, the metabolic profile in a cytokinin-primed plant is significantly distinct from unprimed plants. We propose that cytokinin-induced chromatin regulation and alteration of the metabolomic profile provide insights into the general mechanisms of defense priming against biotic stress.

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Primary Poster Presenter: Kathryn McIntyre

Investigating a conserved low copy number immune gene family (1000-049)
Hall 2

Plant effector-triggered immunity is activated by intracellular nucleotide-binding, leucine rich repeat (NLR) proteins in response to the recognition of pathogen effector proteins. NLRs are made up of a central nucleotide-binding (NB) domain followed by a series of leucine-rich repeats, which serve as key initiators of plant defense responses. NLR proteins can include Toll/interleukin-1 receptor/resistance protein (TIR) domains, which can be either TIR-1 or TIR-2. We are currently interested in two clades of plant TIR-2 proteins referred to as TIR2-X and TIR2-NB, which are retained in both monocots and dicots in spite of their evolutionary divergence. When overexpressed in Nicotiana benthamiana, TIR2-NB genes have been shown to cause cell death, a distinctive characteristic of disease resistance proteins. Previously, all TIR-1 NLRs have been shown to require downstream signaling components eds1 and nrg1 to induce the hypersensitive response. We now look to test what genes are required to trigger cell death by the TIR2-X/NB genes and understand the immune pathway function through means of overexpressing the TIR-2 proteins in eds1 and nrg1 genotypes of N. benthamiana. We are also testing for the suppression of the hypersensitive response by co-expression of effectors with our TIR-2 genes. As we gather a better fundamental understanding of the conserved pathways leading to plant cell death, we will potentially be able to manipulate the TIR2-X/NB clade to improve future crop disease resistance.

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Primary Poster Presenter: Celine Bellegarda

Investigating the role of replication factor C-like genes in arbuscular mycorrhizal symbiosis (1000-050)
Hall 2

The arbuscular mycorrhizal (AM) symbiosis is an endosymbiotic association that occurs between fungi of the Glomeromycotina and the roots of vascular flowering plants. In exchange for carbon, the fungus provides the plant with mineral nutrients, mainly phosphorus. This relationship is ancient, and thus the ability to support the symbiosis is highly conserved in many plant families. A phylogenomic study resulted in a set of genes conserved exclusively in AM plant hosts, two of which were annotated as replication factor C-like genes (RFCa and RFCb) [1]. RFCa and RFCb are homologous to the subunit 3 of the well characterized replication factor C complex (RFC), the complex used to load Proliferating Cell Nuclear Antigen (PCNA) onto the DNA during replication and repair. However, RFCa and RFCb have additional domains of unknown origin, almost doubling their size compared to RFC3.Expression data shows RFCa and RFCb are induced in mycorrhizal roots. The

expression patterns were confirmed using promoter-GUS fusions, which showed GUS expression only in colonized root cells. An RFCa-GFP fusion protein localized to discrete regions near the periarbuscular membrane around the tips of the youngest branches of mature arbuscules. Additionally, in some cells, the fusion protein was visible in the nucleus. We were unable to localize the RFCb-GFP fusion because expression levels were too low. Mutant rfcb plants were inoculated with Glomus versiforme and colonization levels were assessed at 5 weeks post planting. rfcb showed a small but significant reduction in the amount of colonization as compared to wildtype plants [1]. A double rfca rfcb mutant was generated, and mycorrhiza phenotyping experiments are in progress. The replication factor C complex consists of 5 subunits. We are currently evaluating the ability of RFCa and RFCb to interact with other subunits of the replication factor C complex.[1] Bravo et al. (2016) Nature Plants, 2:1-6.

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Primary Poster Presenter: Cassandra Proctor

It´s a matter of time: circadian clock and defense responses crosstalk in Arabidopsis thaliana (1000-058)
Hall 2

In an ever-changing world, possessing the capability to adapt and even predict daily and seasonal environmental changes presents a valuable feature for living organisms. Subsequently, in most organisms multiple physiological and developmental processes are driven by an internal timekeeping mechanism known as the circadian clock, which accurately tuning contributes to an enhanced fitness. In plants, the endogenous biological clock regulates multiple processes, and in turn, several clock regulated signaling pathways feedback to control clock function. Given that plants do not have any specialized immune cells, each individual cell must regulate and balance the high energy consuming stress responses with other cellular functions, such as growth. The circadian clock has been shown to modulate plant immunity and the role of several clock genes in the control of biotic stress responses has been addressed, but whether plant-pathogen interactions modulate clock function is still unclear. In the current investigation, we found that an enhanced disease susceptibility (eds) mutant displayed alterations in circadian rhythms and clock associated responses. Also, by implementing a mapping by sequencing approach we were able to determine the identity of the mutation responsible for the eds phenotype, which had remained unknown for more than 20 years. Simultaneously, we found that an infection with Pseudomonas syringae strongly alters the expression of most core clock genes, as early as 1h post-infection in wild type (wt) plants and that this effect was attenuated in the eds mutant. Furthermore, we identified new clock mutants that turned out to be more susceptible to Pseudomonas syringae infection. Thus, these results comprehend a novel example of the relevance regarding correct circadian function in defense

responses and reinforce the idea of strong crosstalk between biotic stress stimulus and the Arabidopsis circadian clock.

Primary Poster Presenter: María José de Leone

Knockout of Os8N3 for resistance to rice bacterial blight using CRISPR/Cas9 system (1000-067)
Hall 2

Rice (Oryza sativa) is one of the most important cereal crops in the world. Molecular and conventional breeding approaches have been applied to develop disease resistant rice cultivar. Genome-editing, one of those approaches, is important for functional genomics research. Recently, the CRISPR/Cas9 system for gene knockout has emerged as the most effective genome-editing tool. It has previously been reported that, in rice plants, knockdown of the Os8N3 gene resulted in enhanced resistance to Xanthomonas oryzae pv. oryzae (Xoo), while displaying abnormal pollen development. Bacterial blight, caused by Xoo, is a prevalent and destructive rice disease that causes serious production loss worldwide. We employed the CRISPR/Cas9 system to knockout rice Os8N3, in order to confer enhanced resistance to Xoo. The OsU6a promoter from Kitaake, a Japonica rice cultivar, was amplified and replicated with Arabidopsis U6 promoter in the CRISPR/Cas9 vector, pHAtC. The resulting vector was used for rice CRISPR/Cas9-mediated Os8N3 mutagenesis. Analysis of the genotypes and edited Os8N3 in transgenic rice plants showed that the mutations were transmitted to subsequent generations, and homozygous mutants displayed highly robust resistance to Xoo. Stable transmission of CRISPR/Cas9-mediated Os8N3 editing without the T-DNA was confirmed by segregation in the T1 generation. With respect to all investigated agronomic traits including pollen development, there was no significant difference between homozygous mutants and non-transgenic control plants under greenhouse growth conditions. In summary, the T-DNA-free homozygous Os8N3 mutants generated using the CRISPR/Cas9 system displayed significantly enhanced resistance to Xoo and normal pollen development. This study provides a successful example of improving bacterial blast resistance using CRISPR/Cas9 technology and indicate that the CRISPR/Cas9-mediated Os8N3 edition can be employed for non-transgenic crop improvements.

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Lysin Motif Receptor-Like Kinase 4, participates in early signal perception and regulates the defens (1000-056)
Hall 2

Receptor-like kinases (RLKs) also known as Pattern-recognition receptors (PRRs) are transmembrane proteins, involved in detecting pathogen associated molecular

pattern (PAMP) which then culminate into PAMP-triggered immunity (PTI). RLKs are known to play significant role in various biotic and abiotic stress responses. In the present work, we tried to investigate the role of RLKs involved in resistance against the necrotrophic fungal pathogen Alternaria brassicicola and how the response varies between susceptible Brassica juncea and non-host resistant Sinapis alba. Comparative transcriptomics between the two species was carried out using Next Generation Sequencing (NGS) to understand the trends of differential gene expression during Alternaria infection. Expression data revealed 28 differentially expressed receptor-like kinase genes of which Lysin motif rich receptor-like kinase (LYK 4 and 5) genes were expressed at significantly higher level. Quantitative gene expression analysis revealed significant up-regulation of (LYK4) transcript in S. alba both during Alternaria infection and chitin treatment in contrast to the unchanged expression in B. juncea. Isolation of 1500 bp upstream element from S. alba (LYK4) followed by in silico analysis of the sequence revealed several putative cis elements including multiple W-boxes and fungal elicitor motifs which were also in agreement with the experimental observations. Furthermore, over-expression and antisense transgenic lines of BjLYK4 were generated, which showed distinct expression pattern of a number of defense related marker genes which are likely regulated by LYK4 during Alternaria infection. Subcellular localization of BjLYK4 using GFP fusion protein indicated that LYK4 is localized in the plasma membrane, suggesting it might have some role in the early signaling event. Together the results provide insight into the possible involvement of LYK4 in defense against the fungal pathogen Alternaria brassicicola.

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Primary Poster Presenter: AISHEE DE

microRNA156 controls age-dependent resistance to bacterial pathogen Pseudomonas Syringae (1000-062)
Hall 2

During development, plants cannot move to escape the bombardment by a battery of pathogens. Many plants evolve to enhance disease resistance against various pathogens during maturation. This is known as age-related resistance (ARR). ARR occurs during vegetative phase change, a developmental transition from juvenile to adult phase. Temporal reduction of microRNA156 (miR156) regulates the timing of morphological changes during vegetative phase change. Nevertheless, it is still puzzling that how defence strength is differentially determined at juvenile and adult stages. Here, we show that, a subclass of miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPLs), SPL2, SPL10 and SPL11, are responsible for promoting ARR against both oomycete and bacterial pathogens. First, an increase

of disease resistance to Pseudomonas Syringae pv. tomato DC3000 was associated with the reduction of miR156. Second, plants expressing miR156-resistant SPL2/10/11s displayed enhanced disease resistance. Third, SPL10 was likely to be required for full strength of salicylic acid (SA) response. Using inducible over-expression of SPL10, we demonstrated that the SPL-mediated resistance can be decoupled from its function in morphogenesis. We will discuss how decoupling the immune responses from developmental transition help resolve the growth-defense tradeoffs. Simultaneously improving disease resistance and developmental traits in planta would be exciting future goals.

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Primary Poster Presenter: Lanxi Hu

Molecular characterization of rhizobial volatile organic compound impact on root architecture (1000-066)
Hall 2

Plant-microbe associations may provide both benefit and harm to plants, yet much of how plants distinguish between beneficial associations and infections remains unknown. Some microbes are capable of influencing plant health without coming in direct contact with the plant by secreting volatile organic compounds (VOC). These compounds may affect plant health by modifying the composition of soil and making nutrients more readily available, interaction with other microbes in the soil, or by directly modifying plant growth by affecting plant hormonal signaling. While the ability of VOC to alter plant hormonal signaling suggests a pathway by which growth can be modified, specific mechanisms by which VOCs impact hormone regulation and response, or how these VOC are perceived are unknown. To uncover such mechanisms, ten VOC compounds were screened to characterize their impact on Arabidopsis thaliana root architecture. Treatment of natural accessions with one VOC produced by fungi, 1-octen-3-ol, uncovered natural variation of the root growth response to it. To map genes and their variants that underlie this response, 900 Arabidopsis natural accessions were screened for response to 1-octen-3-ol and root growth traits were quantified. Genome-wide association studies (GWAS) were carried out using primary root length upon 1-octen-3-ol treatment. We identified multiple significant associations and currently are molecularly characterizing candidate genes to determine their involvement in 1-octen-3-ol perception and response to environmental stimuli.

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Primary Poster Presenter: Nicole Gibbs

Molecular Interactions between Fusarium graminearum and Host Crops (1000-079)
Hall 2

The ascomycete fungus Fusarium graminearum not only infects wheat to cause Fusarium head blight and seedling blight, but also infects maize to cause Gibberella stalk rot. In addition, it can also grow in axenic media. To comprehensively understand how F. graminearum invades various hosts, various tissues and causes different diseases, we take an approach of cellular tracking and gene profiling of fungal infection process. We tracked F. graminearum growth inside host plants during disease development, and found fungal growth inside hosts has different paths: intercellularly and intracellularly. Using laser microdissection, we profiled in planta fungal gene expression during wheat seedling coleoptile infection and during maize stalk infection in a stage-specific manner, and started to elucidate its molecular strategies in confronting the host environments. In wheat infection, we identified that a nonribosomal peptide, as a product of secondary metabolite biosynthesis cluster of F. graminearum, facilitates cell-to-cell invasion in wheat, probably through manipulating plasmodesmal permeability and/or chloroplast activity. In maize stalk disease progression, we showed that F. graminearum remodels membrane lipids to overcome the phosphate limitation in the intercellular space of maize stalks.

Primary Poster Presenter: Weihua Tang

New insights to N-hydroxy-pipecolic acid induced defense priming across the plant kingdom (1000-078)
Hall 2

Signal propagation and coordination of whole-organism responses in plants rely heavily on small molecules. Systemic acquired resistance (SAR) is one such process in which long-distance signaling activates immune responses in uninfected tissue as a way to limit the spread of a primary, localized infection. Despite the importance of defense priming, the identity of the mobile defense signal that moves systemically throughout plants to initiate SAR has remained elusive. In this work, we report the discovery of N-hydroxy-pipecolic acid (NHP), a metabolite that plays a key role in initiating and amplifying SAR signaling in Arabidopsis. We show that Arabidopsis FMO1 (FLAVIN-DEPENDENT MONOOXYGENASE 1) synthesizes NHP from pipecolic acid and exogenously applied NHP moves systemically in Arabidopsis plants. We also provide evidence that NHP is conserved across the plant kingdom and demonstrate a role for NHP in mediating SAR responses in important crop plants. We used heterologous expression in Nicotiana benthamiana to identify a minimal set of genes required for NHP biosynthesis. Expression of these genes in tomato is sufficient to trigger SAR. Our results suggest chemical application or engineering strategies to induce NHP-mediated SAR are promising routes to improve broad-spectrum pathogen resistance in crops.

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P. syringae PtoDC3000 produces IAA and PAA via the Indole Acetaldehyde Dehydrogenase AldA (1000-076)
Hall 2

The bacterial pathogen Pseudomonas syringae uses several strategies to manipulate auxin physiology in Arabidopsis thaliana to promote pathogenesis, including synthesis of indole-3-acetic acid (IAA), a predominant form of auxin in plants, and production of virulence factors that alter auxin responses in the host. However, the role of pathogen-derived auxin in P. syringae pathogenesis is not well understood. Previously, we demonstrated that P. syringae strain PtoDC3000 produces IAA using a novel NAD-dependent indole-3-acetaldehyde (IAAld) dehydrogenase, AldA, that catalyzes the formation of IAA from IAAld (McClerklin et al, 2018 PLoS Pathogens). Further biochemical analysis has revealed that AldA can also utilize phenylacetaldehyde (PAAld) as a substrate to produce the natural auxin phenylacetic acid (PAA). Disruption of aldA leads to reduced IAA and PAA production in culture and reduced virulence on A. thaliana, and both IAA and PAA promote virulence of PtoDC3000 when applied exogenously. Thus, auxin can be added to the list of PtoDC3000 virulence factors. To investigate the regulation of auxin production by PtoDC3000 we are currently investigating the expression of the aldA gene during growth in culture and in planta. A working model for the roles of different forms of auxin during pathogenesis will be discussed.

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Primary Poster Presenter: Barbara Kunkel

PDBP1 is a novel regulator of plant elicitor peptide induced ethylene signaling and plant immunity (1000-082)
Hall 2

Plants have evolved specialized plasma membrane-localized receptor proteins to perceive microbial pathogens, thus enabling them to respond to the threats in a timely and specific manner. An early response upon pathogen attack is activation of endogenous plant elicitor peptides (PEPs) which bind to PEP Receptors (PEPRs), recruiting additional co-receptors (e.g. BAK1) required for full signaling capacity. Subsequently a signal transduction cascade leads to phosphorylation of target proteins to modulate their function. In a phosphoproteomics screen evaluating PEP-dependent phosphorylation patterns in maize and Arabidopsis, we identified a DNA-binding protein (PDBP1) with increased phosphorylation after ZmPep3 and AtPep1 treatment, respectively.We created overexpression lines abolishing or mimicking phosphorylation of PDBP1 in Arabidopsis to elucidate the molecular function of this

protein. Phospho mimetic variants show elevated responses to AtPep1-treatment, with stronger inducible gene expression (e.g. ERF-1, TAT3), enhanced ethylene emission and, elevated reactive-oxygen species generation, indicating a potential role for PDBP1 as a positive regulator of the PEP response. A yeast two-hybrid screen identified a major facilitator of the ethylene signaling pathway EIL1 (ethylene-insensitive3-like1) but not EIN3 (ethylene-insensitive3) as an interactor of PDBP1, supporting AtPep1-induced modulation of ethylene signaling through PDBP1. This specific interaction could be confirmed in planta via CoIP and BiFC. Currently, we are elucidating PDBP1-binding sites in Arabidopsis via ChIPseq analysis, where preliminary results indicate upstream regulators of the EIL1/EIN3 signaling pathway as putative targets of PDBP1 in Arabidopsis. Bioassays reveal a role for PDBP1 in fungal resistance against Botrytis cinerea. We hypothesize that phosphorylation of the DNA-binding protein PDPB1 stimulates plant immune responses by modifying the transcriptional output after pathogen attack.

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Phenotyping growth dynamics of sorghum lines inoculated with diazotrophs (1000-045)
Hall 2

Diazotrophs are the microbes that are capable of reducing gaseous nitrogen (N) into ammonia,making N available for plant uptake. Diazotrophs mitigate N stress in plants by interactingintimately with plants, yet the mechanisms for how diazotrophs relieve N stress may go beyondjust N fixation. This research aims to study the changes in plant traits and N accumulation inducedby a diverse set of diazotrophs over time and ultimately establish a stable relationship betweenplants and diazotrophic microbes under N stress. Sorghum bicolor is used in this study because itis widely grown in developing countries and its increasing importance in biofuel production. Todetermine if diazotrophs enhance sorghum growth under low N conditions, plants were inoculatedwith synthetic communities (SynComs) consisting of at least nine different diazotrophs associatedwith sorghum and grown in an automated high-throughput phenotyping facility. An RGB (RedGreen Blue) camera was used to monitor biomass production, whereas a hyperspectral camera wasused to assess nitrogen and chlorophyll content in plants once every two days. After two monthsof growth, SynCom inoculations did not improve plant growth significantly but the four sorghumgenotypes used in this experiment had distinct growth responses to the different SynComs thatdiffered depending on nitrogen treatment. Next-generation sequencing of 16S amplicons fromrhizosphere and root revealed that the microbiome composition was distinct among differentgenotypes, suggesting that these sorghum varieties have their own unique way of recruitingmicrobes. The results that will be presented from this study will allow for

a deeper understandingof the interaction between microbes and plants following inoculation by bacteria.

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Pipecolic acid confers systemic immunity by regulating free radicals (1000-024)
Hall 2

Systemic acquired resistance (SAR), initiated by a plant upon recognition of microbial effectors, involves generation of mobile signals at the primary infection site, which translocate to and activate defense responses in distal tissues. Among the signals contributing to SAR include nitric oxide (NO), reactive oxygen species (ROS), glycerol-3-phosphate (G3P), salicylic acid (SA) and etc. Our previous studies suggest that G3P is a downstream signal induced by NO/ROS, and NO/ROS-G3P signaling functions as a parallel branch as SA signaling during SAR. Pipecolic acid (Pip), a non-proteinaceous product of lysine catabolism, is an important regulator of immunity in plants and humans alike. In plants, Pip accumulates upon pathogen infection and has been associated with SAR. To better understand the role of Pip in SAR and the molecular mechanisms underlying Pip-mediated signaling, we investigated its relationship with two branches of SAR signaling. Our results show that Pip confers SAR by increasing levels of NO, ROS, and G3P but not SA which suggest that Pip-mediated SAR is dependent on the NO/ROS-G3P branch of the SAR pathway. However, plants defective in G3P or SA biosynthesis accumulate both reduced Pip in their distal uninfected tissues although they contain wild-type-like levels of Pip in their infected leaves. These data indicate that de novo synthesis of Pip in distal tissues is dependent on both SA and G3P and that distal levels of SA and G3P play an important role in SAR.

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Plant metabolism of nematode pheromones mediates plant-nematode interactions (1000-034)
Hall 2

Microorganisms and nematodes in the rhizosphere profoundly impact plant health, and small-molecule signaling is presumed to play a central role in plant rhizosphere interactions. However, the nature of the signals and underlying mechanisms are poorly understood. Here we show that the ascaroside ascr#18, a pheromone secreted by plant-parasitic nematodes, is employed by plants to generate chemical signals that repel nematodes and reduce infection. Comparative metabolomics of

plant tissues and excretions revealed that ascr#18 is converted into shorter side-chained ascarosides that confer repellency. An Arabidopsis mutant defective in two peroxisomal acyl-CoA oxidases does not metabolize ascr#18 and thus does not repel nematodes, indicating that plants, like nematodes, employ conserved peroxisomal β-oxidation to edit ascarosides and change their message. Our results reveal plant-editing of nematode pheromones as a defense mechanism that acts in parallel to conventional pattern-triggered immunity, demonstrating that plants may actively manipulate chemical signaling of associated microbiota.

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Plant secondary siRNAs contribute to host-induced gene silencing in oomycete pathogens (1000-071)
Hall 2

Constantly facing challenges from potential pathogens in the environment, plants have evolved a myriad of defense mechanisms. Increasing evidence has accumulated to suggest that a role of small RNAs (sRNAs) in plant immunity. We identified a specific sRNA pathway that is important for defense response in Arabidopsis against the fungi-like filamentous pathogen Phytophthora capsici. In particular, secondary small interfering RNAs (siRNAs) generated from a subset of transcripts from pentatricopeptide-repeat protein (PPR)-encoding genes function as antimicrobial agents and silence specific target genes in P. capsici during infection. PPR-derived secondary siRNAs are triggered by the parent microRNA miR161, which is induced during Phytophthora infection as a defense response. Mutants of both MIR161 and the secondary siRNA-generating PPR genes were hypersusceptible to P. capsici; however, this phenotype could be complemented by transcripts that do not encode functional PPR proteins. Furthermore, PPR-derived siRNAs are found to be cargos in extracellular vesicles (EVs) isolated from the apoplasts of plant leaves. This study highlights the secondary siRNA pathway as an integral component of plant defense through host-induced gene silencing in an invading eukaryotic pathogen. EVs could be involved in shuttling antimicrobial siRNAs from plants to pathogen cells.

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Pseudomonas spp. stimulate plant growth under multiple abiotic stresses (1000-013)
Hall 2

Abiotic stresses can negatively impact horticulture crop quality by causing stunted growth, reduced flowering, and early senescence. Plant growth promoting rhizobacteria (PGPR) can stimulate plant growth during different abiotic stresses by enhancing nutrient uptake and regulating plant hormone levels. In this study, a high-throughput bioassay identified 14 Pseudomonas strains that could grow in 30% polyethylene glycol, a trait correlated with the ability to confer drought tolerance in plants. Independent greenhouse trials were developed to evaluate the lab-selected strains for their ability to stimulate plant growth and flowering under drought and low-nutrient conditions. Three of the 14 strains increased flower number and plant biomass when applied to Petunia × hybrida under both abiotic stress conditions. These three strains were validated in a greenhouse production experiment to evaluate their effects on growth promotion under abiotic stress using three economically-important species: P. hybrida, Impatiens walleriana, and Viola × wittrockiana. Independent drought and low nutrient experiments were conducted on plants treated with each of the three bacteria strains. Negative control plants for both experiments were treated with uninoculated LB media. Each species was grown to flowering and flower number and shoot biomass were recorded as indicators of plant growth promotion. Application of each of the three Pseudomonas strains resulted in an increase in shoot biomass for all three species under both drought and low nutrient conditions compared to the negative controls. The bacterial treatments resulted in higher tissue macronutrient content compared to untreated plants when grown under nutrient stress. All three bacteria strains also increased flower number in P. hybrida after recovery from drought stress. This work provides a unique system to increase the quality of horticulture crops grown under different abiotic stresses in a greenhouse production system.

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Quantification and spatial localization of amine containing root exudates in maize (1000-030)
Hall 2

Root exudates are complex mixtures of primary metabolites, secondary metabolites, and organic acids. They are also the primary communication medium of plants with the surrounding rhizosphere. They act as chemical signals to both recruit helpful microbes as well as deter pathogens. It is known that exudate

expression varies at different regions of the root, with the primary source of exudation being directly behind the growing root tip. However, the exact nature of spatiotemporal variation in root exudation has yet to be determined. Using ninhydrin, a chemical indicator specific for amine containing compounds, we are developing a high throughput method to localize and quantify amine containing exudates. Plants are grown vertically on absorbent blotting paper, the bottom of which is submerged in a CaCl2 solution. Maize seedlings are allowed to grow up to two weeks in controlled growth chambers before capture of exudates. Exudates are absorbed onto a substrate using a simple blotting method. Once roots are blotted, substrates are placed in a dark cool environment to allow exudate to dry. At the time of blotting mock exudate standards are printed onto the substrate. These standards are of known concentrations of analyte and allow for creation of a standard curve to quantify exudate absorbed onto the substrate surface. Once the curing period has concluded, ninhydrin is printed onto the substrate. The blots are then left to develop over the course of 24 to 36 hours before being scanned on flatbed scanners. A quality control process has been designed to detect aberrations in both the printing and imaging processes. Additionally, an automated image processing platform has been created to build standard curves and quantify root exudation signals. This method is currently being applied to investigate spatial variation in exudate production in maize genotypes.

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Primary Poster Presenter: Michael Israel

RD29A and RD29B are Putative Drought Tolerance Genes Which do not Compromise Plant Growth (1000-015)
Hall 2

In recent decades, the scarcity of water referred to as ‘drought’ has become a global problem of food security and agricultural production. A variety of strategies are being developed to engineer tolerance and avoidance in plants. However, most confer growth retardation via e.g. stomata closure and canopy decrease, underscoring the need of exploring other avenues to protect plants against drought. We thus have isolated a strain of plant growth promoting rhizobacteria (PGPR), Panebacillus polymyxa, that is capable of priming drought tolerance, and

concurrently stimulating plant growth and development. The drought priming occurs in parallel to the upregulation of two abscisic acid (ABA)-responsive genes, RESPONSIVE TO DESICCATION 29A and 29B (RD29A and 29B), by P. polymyxa, of which expressions are oscillated diurnally under the control of a circadian rhythms. Interestingly, RD29A and 29B appeared as the 'memory gene' of drought and/or P. polymyxa treatments; their transcription levels are upregulated to a greater extent, when plants encountered drought and/or P. polymyxa for the second (vs. the first) time. Hence, T-DNA insertion KO mutant plants disrupting RD29A or 29B expression (rd29a and rd29b-1 and rd29b-2) exhibited enhanced susceptibilities and stomata responses towards drought stress, as well as overall growth reductions. These results together elucidate a discovery of unique, generic repertoires that potentially aid in upgrading plants' own survival capacity against drought conditions without trading off their yields and productions.

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Primary Poster Presenter: wenshan liu

REL2 Acetylation in Plant Pathogen Interactions (1000-059)
Hall 2

Plant pathogens are some of the most devastating crop stressors. Protein acetylation has emerged as a major post-translational modification that modulates many different cellular processes, including plant immunity and stress responses. Acetylation and deacetylation alter the state of defense gene promoters, promoting susceptibility or resistance. Cochlibolus carbonum, Northern Leaf Spot, produces an effector molecule called HC-Toxin that functions as a histone deacetylase inhibitor, which is required for pathogen virulence. ZmREL2, ramosa1 enhancer locus, encodes a transcriptional corepressor that is homologous to the TOPLESS (TPL) gene in Arabidopsis. Furthermore, expression of ZmREL2 in Arabidopsis rescues developmental defects in tpl mutants. The TPL family acts as corepressors in many different pathways including auxin (TPL-IAA-ARF) and jasmonate (TPL-JAZ-MYC2) signaling. We identified a site of lysine acetylation on REL2 using global acetylome profiling of corn plants treated with HC-Toxin or infected with C. carbonum. We have found that rel2 mutant corn plants are susceptible to C. carbonum infection, unlike their B73 counterparts, which demonstrates that this gene is directly related to plant immunity. In addition, using Yeast Two Hybrid assays, we have shown that mutations of REL2 that mimic acetylation result in reduced interaction of REL2 with transcription factors containing DLN and RLFGV repression motifs. Finally, we confirmed using luciferase corepression assays that REL2 acts as a corepressor. Furthermore, REL2 acetylation null mutations abolish the repression activity of REL2. Ultimately the goal of this work is to elucidate how hyperacetylation impacts the biological activity of REL2 and its roles in plant pathogen interactions.

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Response of potato (Solanum tuberosum) microbiome to seasonal development and fertilizer treatment o (1000-092)
Hall 2

In previous experiments, Berg and Koskella (Current Biology 28: 2487, 2018) have shown that phyllosphere (above ground) microbiota of tomato provide protection against plant pathogens in nutrient- and dose-dependent manner. We have investigated whether the microbes from field-grown potatoes changes during the growing season and whether there is an effect of fertilizer regime on the abundance and diversity of the microbiome in the soil, rhizosphere, endosphere and phyllosphere, and on the yield of potato tubers. Changes in microbiome associated with improved potato yield is expectant as a result of improved nutrient assimilation and abiotic and biotic stress resistance. We have extended the experiments to compare the effects both of standard Nitrogen fertilization and Azomite® volcanic ash fertilizer (AZOMITE soil products, LLC). We have conducted 16S rRNA gene sequencing to determine microbiome dynamics between soil, rhizosphere, root endosphere and phyllosphere. These results will be used to analyze any functionally significant correlations between the different microbiomes during the growing season, and plant assimilation of available nutrients from soils treated with or without Azomite® volcanic ash fertilizer, potentially providing novel methodologies to increase yield in agricultural context.

Primary Poster Presenter: Kent F. McCue

Rice arbuscular mycorrhizal symbiosis establishment depends on the removal of the suppressor SMAX1 (1000-080)
Hall 2

Under nutrient deficient condition, more than 80% terrestrial plants are engaged with arbuscular mycorrhizal (AM) fungi to obtain minerals such as phosphate and nitrogen. AM symbiosis begins with a bidirectional chemical dialogue where both symbiotic partners perceive diffusible signaling molecules to recognize each other prior to physical contact. Sensing of AM fungi by rice requires the alpha-beta fold hydrolase Dwarf14-like (D14L) and the F-box protein Dwarf3 (D3). The same receptor complex has previously been described for the perception of the smoke derived-compound karrikin, triggering seed germination and seedling growth post wildfire. I investigated the symbiosis signaling pathway downstream of D14L/D3 and identified Suppressor of MAX2 -1 (SMAX1) as an essential component. Mutation of SMAX1 led to a higher level of colonization than wild-type functions suggesting SMAX1 functions as a negative regulator of AM symbiosis. Indeed, absence of fungal colonization in the rice receptor mutants d14 or d3 was suppressed in d14l/smax1 and d3/smax1 double mutants. SMAX1 operates therefore epistatically downstream of the two known signaling components. The subcellular localization of

SMAX1 in the nucleus, suggested that SMAX1 suppresses transcription of AM symbiosis genes. RNAseq analysis of non-colonized smax1 mutant indeed uncovered a suite of AM symbiosis-induced genes. In summary, we found that successful AM colonization depends on the removal of the SMAX1 suppressor for transcriptional reprogramming rice roots for symbiosis.

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Primary Poster Presenter: Jeongmin Choi

Role of Bacterial Volatiles in Plant Defense Signaling and Disease Resistance (1000-086)
Hall 2

Volatile Organic Compounds (VOCs) isolated from plant growth promoting rhizobacteria (PGPR) have been shown to confer resistance in plants to a variety of pathogens, leading to reduced disease incidence and severity. However, the molecular mechanisms of VOC-mediated plant defense responses are rudimentary. We hypothesized that the bacterial VOCs act as elicitors of defense signaling by triggering the expression of an array of defense-related genes in plants that play a role in either salicylic acid (SA)- or jasmonic acid (JA)-mediated defense signaling pathways. The role of VOCs as elicitors of defense signaling was investigated through gene expression studies (reverse transcriptase-PCR and promoter-GUS assays). We also performed pathogenicity assays to evaluate the role of bacterial VOCs in conferring resistance to soybean white mold caused by Sclerotinia sclerotiarum. To analyze VOC-mediated defense signaling, we used Arabidopsis thaliana, Medicago truncatula (dicots) and rice (a monocot) as model systems. Ten different bacterial VOCs were tested for their role as elicitors of defense signaling. The expression profile of genes that are known to be involved in the SA- or JA-mediated defense signaling pathway were monitored in each model plant system. In addition, we used A. thaliana seedlings stably transformed with the promoter of defense-related genes fused to the coding sequence of beta-glucoronidase (GUS) to monitor the expression of VOC-induced defense genes using colorimetric assays. Using gene expression studies, plant morphometric analyses and pathogenicity assays, we have identified a set of promising VOCs that can be used as elicitors of plant defense. Using nanoparticles of poly-lactic-co-glycolic acid (PLGA) encapsulated with 2-octanone, we explored the scope of using biopolymers for the controlled delivery of VOCs to induce plant defense against pathogens.

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Role of PHENYLALANINE AMMONIA LYASE (PAL) in antiviral defenses in Brachypodium distachyon (1000-046)
Hall 2

Our understanding of the genetic basis of antiviral defenses in monocots has been lagging compared to dicots, due to the lack of a suitable monocot model. Brachypodium distachyon (Brachypodium) has recently emerged as a premier model plant for monocot biology. We previously reported genome-wide transcriptomic and alternative splicing changes occurring in Brachypodium during compatible infection with Panicum mosaic virus (PMV), an important pathogen of bioenergy and food related grasses. Here, we studied the extent of metabolic changes altered in Brachypodium during PMV infection and dissected the role of PHENYLALANINE AMMONIA LYASE (BdPAL), a key enzyme in phenylpropanoid and salicylic-acid biosynthesis, in antiviral defenses. Metabolic profiling of PMV-infected Brachypodium, using LC-MS/MS tools, revealed enhanced levels of cinnamic acid (CA) and salicylic acid (SA). In contrast, levels of jasmonic acid (JA) and fatty-acid precursors (linoleic acid,18:2 and α-linolenic acid, 18:3) were lowered in PMV-infected Brachypodium. The altered profiles correlated with changes in transcript abundance of putative genes in the respective metabolic and hormone biosynthetic, as well as signaling pathways. Knockdown of BdPAL using RNAi resulted in enhanced susceptibility of plants to PMV, when compared to wild-type (WT) plants. Although basal SA levels are unaltered in mock-infected plants, SA accumulation was significantly lower in BdPAL-RNAi compared to WT. Furthermore, exogenous application of SA alleviated the disease severity of PMV-infected BdPAL mutants. Together, these results suggest that BdPAL contributes to SA accumulation and antiviral defenses in compatible grass-virus interactions.

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Root Cell Type Signature of Sugar Beet Cyst Nematode Induced Syncytia in Arabidopsis (1000-060)
Hall 2

One of the most fascinating adaptations of plant-parasitic cyst nematodes is their ability to induce the formation of a feeding site called a syncytium. During infection, the nematode selects a single cell within the host vascular cylinder as the initial syncytial cell (ISC), and secretes a suite of effectors to re-direct its development. Under the influence of these effectors, the host cell undergoes dramatic changes, including an increase in the number of organelles, dissolution of the central

vacuole, enhanced metabolic activity, and incorporation of hundreds of adjacent cells by partial cell wall breakdown and cell fusion. In Arabidopsis roots infected by the sugar beet cyst nematode (Heterodera schachtii), the syncytium originates from procambial/cambial and pericycle cells, both of which serve as lateral meristems that give rise to conductive tissues (xylem and phloem) and lateral root primordia, respectively. To successfully establish and maintain the syncytium, H. schachtii has to co-opt developmental programs of selected host cells and re-route them into a cell type that is normally not seen in the host. To explore the cellular identity of the syncytium, we profiled the early transcriptome of H. schachtii-induced syncytia in Arabidopsis by coupling Laser Capture Microdissection (LCM) and RNA-seq, and then examined the enrichment of genes associated with different cell types within the Arabidopsis root system among syncytium up-regulated genes. Both hypergeometric and Fisher's exact tests showed that several groups of cell-type specific genes were significantly enriched in the syncytium. These data provide new insight into how cyst nematodes commandeer root developmental programs for parasitic success.

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Root-Associating Bacteria Cloak Maize Roots from Detection by the Specialist Herbivore, the Western (1000-036)
Hall 2

Insect herbivores often rely on plant-borne chemicals to identify host plants for feeding. Western corn rootworm (WCR) larvae use the DIMBOA-Fe(III) complex as a soil foraging cue to identify maize roots. DIMBOA, a benzoxazinoid secondary metabolite, is produced and excreted from maize roots for generalist herbivore protection. It also acts as a plant siderophore, chelating with Fe(III) in the soil to facilitate uptake of mineral nutrients. Here, we show Azospirillum brasilense, a prolific grass root colonizing bacteria, alters host physiological and metabolic status resulting in increased iron assimilation. Due to greater assimilation of iron, less is available to form the WCR foraging cue, DIMBOA-Fe(III) complex. In greenhouse studies, WCR susceptible maize lines inoculated with these bacteria exhibited significantly lower body masses, fewer recovered larvae, and significantly less root damage suggesting the larvae are not locating the roots under bacterial treatment. Using nuclear-based technologies, radioactive 59Fe (t1/2 = 44.5 d) administered to roots enabled measurement of assimilation and whole-plant iron transport. Bacteria inoculated maize exerted significantly different host iron assimilation responses relative to non-inoculated controls. Radioactive 11CO2 (t1/2 = 20.4 m) was also administered to leaves to examine changes in host physiology and metabolism in response to inoculation. Again, inoculated maize exerted significantly different host responses in their carbon utilization relative to non-inoculated controls. These results provide a mechanistic understanding for how A. brasilense can down-regulate soil DIMBOA-Fe(III), thus impacting WCR feeding behavior. This research

is supported by Agriculture and Food Research Initiative Award No. 2017-67013-26216 from the USDA National Institute of Food and Agriculture.

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Primary Poster Presenter: Alexandra Bauer

StbHLH94: A MYC transcription factor associated with differential susceptibility to PVY in potato (1000-057)
Hall 2

Potato (Solanum tuberosum) is the fourth most cultivated crop after rice, wheat, and corn. Potato Virus Y (PVY) is a significant burden on potato production that can reduce yields or render the crop unmarketable. Cultivars that resist specific strains of PVY are often used in potato production. These cultivars recognize viral components and initiate a hypersensitive response (HR) manifesting as necrotic lesions at the point of infection. Recombinant strains of PVY such as PVYN-Wilga and PVYNTN have become increasingly prevalent in US production areas in recent years and these strains can circumvent many of the strain-specific resistances used in potato production. Systemic infections can even develop when HR is elicited. There is a need to better understand the strain-specific interaction between potato and PVY. The MYC family of transcription factors (TFs) are beta helix-loop-helix (bHLH) TFs that are involved in many biological processes including defense responses. Previous transcriptomic work identified a MYC gene, StbHLH94 (PGSC0003DMG400012237), that was significantly differentially expressed early in the interaction between the variety Premier Russet (PR) and PVYO, a strain that PR resists and displays HR to. We hypothesize that StbHLH94 plays an important role in the establishment of the strain-specific resistance response. To better understand its function, transgenic PR plants overexpressing (OE) StbHLH94 under the control of the CaMV35S promoter and non-modified control PR were grown in a greenhouse and mechanically inoculated with PVY strains O, N-Wilga, NTN or a mock inoculation. HR symptom development was monitored weekly and systemic PVY infection was tested by RT-PCR. The OE lines displayed lower rates of systemic

infection with strains of PVY for which PR displays HR (PVYO and PVYN-Wilga) when compared to non-transgenic control plants, but not against NTN. These findings suggest that StbHLH94 plays a role in HR to restrict systemic infection of PVY.

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Primary Poster Presenter: Max Combest

Study on the Pathogenesis of Fusarium fujikuroi Causing Rice Bakanae Disease (1000-044)
Hall 2

Rice bakanae disease is caused by Fusarium fujikuroi. This seed-borne disease widely occurs in rice-growing countries and can cause yield losses ranging from 30-95%. The infected seedlings are thinner, higher or paler than healthy ones. The infected adult plants show adventitious roots on the node or empty panicles. Due to the lack of knowledge on the rice-F. fujikuroi pathosystem, this study aims to uncover the pathogenesis of F. fujikuroi during different infection stages. Artificially inoculated rice seedlings and naturally infected adult plants were investigated by hand sectioning as well as real-time quantitative PCR. A fluorescent gene-transformed F. fujikuroi generated by protoplast-PEG transformation method was used for artificial inoculation. As detected by fluorescence microscopy of consecutive 3-cm segments of the whole plants, we observed intra- and intercellular hyphae expanding from the embryo to the basal part of rice stem and root during early infection. In the infected plants at the tilling or booting stage, the hyphae were found heavily colonizing the xylem vessels, suggesting that F. fujikuroi may spread through the vascular bundles. At the upper stem node, the emergence of adventitious roots may be earlier than the invasion of F. fujikuroi. The qPCR analysis of artificially inoculated seedlings revealed that the biomass of F. fujikuroi in the root were usually higher than that in the stem. During 3 to 10 days after inoculation, the amounts of F. fujikuroi in resistant and susceptible cultivars did not differ significantly. This implies that macroscopic bakanae phenotypes were not necessarily correlated with the pathogen quantity. We hope this study can offer new insights into the interactions between rice and F. fujikuroi.

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Primary Poster Presenter: Chieh-Yi Chen

The early transcriptomes of M. truncatula wild type and hypernodulation mutant plants inoculated wit (1000-055)
Hall 2

The molecular signaling that underlies the ability of legumes to host rhizobia and benefit from nitrogen fixation by the bacteria is a topic of intense study. The converse process, the need of the legume plant to regulate the number of rhizobia that colonize the roots or to eliminate colonization altogether when adequate nitrate is available in the soil (AON) also requires complex signaling. Split root experiments have determined that AON in Medicago truncatula occurs by 48-72 hours after inoculation with rhizobia; in effect both development of rhizobial colonization and limitation of further colonization are occurring at the same time in the same cells. In order to understand the nature of this signaling, we performed RNASeq on libraries derived from the zone of developing nodules and compared the results with libraries made from comparable tissue derived from uninoculated roots at 0, 12, 24, 48, and 72 hours post inoculation. We also sequenced libraries made from sunn and rdn1 hypernodulation mutants as well as shoots of plants following the same time course. Examination of differentially expressed genes at various time points and identification of modules of genes that are co-regulated across time points in both the wild type and the mutants has allowed us to begin to unravel the complex molecular signaling behind AON. In addition to nodulation genes already known from the literature, we have identified several modules of genes related to pathogenesis, lipid biosynthesis and hormone response that are co-regulated during early nodule development. Comparison of wild type and hypernodulation mutants reveals known nodulation genes that are differentially regulated in the mutants versus wild type undergoing nodulation, suggesting underlying mechanisms for the phenotypes observed. This work is supported by NSF IOS#1444461 to Frugoli and Feltus.

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The Effect of Lactic Acid Bacteria (LAB) on Terpene content of Cannabis sativa 'Wife' female flowers (1000-023)
Hall 2

Cannabis sativa is of growing interest to industry and consumers. In whole flower products, consumers are interested not only in cannabinoid content, but also terpene profile and content. Terpenes are a large class of volatile molecules found in many plants and are found in high concentrations in the unfertilized female flowers of C. sativa, giving the plants their characteristic aroma. A means to increase the content of these terpenes is desirable among Cannabis producers. A common, however, under-studied, technique practiced by growers is the application

of natural farming (NF) inputs to increase terpene content. In this study, one such NF input, lactic acid bacteria (LAB), were cultured from rice and milk. The preparation of lactic acid bacteria was applied at a rate of 1:1000 in deionized water. We hypothesized that Cannabis plants treated with LAB would have a higher floral terpene content than control plants' flowers. Three treatments were made: a live LAB treatment, an autoclaved LAB treatment, and a water control. Treatments were applied as weekly soil drenches to Cannabis plants grown in a greenhouse under artificial lights. C. sativa variety 'Wife' clones were grown vegetatively for 7 weeks under 18 h light. The photoperiod was changed to 12 h to induce flowering for 8 weeks, and treatments were made once per week to the plants. Apical female flowers were harvested from each plant, and were cured for two weeks. Terpenes were extracted using 100% ethanol. The presence and amounts of nineteen terpenes were assessed using gas chromatography-mass spectrometry. We found a consistent trend where plants that had received a weekly application of autoclaved LAB solution had the highest content of each terpene, followed by the control, and then the plants' flowers that received the live LAB application. Further research will analyze the cannabinoid content variation among these samples.

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The endophytic fungus Piriformospora indica increase rice plant resistance against insect herbivores (1000-089)
Hall 2

Piriformospora indica (P. indica) is a beneficial root endophytic fungus which can increase plant biomass and crop production, and it has also been observed to enhance plant tolerance toward to abiotic stresses such as high salinity environments. In this study, it was focused to reveal the effect and mechanisms of P. indica-enhanced rice (Oryza sativa) resistance against insect herbivores [rice leaffolder, Cnaphalocrocis medinalis (Guenée)]. The result of insect-feeding assay showed the level of leaf damage conducted by rice leaffolder larvae was significantly reduced in the P. indica-inoculated rice plants. The growth rate of larvae fed with P. indica-colonized rice seedlings was decreased compared to that were grown on leaves of non-colonized plants. Furthermore, the data showed the rice leaffolder chewing-stimulated trypsin inhibitor activity and jasmonic acid (JA) were obviously increased in P. indica-colonized plants. The P. indica-enhanced trypsin inhibitor activity would be repressed in JA inhibitor-treated plants. It was indicated P. indica enhanced trypsin inhibitor activity were mediated JA signaling. Moreover, P. indica also function to reduce oxidation stresses and regulate flavonoids biosynthesis in larvae-infested rice plants. In conclusion, P. indica can increase rice resistance to against insect infestation mediated enhancing antioxidant activity and promoting trypsin inhibitor activity through jasmonic acid signaling pathway.

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The Role of Agricultural Soil Management Practices in Shaping the Microbiome of Sorghum bicolor (1000-021)
Hall 2

The wealth of nations is closely tied to agricultural productivity, and hence, the soil underfoot. Soils are a living and finite resource that play integral roles in sustaining biological productivity, impeding crop disease and maintaining air and water quality. While recent research has demonstrated the major role that microbes play in shaping soil health, how agricultural soil management regimes shape the microbiomes of the crop host remains largely unexplored. Using a field site that has been managed under conservation till (CT), standard till (ST), and cover-cropping (CC) practices for two decades in California's Central Valley, we employed 16S rDNA and ITS2 amplicon sequencing and meta-transcriptomics to determine how soil management impacts microbial community composition and function in the sorghum root microbiome. Preliminary results show that tillage type and CC both impact microbial composition, with significant shifts in the relative abundance of bacterial and fungal lineages across treatments. We observed that fungal communities appear more dissimilar between CT and ST treatments than bacterial ones, and that changes in alpha diversity across treatments are also greatest for fungi the rhizosphere and soil. Across treatments, we further identified significantly enriched Clusters of Orthologous Groups (COG) categories that highlight the role of lipid and carbohydrate transport and metabolism. Determining the impact of agronomic practices on the soil environment, and subsequently on crop microbiomes, will not only inform efforts to configure trait-associated microbiomes through traditional agricultural techniques, but also allow for the development of an effective beneficial microbiome component to crop production systems.

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The root of it all: Factors influencing plant-associated microorganism communities in Vitis (1000-098)
Hall 2

Plants have multiple organs (e.g. roots, leaves, fruits, etc.) with each acting as a potentially unique habitat for microorganisms due to variation in structural characteristics, environmental conditions, and resource availability. To understand how one organ can influence the microbiome of another, we used grafting, the horticultural technique of joining different plant organs together to form a vascular connection. Grapevines (Vitis spp.) are commonly grown by grafting the shoot

system of a particular cultivar to a different root system (rootstock). We examined 'Chambourcin' (a French-American hybrid grapevine) growing ungrafted and grafted to three different rootstocks ('3309C', '1103P', 'SO4') across three irrigation treatments in Mount Vernon, MO. We sampled soil, roots, leaves and berries from each vine at harvest and used 16S and ITS barcoding to assess the bacterial and fungal diversity. We found significant variation in alpha diversity (Shannon's index) across tissues, with each tissue also having distinct bacterial and fungal taxa. Soil, followed by roots, were consistently the most diverse. For bacterial taxa, leaves and berries had similar levels of diversity, whereas for fungal taxa, berries were more diverse than leaves. Across rootstocks, there was no significant difference in beta diversity metrics (UniFrac and Bray-Curtis), however, individual taxa did differ significantly. Our results indicate that rootstock choice has a subtle but significant impact on microorganism communities. Future work will be required to determine if taxa which are differentially abundant across rootstocks have a functional impact on the plant and lead to variation in agriculturally important traits such as yield.

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The transcriptional landscape of plant pattern-triggered immunity (1000-054)
Hall 2

Plant immunity is initiated upon perception of molecular signatures of microbes or damage by cell-surface pattern recognition receptors, which instigate signaling cascades resulting in a large-scale transcriptional reprogramming. A key question in this process is whether plant cells induce different immune responses depending on the origin or nature of the elicitors that are perceived by distinct receptors. To address this point, we assayed transcriptional responses of Arabidopsis thaliana to an array of seven elicitors comprising a variety of source and receptor categories, across a six point time course focusing on rapid responses. We find an extremely strong correlation among transcription patterns induced by all elicitors, suggesting that plant cells mostly perceive danger. Accordingly, this core response is shared among published responses to abiotic stresses, highlighting the importance of a plant general stress response (GSR). Genetic manipulation of the GSR affects elicitor-induced resistance against bacteria, and gene regulatory network analysis suggests other key regulators which may play a role in this response. Despite the preponderance of the GSR, a subset of genes represents a core immunity response, induced by all elicitors but no tested abiotic stresses. Ongoing work is investigating the role and regulation of these genes during immunity. Through combining a panel of elicitors with an early-focused time series, we have defined the transcriptional landscape of surface immunity, and have identified potential novel regulators and executors of plant immunity.

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Understanding the Role of DAR1, a Putative O-Fucosyl Transferase, in Plant Systemic Immunity and Flo (1000-032)
Hall 2

Abietane diterpenoids accumulate in conifers (gymnosperms) as a component of oleoresin, which is valued as a starting material for pharmacologically active compounds. Although recent studies show that abietane diterpenoids are produced in angiosperms, their biological role in flowering plants was not known. We identified the abietane diterpenoid dehydroabietinal (DA) as a potent activator of systemic acquired resistance, which confers enhanced resistance against a variety of pathogens. DA also promotes transition from the vegetative to reproductive phase of growth (producing flowers) in Arabidopsis by upregulating expression of the flowering time promoter FLOWERING LOCUS D (FLD) and by simultaneously repressing expression of the flowering repressor FLOWERING LOCUS C (FLC). Earlier studies have shown an interconnection between SAR activation and flowering time in Arabidopsis. To further understand the mechanism underlying signaling by DA, we have identified mutants that are resistant to DA. One of these genes, DEHYDROABIETINAL RESISTANT 1 (DAR1) encodes a protein that is homologous to human protein o-fucosyltransferase 2. dar1-1 mutant plants are compromised in SAR induction and exhibit delayed flowering. We hypothesize that DAR1 is involved in the post-translational modification (o-fucosylation) of a protein that is essential for SAR and flowering time. We further suggest that o-fucosylation likely promotes channeling of the DAR1 targeted protein through the ER/golgi network.

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Unraveling metabolic interactions among 'Candidatus Liberibacter asiaticus' and Citrus sinensis (1000-029)
Hall 2

Huanglongbing (HLB) is a worldwide citrus disease associated with the putative bacterial pathogen Candidatus Liberibacter asiaticus (CLas), and vectored by the Asian citrus psyllid (ACP) (Diaphorina citri). This lethal plague affects citrus crops, reducing crop productivity by up to 80%. Although this pathogen has eluded cultivation, recent omic tools have made possible the genome sequencing of multiple CLas strains collected from in vivo environments. Comparative analyses of these genome sequences have provided preliminary insights into the metabolic capabilities of CLas. However, a comprehensive functional characterization of CLas'

metabolism is currently lacking, hindering targeted treatment against the pathogen. We reconstructed and manually curated genome-scale metabolic models for six CLas strains, i.e. A4, FL17, gxpsy, Ishi-1, psy62, and YCPsy. Furthermore, the generated a model of the closest phylogenetically related microorganism to CLas, the culturable bacterium L. crescens BT-1. We successfully validated the growth predictions of BT-1 model. Additionally, CLas models were constrained using expression data obtained from CLas infected citrus plants as well as from the bacteria residing in the psyllid host. The metabolic networks were used to analyze the shared and unique metabolic capabilities for all strain-specific variants cultivable and uncultivable. Our results enabled to identify metabolic bottlenecks that limit CLas growth in vitro as well as similarities and differences between Liberibacter strains that can provide insight into interactions between CLas and its different hosts.

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What effect does organic material have on plant health & productivity and soil microbiology in corn? (1000-096)
Hall 2

Soil nutrient depletion, due to long-term intensive agriculture, has a negative impact on plant health and decreases the ability of the soil to support a rich and abundant growth and yield. Recent studies have shown that biochar, a soil amendment made from biomass pyrolysis, can improve the soil's ability to retain water, stabilize the pH, and prevent habitation by pathogenic organisms. Organic compost has been shown to improve overall soil composition, including increasing the nitrogen and carbon content, but its combination with biochar has relatively unknown effects. In this study, Agrisure® 3000 field corn (Syngenta) was grown under different soil treatments of control (0), compost (C), and compost-biochar (CB) Chlorophyll concentration and peroxidase enzyme levels were measured each week to assess plant health and stress levels respectively over the growing season. Plant productivity was determined by comparing the yield of the corn grown in biochar and compost to that of the control group (0). Results illustrate that both compost and compost-biochar treatments improve plant health and yield while at least somewhat lowering oxidative stress. We have also isolated DNA samples from the plots over the course of the season to assay changes in the microbial population. This work is funded by grant OIA-155741: RII Track 1-Center for Root and Rhizome Innovation from the National Science Foundation (NE EPSCoR) and by a grant from the NU Foundation.

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SDE15 of Candidatus Liberibacter asiaticus suppresses host programmed cell death to facilitate chron (1000-123 (Screen 3))
Hall 2

Citrus Huanglongbing (HLB), caused by the psyllid-transmitted, phloem-living bacteria 'Ca. Liberibacter Asiaticus' (CLas), 'Ca. Liberibacter Africanus' (CLaf), and 'Ca. Liberibacter Americanus' (CLam), is the most destructive citrus disease. The mechanisms of the HLB pathogenicity remain largely unknown because 'Ca. Liberibacter' species have not been cultured. Las is known to secrete putative virulence proteins (effectors) in a Sec-dependent manner. More than 80 SDEs were identified for CLas in previous studies. Here we showed that SDE15 interacts with a well-known negative regulator of plant programmed cell death (PCD) to promote infection. Transgenic expression of SDE15 in citrus promotes Las multiplication and HLB symptom development. SDE15 suppresses not only PCD induced by Xanthomonas citri subsp. citri (Xcc) in citrus, but also PCD induced by AvrBsT (a PCD-eliciting Xanthomonas effector) in tobacco, suggesting that SDE15 is a broad-spectrum suppressor of plant PCD. Characterization of SDE15 unravels an elusive aspect of the mechanism of a major plant disease.

Primary E-Poster Presenter: Zhiqian Pang

Mechanical stress of Arabidopsis upregulates jasmonates and enhances Fusarium oxysporum infection (1000-124 (Screen 2))
Hall 2

Insect herbivory, touch, strong winds and heavy rainfall are natural ubiquitous stimuli that cause mechanical stress (MS) to plants. Prolonged MS can induce plant stress acclimation by priming plant defenses and thigmomorphogenesis; which is characterized by thicker stem, smaller leaves, reduced height and overall increased in tensile strength. We demonstrate that MS of Arabidopsis increased lignin and callose depositions, jasmonic acid (JA) level and repressed salicylic acid (SA). Prolonged MS primed JA-induced resistance against necrotrophs, Botrytis cineria and Alternaria brassicicola. In contrast, MS enhanced disease susceptibility to the hemibiotroph, Fusarium oxysporum. While JA deficient mutants, coronatine-insensitive 1 (coi1) and jasmonate resistance 1 (jar1) attenuated MS-induced F. oxysporum infection, exogenous application of methyl jasmonate (MeJA) promoted susceptibility to F. oxysporum. JA level and JA responsive genes were hyper-induced in MS-infected plants, revealing that F. oxysporum can hijack the JA-induced signalling pathway primed by MS. We show that a short period of MS repressed the induction of JA responsive PLANT DEFENSIN 1.2 (PDF1.2) gene by up-regulating Glutaredoxin 480 (GRX480), a transcriptional repressor, independent of SA-dependent NPR1 signaling. However, prolonged MS instead triggered the up-regulation of PDF1.2 expression and enhanced SA levels as well as PATHOGENESIS-

RELATED GENE 1 (PR1) gene expression in naïve leaf tissues. Exogenous application of SA repressed MS and MeJA-induced PDF1.2 transcript level, confirming that cross talk between JA and SA is differently primed during prolonged MS. We present a model showing how short and prolonged MS differentially regulate PDF1.2 expression via GRX480 dependent and independent pathways, respectively. We conclude that repetitive MS can program and train cellular memory to alter plant immunity in a pathogen-dependent manner.Glossary: Mechanical stress, pathogens, defense, touch.

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Tracking Microbial community succession using multi-generation ecosystem selection in A. thaliana (1000-126 (Screen 11))
Hall 2

Ecosystem selection involves inheritance of specific traits in an organism that is determined by ecology alone. It has been carried out for centuries and is a driving force of natural selection. In terms of plant-microbe interactions, ecosystem selection might involve selection on the microbial community in the soil which in-turn acts on a plant trait (e.g. biomass). Artificial ecosystem selection can be carried out in a laboratory setting, by selecting for an ecosystem associated with a phenotypic trait. For example, Arabidopsis thaliana has been used as a model system to show that plant biomass and flowering time can be altered reproducibly over multi-generation selection of the soil ecosystem (Swenson et. al. & Panke-Buisse et. al). In this study, the soil ecosystem (consisting of complex interactions of bacteria, fungi and protozoa) is selected with respect to the phenotypic trait "above ground plant biomass" (both plants with low biomass and high biomass). The selected ecosystem serves as "parents" for the next generation of soil and over multiple generations, the phenotypic trait (i.e. lower or higher biomass of plants), shifts in the direction of selection. This study shows a preliminary characterization of the microbial community dynamics occurring in the soil ecosystem over five generations of the ecosystem selection regime with respect to plant biomass in Arabidopsis thaliana Cvi and Ler by using a next generation sequencing -16s rRNA metagenomics approach. All data analysis and modeling has been done on QIIME 2 and custom scripts in R. Future studies involve looking at plant gene expression associated with the plants resulting from the ecosystem selection to get an insight into how the microbiome influences specific genes in the plant. This study has the potential to enhance our understanding of both microbial community dynamics and plant genetics and can be applied in agriculture to obtain a desired plant phenotype.

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Transcriptome analysis of Peach-T. deformans interaction in susceptible and resistant genotypes (1000-125 (Screen 5))
Hall 2

Peach (Prunus persica) is an appreciated summer fruit. The "peach leaf disease", caused by Taphrina deformans, affects its production. Although it is controlled with fungicides, to avoid the potential negative impact on health and environment, new farming strategies based on genetically resistant materials are needed. Here, we worked with a resistant genotype (DR) and compared it with a susceptible one (DS). Leaves inoculated with the fungus were collected at 0, 12 and 96 hpi. RNA-Seq analysis was used to identify candidate resistance genes and to dissect the early molecular processes during the interaction in resistant and susceptible genotypes.T. deformans-DR interaction analysis identified 190 and 1080 differentially expressed genes (DEGs) at 12 and 96 hpi with respect to 0 hpi, respectively. Using DS, 357 and 210 DEGs were identified at 12 and 96 hpi, respectively. The analysis over time indicated the most represented functional categories were RNA, protein, hormone, cell wall and secondary metabolisms, biotic and abiotic stress, signalling and transport.Differences regarding phytohormones were found between genotypes; while in DR transcripts related to jasmonate synthesis and responses were induced; in DS those involved in its synthesis were repressed. In DS transcripts encoding ACC synthase and oxidase were repressed; suggesting a decrease in ethylene synthesis. Conversely, in DR a transcript encoding ACC synthase was induced at 96 hpi. With respect to auxins, the repression of transcripts involved in auxin synthesis and in auxin responsive proteins was observed in DR. In contrast, in DS PIN5 was greatly increased.In DR at 96 hpi, it is notable the induction of plant-defense genes such as chitinases, major latex proteins-related and disease resistance proteins (NBS-, TIR-NBS- and CC-NBS-LRR). These findings provide an important basis for understanding the molecular mechanism that leads to the resistance in P. persica

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Cell Biology: General

The auxin transport inhibitor targets villin-generated actin bundles to regulate polar auxin transpo (0800-085 (screen 3))
Hall 2

Plant development and tropisms are largely dependent on the polar transport of the phytohormone auxin. Actin cytoskeleton regulates auxin transport by controlling polar localization of auxin transporters such as PIN2. Inhibitors of polar auxin transport have been essential tools in understanding auxin-dependent plant development. One mode of their inhibitory effect is to affect actin dynamics, however, the underlying mechanisms remain unclear. In this study, we demonstrate that auxin transport inhibitor such as 2,3,5-triiodobenzoic acid (TIBA) target villin-mediated actin bundles in Arabidopsis to inhibit auxin transport. Multiple villins isovariants are targeted by TIBA, among which, villin4 (VLN4) has the highest affinity to this inhibitor. Mutants of VLN4 have significantly reduced TIBA sensitivity. Loss of VLN4 results in low abundance of actin bundles. Furthermore, VLN4-dependent actin bundling controls the plasma membrane presence of auxin exporter and subsequent auxin transport, which is critical for the inhibitory effect of TIBA. Biochemical approaches and docking simulation demonstrate that TIBA directly interacts with the C-terminal headpiece domain of Arabidopsis villins. The VHP-TIBA interaction promotes villin to oligomerize, which facilitate cross-linking of actin filament. Villin C-terminal headpiece confers in-vivo TIBA sensitivity. VLN4 mutant lacking VHP domain fails to mediate the action of TIBA on both actin cytoskeleton and auxin transport in plant. Collectively, our study provides evidence that villins mediates the action of TIBA on actin dynamics in Arabidopsis; Villin-generated actin bundles determine downstream location of auxin efflux transporters and regulate polar auxin transport.

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A receptor and pathways discovered in the lignin-based resistance to Cuscuta campestris in Heinz hyb (0800-086 (Screen 12))
Hall 2

Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. Cuscuta species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Reports have shown a 50–72% reduction in tomato yield when attacked by dodders. The physiological connection between host plants and parasites makes traditional herbicides and control methods ineffective. The Heinz hybrid cultivars H9492 and H9553 exhibit resistance to dodders. The stem cortex in these lines responds with local lignification upon C. campestris attachment causing the C. campestris strand to fall off. To identify the

key resistant genes, we focused on genes that have different expression patterns under C. campestris infestation in the resistant cultivars, compared to susceptible cultivars. Based on these criteria, we identified an AP2-like transcription factor, MYB55, and CC-NBS-LRR as key resistant genes. The transient overexpression of MYB55 and AP2-like induced stem lignification in the susceptible cultivar. These results suggest that MYB55 and AP2-like may directly regulate the biosynthesis of lignin in the cortex. Therefore, we termed this AP2-like protein as LRF1 (Lignin-based resistance factor 1). On the other hand, overexpression of this CC-NBS-LRR only induced lignification upon C. campestris attachments. This result indicates that this CC-NBS-LRR functions as a receptor for receiving C. campestris signals, thereby leading to the lignification-based resistance. Thus, we named it CuLiRR1 (Cuscuta-induced lignin-based resistance receptor). We also identified a transcription factor WRKY16 as a negative regulator of the lignin-based resistance. WRKY16 CRISPRed plants also induced lignification in the cortex and became more resistant to C. campestris. The results of this study provide the starting point for developing a parasitic plant-resistant system in crops.

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Motors take a pause: A new role for myosin XI in secretory vesicle tethering (0800-087 (Screen 6))
Hall 2

Cellulose microfibrils, the major tensile components of the plant cell wall, play essential roles in plant growth and development. In flowering plants, cellulose is synthesized at the cell surface by plasma membrane (PM)-localized cellulose synthase (CESA) complexes (CSCs). Cellulose production is influenced by the abundance of CSCs at the PM which is thought to be coordinated by intracellular trafficking events and the cytoskeleton. The cortical actin cytoskeleton has been implicated in trafficking of CSCs to the PM, but the exact mechanism remains unclear. Here, we demonstrate that myosin XI and the actin cytoskeleton mediate CSC delivery to the PM by coordinating the exocytosis of CESA-containing compartments. Measurement of cellulose content indicated that cellulose biosynthesis was significantly reduced in a myosin xik xi1 xi2 triple knockout (xi3KO) mutant. By combining genetic and pharmacological disruption of myosin activity with quantitative live-cell imaging of functional YFP-CESA6, we observed decreased abundance of PM-localized CSCs and reduced delivery rate of CSCs in myosin-deficient cells. These phenotypes correlated with a significant increase in failed vesicle secretion events at the PM as well as an abnormal accumulation of CESA-containing compartments at the cell cortex. Through high-resolution spatiotemporal assays of cortical vesicle behavior, we identified defects in CSC vesicle tethering and fusion at the PM. Furthermore, colocalization studies showed transient association of MYOSIN XIK with CSCs during vesicle tethering. These findings reveal a previously undescribed role for myosin in vesicle secretion and cellulose production at the cytoskeleton–PM–cell wall nexus.

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Cell wall dynamics influence the formation, function, and aging of stomatal guard cells in Arabidops (0800-088 (Screen 14))
Hall 2

Stomatal guard cells are some of the most dynamic cells in plants due to their ability to expand and contract to control the size of stomatal pores. However, our understanding of how the walls of sister guard cells separate to form stomatal pores, and how they imbue guard cells with the strength and elasticity required to repeatedly inflate and deflate over the lifetime of a leaf, is limited. We applied molecular genetics, cell biology, and mechanical modeling to probe stomatal development, function, and mechanical aging in Arabidopsis thaliana. Using time-lapse microscopy we determined the contributions of wall degradation and mechanical pressure to stomatal pore formation. Our results indicate that pectin degradation is the primary driver of pore initiation, whereas both pectin degradation and cell presurization contribute to pore enlargement. We also discovered that cellulose, xyloglucan, and pectin contribute to stomatal function in distinct ways: mutants with reduced cellulose have enlarged guard cells, display reduced relative increases in pore width during stomatal opening, and, counterintuitively, are modeled as having stiffer walls than wild type, whereas xyloglucan-deficient mutants have smaller guard cells that display normal relative opening, but are modeled as having softer cell walls than wild type. In a mutant with reduced pectin molecular mass, stomata appeared normal in the closed state, but opened much wider than wild type despite a modeled increase in the longitudinal stiffness of the wall. Finally, we found that manipulating the expression of different endogenous pectinases has differential effects on stomatal development and function, implying that pectin autodegradation has complex effects on guard cell size, stomatal pore formation, and the elastic behaviors of guard cells over developmental time. This research will inform efforts to generate crop plants with enhanced control of stomatal dynamics and improved water use efficiency.

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Arabidopsis 3-Phosphoinositide-dependent kinases (PDKs) are important for pollen penetration through (0800-091 (Screen 8))
Hall 2

In Arabidopsis, 39 AGC kinases are involved in regulating processes including growth, reproduction, and stress responses. Two of these AGCs are 3-Phosphoinositide-dependent kinase 1 (PDK1) and PDK2 and are proposed to be "master regulators" activating downstream AGCs. Sharing structural homology with mammals, Arabidopsis PDKs have a phospholipid-binding Pleckstrin Homology (PH) domain and a peptide interacting fragment (PIF) pocket interacting with other AGCs. Here, we report genetic evidence from pollen transmission assays that AtPDK1 and 2 are functionally redundant and important for signaling events that allow pollen penetration through the stigma. Compared to other plant tissues, PDKs are most highly expressed in pollen, suggesting significant roles in rapid cellular growth and fertilization. Pollen harboring a pdk1/2 partial loss-of-function mutation are near-sterile, with a 265-fold reduction in pollen transmission compared to wild-type. This decrease in transmission was largely overcome by eliminating stigma-style barriers and manually pollinating decapitated pistils. Semi-in vivo pollen tube growth assays indicate that mutant pollen tubes have a severely attenuated ability to penetrate through the stigma (> 50-fold impairment compared to wild-type). This near-sterile phenotype was rescued by transgene expression encoding PDK1 or PDK2. Interestingly, an AtPDK2-YFP fusion accumulated with vesicles in the apical dome of a growing pollen tube, as opposed to plasma membrane localization predicted by analogy to mammalian PDK. A deletion of the PH domain (PDK2-ΔPH-YFP) retained tip localization and rescued the near-sterile phenotype, suggesting that the phospholipid binding (PH) domain is not important for pollen tube growth or male fertility. Together, this evidence supports a new working model that AtPDKs function independent of phospholipid signals to regulate cell-cell interactions in growing pollen tubes, enabling penetration through the stigma-style barrier.

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Division Plane Orientation Defects Revealed by a Synthetic Double Mutant Phenotype (0800-090 (Screen 4))
Hall 2

Proper positioning of the new cell wall during cell division is essential for plant patterning and development. TANGLED1 (TAN1) and AUXIN INDUCED IN ROOTS9 (AIR9) are microtubule-binding division site marker proteins. Single tan1 and air9 mutants have no discernable phenotypes in Arabidopsis. However, Arabidopsis tan1 air9 double mutants have a synergistic phenotype displaying altered cell file rotation, root growth, and cell division orientation. Transformation with either full length TAN1 or AIR9 is sufficient to rescue these mutant phenotypes. This suggests that TAN1 works in conjunction with AIR9 to properly orient the division plane and potentially influence the organization of cortical microtubules in nondividing cells. Surprisingly, amino acids 1-132 of TAN1 (TAN1(aa1-132)) are capable of significantly rescuing the double mutant, which suggests that this section of the TAN1 protein plays a crucial role in division plane orientation. The present study

focuses on investigating TAN1(aa1-132) and TAN1(aa1-132) interacting proteins identified previously by yeast two-hybrid screening. After mutagenesis of TAN1(aa1-132), loss of interaction with known interactors will be screened for by yeast two-hybrid. The in vivo relevance of these disrupted interactions will then be assessed by transforming the tan1 air9 double mutant with the mutagenized versions of TAN1(aa1-132). Transformed double mutants will be examined for changes in protein localization as well as the ability of mutagenized TAN1(aa1-132) to rescue. By doing so, interactions required for TAN1 localization, function in division plane orientation, or both will be determined.

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Kinectin is essential for cell expansion in Zea mays (0800-089 (Screen 3))
Hall 2

Cell division and directed cell expansion are essential for normal growth and development. TANGLED1 (TAN1) plays an essential role in division plane maintenance in plants, but other proteins required for division plane maintenance have not yet been identified. We identified a protein similar to an animal protein called KINECTIN (KNN), that interacts with TAN1. In animals, KNN is an endoplasmic reticulum anchored protein that interacts with microtubule associated motor proteins. In silico analysis showed that the transmembrane domain found in animal KNN is not present in plant KNN. This finding suggests that KNN may have a different localization pattern in plant cells. However, domains required for microtubule associated protein interaction are conserved, suggesting that KNN might interact with microtubule associated proteins. We used the CRISPR/Cas9 system to mutagenize KNN in Zea mays. Analysis of knn mutant leaf epidermis showed no apparent defects in division plane orientation and TAN1 localization during mitosis was unaltered. However, knn cells had cell expansion defects and dark grown knn plants had shorter mesocotyls than wild-type siblings. The observed cell expansion phenotype in knn plants may be mediated either by TAN1 interactions or interactions with other microtubule associated proteins in maize. Although the relationship between KNN and TAN1 is not yet clear, KNN plays an important role in cell expansion, which is critical for plant growth and development.

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Primary E-Poster Presenter: Marschal Bellinger

A heterogeneous nuclear ribonucleoprotein (hnRNP) in Chlamydomonas functions as a cell-cycle repress (0800-022)
Hall 2

Coordination of growth and division in eukaryotic cells is thought to be mediated by size checkpoints, but the mechanisms for size homeostasis are largely unknown. The green alga Chlamydomonas divides by a multiple fission cell cycle, where the Commitment checkpoint ensures enough growth for completion of at least one division, and the DNA synthesis/mitosis checkpoint ensures mother cells undergo the correct number of divisions to produce uniform-sized daughters. tny1-1 was identified in a insertional mutagenesis screen and exhibits a recessive small phenotype due to defects at both checkpoints. TNY1 encodes a predicted hnRNP A-related RNA binding protein with two N-terminal RNA recognition motifs and a low complexity glycine-rich C-terminus—a structure shared by many eukaryotic hnRNPs. Microscopy showed that TNY1 is cytosolic throughout the cell cycle. Immunoblotting revealed that daughter cells are born with a fixed amount of TNY1, whose absolute abundance remains constant on a per-cell basis during G1 phase, but whose overall cellular concentration decreases as cells grow. TNY1 mRNA and protein levels peak during cell division and are reset to the highest concentration in newly-formed daughters. Altering the dosage of TNY1 in diploids impacted daughter cell size, indicating that TNY1 is limiting for size control. Epistasis experiments placed TNY1 upstream of cyclin dependent kinase CDKG1, one of whose substrates is MAT3/RB (retinoblastoma tumor suppressor homolog). In wild-type cells CDKG1 is produced before division and eliminated upon mitotic exit, but in post-mitotic tny1-1 mutants CDKG1 remains detectable, suggesting TNY1 inhibits CDKG1 accumulation. North-Western assays showed that TNY1 binds to the unusually long and uridine-rich 3′ UTR of CDKG1 mRNA but not to its CDS or 5′ UTR. Taken together, our data suggest a model where TNY1 influences size homeostasis through dosage-dependent repression of CDKG1, possibly through direct binding to the CDKG1 3′UTR.

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A hybrid approach enabling large-scale glycome analysis of post-Golgi vesicles reveals a transport r (0800-032)
Hall 2

The plant endomembrane system facilitates transport of polysaccharides, associated enzymes and glycoproteins through its dynamic pathways. Although enzymes involved in cell wall biosynthesis have been identified, little is known about the endomembrane-based transport of glycan components. This is partially attributed to technical challenges in biochemically determining polysaccharide cargo in specific vesicles. Here we introduce a hybrid approach addressing this limitation.

By combining vesicle isolation with a large-scale carbohydrate antibody arraying technique, we charted an initial large-scale map describing the glycome profile of the Syntaxin of Plants 61 (SYP61) trans-Golgi network (TGN) compartment. A library of antibodies, recognizing specific non-cellulosic carbohydrate epitopes, enabled us to identify a range of diverse glycans, including pectins, xyloglucans (XyGs) and arabinogalactan proteins (AGPs) in isolated vesicles. Changes in XyG- and pectin-specific epitopes in the cell wall of the Arabidopsis SYP61 mutant corroborate our findings. Our data provide evidence that SYP61 vesicles are involved in transport and deposition of structural polysaccharides and glycoproteins. Adaptation of our methodology can enable studies characterizing the glycome profiles of various vesicle populations, in plant and animal systems and their respective role in glycan transport defined by subcellular markers, developmental stages or environmental stimuli.

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An Efficient Transient Expression Method for Catharanthus roseus Seedlings and its Applications (0800-005)
Hall 2

The medicinal plant Catharanthus roseus produces two terpenoid indole alkaloids (TIAs), vinblastine and vincristine, used as anti-cancer drugs. However, vinblastine and vincristine are only produced at less than 0.001 % by weight in the plant. The expression of this pathway is tightly regulated and understanding this regulation can lead to strategies that increase TIA production. Studying gene function in C. roseus is limited by the availability of methods as no reliable method for overexpressing genes of interest in leaves exists for C. roseus. Previous transient transformation methods for seedlings showed an uneven and faint expression of the transgene due to the waxy surface of C. roseus leaves. We systematically optimized the transient expression method by investigating: 1) the infiltration method and age of seedlings, 2) the introduction of a constitutively active VirG gene, and 3) improvement of construct design by implementing a dual-luciferase system. Routinely, 100 % of the seedlings show transgene expression, and cotyledons are uniformly transformed. As a proof-of-concept, this method was used for transactivation assays of the STR1 promoter with its known activator, ORCA3, and its known repressor, ZCT1. The published method is available at doi: 10.3389/fpls.2019.00755. Furthermore, we used this method to investigate the regulation of ZCT1 by performing transactivation and promoter deletion studies, highlight the importance of an activation sequence-1 (as-1) element within the

ZCT1 promoter. In summary, I will present an efficient and reliable seedlings transformation protocol and its application to studying the transcriptional regulation of alkaloid biosynthesis in C. roseus.

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Analyzing the role of cell shape in division plane orientation (0800-033)
Hall 2

How cells within multicellular organisms coordinately grow and divide are fundamental questions in plant biology. Pattern formation within tissues and organs relies on proper integration of cell-cell communication and developmentally regulated growth and division. We developed a cell-shape based model to predict division planes by using a soap-film minimizing approach. This model produces two equal-sized daughter cells while minimizing the surface area of the division plane similar to soap-films. In wild-type cells, the geometry of the cell is almost always sufficient to generate predictions that closely match in vivo division planes across developmental stages and in both plant and animal cells. However, when we used our model on a mutant with defects in division plane orientation, tangled1, we noticed that most divisions aligned well with predicted divisions, but some did not. Preliminary evidence showed a positive correlation between irregular cell shape and division plane offset from the prediction in the tangled1 mutant. This suggests that aberrant cell shape itself may promote improper division plane placement. When we searched for aberrantly shaped wild-type cells, they also had division planes that did not align with our predicted divisions from modeling. Together, these data suggest that the mechanism promoting soap-film minimization in plant cells is more accurate in regularly-shaped and less accurate in irregularly-shaped cells. Future studies will provide insight into how microtubule organization contributes to division plane orientation in maize and other plants.

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ARPF2 is involved in ribosomal RNA processing and ribosome biogenesis through interaction with ARRS1 (0800-003)
Hall 2

Yeast Rpf2 plays a critical role in 5S ribosomal RNA (rRNA) incorporation into pre-ribosomes by forming a binary complex with Rrs1. Arabidopsis RPF2 (ARPF2) interacts with ARRS1 and binds to specific rRNA sequences. Overexpression of ARPF2 and ARRS1 causes the extension of plant life span. However, loss-of-function phenotypes of ARPF2 have not been examined due to the embryo-lethality of its null mutations. In this study, using virus-induced gene silencing, we investigated ARPF2 function in the regulation of pre-rRNA processing and ribosome biogenesis. ARPF2 silencing in Arabidopsis led to pleiotrophic developmental defects including growth retardation, short inflorescences, and abnormal flowers. RNA gel blot analyses and circular RT-PCR revealed that depletion of ARPF2 delayed pre-rRNA processing, resulting in accumulation of multiple processing intermediates. Metabolic rRNA labeling and ribosome profiling suggested that ARPF2 deficiency mainly affected 25S rRNA synthesis and 60S ribosome biogenesis. ARPF2 interacted with ARRS1, RPL5, and RPL11, and furthermore with diverse nucleolar proteins involved in ribosome biogenesis. ARPF2 and ARRS1 co-fractionated primarily with the 60S ribosomal large subunit, and ARRS1 silencing caused similar growth defects, similar accumulation patterns of processing intermediates, and similar ribosome profiling to those of ARPF2-silenced plants. ARPF2-deficiency caused nucleolar stress, leading to localized cell death and excessive accumulation of anthocyanin pigments and reactive oxygen species. Taken together, these results suggest that the ARPF2-ARRS1 complex play a crucial role in plant growth and development by modulating ribosome biogenesis.

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Primary Poster Presenter: ILYEONG CHOI

AtTRAPPC11/ROG2: a role for TRAPPs in maintenance of plant TGN/EE organization and function (0800-010)
Hall 2

The dynamic trans-Golgi network (TGN) facilitates cargo sorting and trafficking and plays a vital role in plant development and environmental response. Transport protein particles (TRAPPs) are multi-protein complexes acting as guanine nucleotide exchange factors (GEFs) and possibly as tethers, regulating intracellular trafficking. TRAPPs are essential in all eukaryotic cells and are implicated in a number of human diseases. It has been proposed that they also play crucial roles in plants; however, our current knowledge about the structure and function of plant TRAPPs is very limited. We identified and characterized AtTRAPPC11/Response to Oligogalacturonide-2 (AtTRAPPC11/ROG2), a TGN-associated, evolutionarily conserved TRAPP protein in Arabidopsis. AtTRAPPC11/ROG2 regulates TGN integrity, as evidenced by altered TGN association of several TGN residents, including SYP61, and altered TGN vesicle morphology in attrappc11/rog2 mutants. Further, endocytic uptake and BFA body formation are perturbed in attrappc11/rog2, suggesting a role of AtTRAPPC11/ROG2 in regulation of endosomal function. Proteomic analysis showed that AtTRAPPC11/ROG2 defines a hitherto uncharacterized TRAPPIII complex in plants. In addition, attrappc11/rog2

mutants are hypersensitive to salinity, indicating an undescribed role of TRAPPs in stress responses. Overall, our study illustrates the plasticity of the endomembrane system through TRAPP protein functions and opens new avenues exploring this dynamic network.

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Autophagy development in Arabidopsis thaliana under microgravity (0800-001)
Hall 2

Autophagy is involved in the plant adaptation to different stress factors. Thus, the purpose of our study was assessment of basic parameters of autophagy development in A. thaliana under the influence of simulated microgravity. After sterilization the seeds of A. thaliana ecotype Col-0 were planted on a basal growth Murashige and Skoog medium in a Petri dishes. A part of experimental plant material was placed in a clinostat with a rotational mode of 2 rpm for 1-15 days at 22 ° C with 14/8 photoperiod. Seeds of control plants were germinated and cultivated in a grow room. Autophagosome visualization was performed using fluorescence microscopy after cell dying with monodansylcadaverine. The level of cell acidification was evaluated using acridine-orange dying. The preliminary results obtained during the 6-10 days of cultivation showed the development of autophagy. Autophagosomes in root cells of A. thaliana seedlings were identified starting from the 6th day of cultivation, but the most active development of autophagy occurred on the 9th day of the microgravity simulation. Data obtained between 10 -15 days of experiments showed that the root cells become adapted to the stress on 11th day. The level of acidification of cells was high on 10-11 day of cultivation, and decreased starting from 12th day. These results demonstrate that microgravity is a stress factor that activates autophagy development in A. thaliana seedlings. For future investigation genes related to autophagy and microtubular cytoskeleton will be studied in A. thaliana seedlings under microgravity condition.

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Auxin-induced actin cytoskeleton rearrangements require AUX1 (0800-026)
Hall 2

Many plant cells begin life with an isotropic shape; establishing a “polar” growth pattern requires the actin cytoskeleton and the phytohormone auxin. Several

models link specific actin arrays to cell growth, but no consensus prevails on whether longitudinal bundles inhibit or stimulate growth. Polarity requires auxin gradients formed by asymmetric flow of auxin, which relies on a functional actin cytoskeleton. Studies have examined actin reorganization after long-term auxin treatments, but plants respond to auxin in minutes. How short-term auxin–actin interactions affect growth, and the molecular players involved, are largely unknown. With quantitative tools, I correlated actin array organization with degree of axial cell expansion, establishing a baseline for actin organization in wildtype Arabidopsis root epidermal cells: cell length was highly predictive of actin array, and rapidly expanding cells had clearly different actin organization than slowly expanding cells. Within 20–30 minutes of growth-inhibitory doses of natural auxin and known root growth inhibitor, indole-3-acetic acid (IAA), actin filaments became more dense, parallel, and longitudinally oriented. Actin filament organization increased after a treatment to stop elongation, demonstrating there is no direct relationship between actin organization and cell expansion, and refuting the hypothesis that "more organized" actin correlates with rapidly growing root cells. AUXIN RESISTANT 1 (AUX1), a plasma membrane-bound auxin influx protein, binds IAA with high affinity and is responsible for 80% of IAA uptake by root hairs. aux1 mutant roots grow in the presence of IAA, but are growth-inhibited by the membrane permeable auxin 1-naphthylacetic acid (NAA). Actin arrays in aux1 mutants failed to reorganize in response to short-term IAA treatments, and reorganization was only partially restored by NAA. These are the first data to demonstrate that AUX1 is critical to auxin signaling to the actin cytoskeleton.

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Primary Poster Presenter: Ruthie Arieti

Characterization and localization of COPI subunits during compatible pollinations in A. thaliana (0800-015)
Hall 2

Coat Protein I (COPI) is a protein complex that consists of seven subunits forming two subunit complexes. It is involved in retrograde transport from the cis-golgi to the endoplasmic reticulum, medial-golgi transport, and maturation of early endosomes. Multiple isoforms exist for each subunit, except for the δ-COP subunit, which may imply roles for functional diversity in the recruitment of type 1 transmembrane proteins. Our goal is to examine the role of COPI and its subunit isoforms during the early events of a compatible pollination in Arabidopsis thaliana. The acceptance of a compatible pollen grain requires water to allow for pollen hydration. Germination and subsequent growth of the pollen tube relies upon vesicle recruitment to the pollen contact point. Using a non-quantitative proteomic experiment we identified the COPI complex as an important candidate in this process. Preliminary results under revision have demonstrated that T-DNA insertion mutants of the α1-COPI subunit exhibit reduced pollen grain adherence, germination, tube growth, and significantly decreased seed set. The α1-copi mutant, however, does not display a dwarf phenotype or loss of function like the

α2-copi. The γ-copi and □-copi mutants also showed defects in early pollination events but weaker when compared to α1-copi. Pollination phenotypes for α2-copi will be characterized and confocal fluorescent microscopy will be utilized to observed where the subunits are localizing during transient expression in Nicotiana benthamiana, using a constitutive 35S promoter, and 10 minutes after a compatible pollination event in A. thaliana using a stigma specific SLR1 promoter, with known membrane markers.

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Characterization of the ATI1 selective autophagy route in Arabidopsis thaliana (0800-011)
Hall 2

Macroautophagy (hereafter referred to as autophagy) is a conserved eukaryotic cellular recycling pathway that delivers unneeded cytoplasmic components in de novo formed double-membrane autophagosomes to the lytic organelle for degradation. In plants, autophagy plays an important role in maintaining cellular homeostasis and overcoming stress. It can be highly selective, targeting specific proteins, protein aggregates, organelles, or other cellular components for degradation. Selective autophagy typically utilizes cargo receptors that directly or indirectly bind specific cargo, and tether it to the forming autophagosome via interaction with core autophagy proteins, mainly ATG8. The ATI1 protein was identified as a plant specific ATG8-interacting protein that is associated with the ER and chloroplasts. ATI1 was shown to be recruited to stress-induced bodies (ATI-bodies) that are distinct from autophagosomes, but are delivered to the vacuole in a process that requires the autophagy machinery. As ATI1 specifically binds several chloroplast proteins, and carries a chloroplast marker to the vacuole, it was suggested that it may function as a selective autophagy receptor. Here we demonstrate that ATI1 is also involved in TOR-dependent dark-induced, but not in ER-stress induced, ER-phagy. We also further characterize ATI1-ATG8 interactions, the link between ATI-bodies and autophagosomes and ATI1 delivery to the vacuole.

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COPII Coated Vesicle Components in Chlamydomonas reinhardtii (0800-025)
Hall 2

The secretory pathway has an essential role in the localization of proteins to correct subcellular compartments in all eukaryotes. An initial step in cargo sorting is the

budding of COPII coated vesicles from the ER and their trafficking to the cis Golgi network in a process called anterograde transport. The inner layer of the COPII coat contains Sar1 GTPase, SEC23 and SEC24 proteins. While Chlamydomonas is an excellent model organism for cell biology, it has not been used for functional analyses of components of COPII vesicles. Chlamydomonas has paralogs of both the SEC23 and SEC24 genes. Our objective is to determine the functional differences among these parologs. We plan to use CRISPR technology to generate mutations in each paralog. In addition, we have recently begun to characterize insertional mutant lines in each of these SEC genes; phenotypic analyses of these mutants will provide insights into the functions of each of them in the trafficking of proteins to their sites of function.

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Deep analysis of protein networks using proximity labeling mass spectrometry (0800-029)
Hall 2

Cellular functions are organized and regulated through specific protein-protein interactions. Identifying these interactions are challenging, particularly for the transient and dynamic interactions such as those between protein-modifying enzymes and their substrates. Proximity labeling has immerged as a powerful method for studying subcellular proteomes and protein-protein interactions. Here we apply a proximity dependent biotin identification (BioID) system using a mutated biotin ligase (BirA) that display enhanced enzymatic activity, which is named TurboID. Using a pair of known interacting proteins of the brassinosteroid (BR) signaling pathway, the GSK3-like kinase BIN2 and its substrate BZR1, we showed that BIN2-TurboID enriches BZR1 about 10x more effectively than the traditional co-immunoprecipitation method. Using the homologs of the BIN2 family and PP2A family that function in the BR pathway, we showed specific interaction of BZR1 with the protein family members that are involved in BR signaling but not those that do not play an important role in BR signaling, demonstrating good specificity of TurboID. We have used TurboID to identify interacting proteins of known kinases and phosphatases, and this has led to an expanded protein networks that regulate plant growth responses to hormonal and environmental signals.

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Primary Poster Presenter: Zhiyong Wang

Elucidating the function of NORTIA in Arabidopsis pollen tube reception (0800-016)
Hall 2

The fertility of plants is dependent upon a complex series of events from pollination to fertilization. Upon pollen tube arrival at the female gametophyte, a complex series of molecular events, known collectively as pollen tube reception, must occur before the pollen tube can burst and release two sperm cells to complete double fertilization. Pollen tube reception is controlled by the synergid cells of the female gametophyte. FERONIA (FER), LORELEI (LRE), and NORTIA (NTA) are synergid-expressed genes that have been shown to participate in signaling between the synergid and the pollen tube. FER acts in coordination with LRE at the filiform apparatus to control rupture of the pollen tube and release of the sperm cells. NTA is localized in Golgi-associated compartments before pollen tube arrival and redistributes to the filiform apparatus, a region of highly invaginated membranes at the pollen tube entry point, upon pollen tube arrival in a FER-dependent process. However, the molecular pathway that NTA belongs to and how NTA executes its function still remains a mystery. This project is aimed at defining the timing and functional significance of NTA redistribution during pollen tube reception and at identifying other molecular components involved in this biological process.

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Primary Poster Presenter: Yan Ju

Enzymatic activities of protein complexes could affect their cellular trafficking in plants (0800-009)
Hall 2

Like in other eukaryotic cells, plant membrane trafficking pathways transport proteins among organelles and play essential roles in growth and development. The cargo proteins for membrane trafficking have diverse functions such as cell wall biosynthesis, signaling, and nutrient uptake. How plant cells accurately control protein transport in a spatiotemporal manner has not been well characterized. Cellulose synthase complexes (CSCs) are large membrane-associated protein complexes that catalyze the synthesis of cellulose at the plasma membrane. CSCs are delivered to the plasma membrane through membrane trafficking pathways for their proper functions. Using chemical genetic approach, we identified a small molecule that targets the catalytic domain of plant cellulose synthases. Combining small molecule treatment, live cell imaging and quantitative image analysis, we found that the catalytic activity of CSCs affected efficient exocytic transport of these large protein complexes. Inhibition of CSCs catalytic activity reduced the transport of CSCs at early steps of exocytic trafficking, although these protein complexes

might have been assembled properly. Our results add to current understanding of how plant membrane trafficking machineries regulate spatiotemporal delivery of proteins for plant growth and environment adaptation.

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Primary Poster Presenter: Chunhua Zhang

Golgi-localized LOT regulates trans-Golgi network biogenesis in plants (0800-013)
Hall 2

The trans-Golgi network (TGN) is an essential tubular-vesicular organelle derived from the Golgi and functions as an independent sorting and trafficking hub within the cell. However, the molecular regulation of TGN biogenesis remains enigmatic. Here we identified an Arabidopsis mutant loss of TGN (lot) that is defective in TGN formation and sterile due to impaired pollen tube growth. The mutation leads to overstacking of the Golgi cisternae and significant reduction in the number of TGNs and vesicles surrounding the Golgi in pollen, which is corroborated by the dispersed cytosolic distribution of TGN-localized proteins. Consistently, deposition of extracellular pectin and plasma membrane localization of receptor kinases and phosphoinositide species are also impaired. Subcellular localization analysis suggests that LOT is localized on the periphery of the Golgi cisternae, but the mutation does not affect the localization of Golgi-resident proteins. Furthermore, the yeast complementation result suggests that LOT could functionally act as a component of the guanine nucleotide exchange factor (GEF) complex of small Rab GTPase Ypt6. These findings suggest that LOT is critical for TGN biogenesis in the plant lineage, distinct from the function of its non-plant organisms (Jia et al., PNAS, 2018). Study of the lot homolog plants suggests that LOT also regulate TNG biogenesis and Golgi structure in root and hypocotyl (Jia et al., 2019). Transmission electron microscopic examination suggests that LOT regulate the very early generation of Golgi-associated TGN formation from the Golgi cisterna. Using biochemical and genetic strategies, substrates of LOT and components in the related signaling pathways are investigated. Proteomic analysis was used to discriminate the cargos of TGN-dependent and –independent trafficking pathways. We also find that GA and BR hormone signaling pathways are disrupted which may contributes to the plant vegetative growth defect.

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Primary Poster Presenter: Hong-Ju Li

Identification of CSLD3 as a β-1,4-glucan Synthase that Functions during Plant Cell Wall Synthesis (0800-008)
Hall 2

Plant cell expansion is a dynamic process which is physically constrained by deposition of the load-bearing plant cell wall. During tip-growth, CSLD proteins specifically localize to apically-localized plasma membranes in these cells and synthesize polysaccharides at the growing tip of root hair cells. The nature of the polysaccharides generated by CSLD proteins has remained controversial. Here we use genetic, biochemical, and computational approaches to characterize the catalytic activity of members of the CSLD protein family. Genetic complementation of a cesa6 mutant with a chimeric CESA6 protein containing a CSLD3 catalytic domain demonstrated that the CSLD catalytic domains can successfully generate β-1,4-glucan polymers for cellulose synthesis during diffuse growth. Time-lapse fluorescence microscopy demonstrated that these CESA6-D3CD chimeric proteins assembled into CSC complexes with similar mobility characteristics as EYFP-CESA6-labeled complexes in hypocotyl cells. In addition, β-1,4-glucan breakdown products were detected by MALDI-TOF mass spectroscopic analysis of yeast microsomal membrane fractions expressing CSLD3 after treatment with specific β-1,4-endoglucanases. Purified, detergent-solubilized CSLD3 and CESA6 proteins were reconstituted into proteoliposomes that could utilize UDP-glucose, but not GDP-mannose as enzymatic substrates. Finally, computational modelling of CESA6, CSLD3, and CSLA9 protein structures showed that CESA and CSLD protein families share conserved catalytic residues, as well as other conserved substrate-binding pocket residues that distinguish CESAs and CSLDs from the CSLAs, consistent with the ability of these glycan synthase families to distinguish UDP-Glucose from GDP-Mannose within their active sites. Taken together, these data strongly support the conclusion that CSLD proteins represent a distinct family of β-1,4-glucan synthases.

Primary Poster Presenter: Jiyuan Yang

Identification of novel components involved in regulating plasmodesmata
(0800-092 (Screen 7))
Hall 2

Cell-to-cell communication is crucial for developmental regulation and stress adaptation in multicellular organisms. In plants, symplastic transport of signaling molecules between cells is mediated by membrane-lined channels termed plasmodesmata (PD). Despite their crucial roles in plants, molecular mechanisms underlying the PD-mediated cell-to-cell communication are largely unknown. It is widely accepted that the PD aperture determines its function, whereas callose deposition at the PD neck region is the major mechanism in controlling the aperture. Callose synthases and ß-1,3-glucanases are known to involved in callose deposition and hydrolysis, respectively. To uncover novel components involved in regulating callose homeostasis at the PD, we screened a library of dominant-negative ‘decoy’ E3 ubiquitin ligases (Feke et al., 2019; eLife 8:e44558 DOI: 10.7554/eLife.44558). The decoys lack F-box or U-box domain, which are unable to ubiquitinate and degrade the target proteins, but retain the ability to bind them. To identify the ligases involved in regulating callose homeostasis at the PD, we transiently express the decoys in Nicotiana tabacum and examine the callose

accumulation at the PD. Using confocal microscopy, we have screened 556 Agrobacterium strains carrying the decoys. We have isolated 44 putative decoys, which exhibit overaccumulation of callose at the PD. The phenotype will be confirmed in Arabidopsis transgenic plants expressing the candidate decoys. Target proteins of the ligases will be identified using immunoprecipitation-mass spectrometry. This work will uncover the regulatory proteins and novel components involved in maintaining callose homeostasis at the PD.

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Ionome patterning in Arachis hypogaea root (0800-019)
Hall 2

Element distribution in primary roots of Arachis hypogaea (peanut) was investigated using SEM/EDS and their concentrations were estimated using ICP-OES. Resin embedded thin sections of root were prepared using an ultramicrotome and observed with a light microscope to understand element distribution in relation to root anatomy. Tissue specific accumulation of potassium, phosphorous and sulfur transported along the length of roots from an endogenous source (i.e. the seed) was assessed. Peanut roots grown in calcium nitrate or carboxyfluorescein diacetate solutions were analyzed for distribution patterns. Our results showed tissue specificity in element accumulation and the element concentration varied along the length of peanut root. Carboxyfluorescein and calcium accumulation from exogenous sources varied in the cortex along the length of root. Exogenous calcium was excluded from lateral root primordia. Root tips had much higher concentration of endogenous elements than any other parts of root. These data indicate that the distribution patterns differ between endogenous and exogenous ions and charge-neutral indicators. Similar studies have been done using Pinus pinea roots (Pesacreta et al. 2018). We speculate that, similar to cellular patterning which dictates the body plan of a plant, ionome patterning may have a critical role in growth, development, physiology and adaptation of plants. Understanding these patterns can explain the nutritional status and requirement of a plant

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Primary Poster Presenter: Aniruddha Acharya

Live imaging the spatiotemporal dynamics of Arabidopsis leaf growth (0800-018)
Hall 2

Reliably producing organs of defined size and shape is an essential activity of living organisms. Macroscopic shapes are thought to arise from the concerted development of spatially distinct zones with defined growth rates. However, the

mechanisms which determine the size and location of these growth zones as well as how they work together to create ultimate organ shape remain elusive. Here, the zone of fast growth at the base of Arabidopsis leaves is studied across early development to begin examining its ability to impact ultimate leaf organ shape. Methods of confocal live imaging over the course of early developmental stages were analyzed for their ability to capture the basal growth zone. Across these experiments, lines with fluorescently labeled plasma membranes were imaged every 24 hours for 3-5 days. Either the first or eighth leaf was imaged following protocols inspired by previous work(1,2). Image series were processed in the MorphoGraphX software package to track individual cellular lineages over each time point revealing the spatiotemporal dynamics of the fast growth zone in the Arabidopsis leaf base. I extended these methods by positioning leaves beneath glass coverslips, allowing them to grow submerged in a high refractive index liquid in which oxygen and carbon dioxide can dissolve (perfluorodecalin) and dissecting either one or both cotyledons for increased resolution of the first leaf margins. These different conditions revealed various compromises in the extent to which the basal growth zone could be captured relative to the context of the whole leaf, such that the method employed must be matched to the specific question of interest. We look forward to using these methods in concert with synthetic approaches to begin to dissect the temporal dynamics of the fast growth zone across different leaves and its ability to modulate final leaf organ size and shape.1. Fox S, et al. (2018) PLoS Biol 2. Vuolo F, et al. (2018) Genes Dev

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Primary Poster Presenter: Kate Harline

Microfluidic Capture of Individual Plant Cells in Mechanically Tunable Hydrogel Microspheres (0800-030)
Hall 2

Protoplast technologies and in vitro culturing techniques have facilitated a great deal of progress in studies of plant development. However, these techniques still do not offer researchers the ability to control the physical environment of individual plant cells. The development of hydrogel microbeads and microcapsules is of significant interest with applications in many fields including bioengineering and biomedicine. This technology has not found significant application in plant sciences, however. We introduce a developing method for generating hydrogel microcapsules containing living plant protoplasts. Individual cells were isolated by cell wall digestion from a suspension culture of Nicotiana tabacum cv. BY-2 cells. Microdroplets around 70 micrometers in diameter were generated from a stream of liquid agarose containing plant protoplasts using a commercial microdroplet chip. These droplets were then solidified into microbeads and coated with a shell of poly(sodium 4-styrenesulfonate) (PSS) and poly(diallyldimethylammonium chloride) (PDADMA). Producing multiple layers of these shells on microbeads allows the thickness to be tuned. This method has the potential to conduct novel studies in

plant cell biomechanics and other areas of plant science and may continue to benefit from technical advances in the field of microsphere fabrication.

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Primary Poster Presenter: Matthew Grasso

Multiple growth modes regulated the expansion of single-celled cotton fibers (0800-017)
Hall 2

Single-celled, cellulose-rich, cotton fibers are extensions of the Gossypium seed epidermis. They provide our most important renewable textile fiber and have qualities important to industry that are established by poorly-understood cellular morphogenesis process(es). Contrary to prior perceptions, not all cotton fibers are the same, as variations exist within and between the fibers of the two most important commercial cotton species (G. hirsutum and G. barbadense). However, multiple fiber types share the feature of extreme anisotropic elongation, including apical elongation, together with diametric expansion behind the apex, which is different than classical tip-growing cells. Where in the cell the diameter of cotton fibers is controlled will be described, along with the characteristics of the microtubule array and cell wall composition in this region. The effects of microtubule antagonists on fiber shape will also be described. Three antagonists impacting microtubules in different ways were added to the media of cotton ovules cultured shortly after flowering when fiber morphogenesis is at an early stage, followed by analysis of potential fiber shape changes compared to the controls. The results reinforced the differences between fiber types, confirmed the existence of two growth modes close to the cotton fiber tip, and expanded our knowledge of how microtubules regulate cotton fiber morphogenesis. For research support, we thank Cotton Incorporated, Cary, NC.

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Primary Poster Presenter: Candace Haigler

Nucleo-cytoplasmic partitioning of ARF proteins controls auxin responses in Arabidopsis thaliana (0800-027)
Hall 2

The phytohormone auxin plays crucial roles in nearly every aspect of plant growth and development. The AUXIN RESPONSE FACTOR (ARF) family of transcription factors regulates auxin-responsive gene expression and exhibit nuclear localization in regions of high auxin responsiveness. Here we show that the ARF7 and ARF19 proteins accumulate in micron-sized assemblies within the cytoplasm of tissues with attenuated auxin responsiveness. We found that the intrinsically disordered middle region and the folded PB1 interaction domain of ARFs drive protein assembly

formation. Mutation of a single lysine within the PB1 domain abrogates cytoplasmic assemblies, promotes ARF nuclear localization, and results in an altered transcriptome and morphological defects. Our data suggest a model in which ARF nucleo-cytoplasmic partitioning regulates auxin responsiveness, thus providing a mechanism for cellular competence for auxin signaling.

Primary Poster Presenter: Lucia Strader

Prenylation is required for establishment of multicellularity and cell differentiation in moss (0800-020)
Hall 2

A key milestone in the history of life on earth was the transition of plants from water to land. The moss Physcomitrella patens is an exceptional model system for understanding the processes that aided the transition to terrestrial environments, due to its simple body plan and limited number of tissue and cell types, its sequenced and annotated genome and the ability to perform stable and efficient targeted genes knockouts. We are interested in protein prenylation, as it is key to many plant growth developmental processes. Knockouts of all eight P. patens putative protein prenyltransferase alpha and beta subunits have resulted in either lethality or mutants with developmental defects. Among the ones that resulted in lethality are PpPPAL1 and PpPPAL2, which suggests that these genes are essential for viability. In order to determine the functional role of PpPPAL1 and PpPPAL2, gene knockdown approaches have been implemented. Preliminary results have shown that PpPPAL1 and PpPPAL2 knockdowns exhibited inhibition of growth but remain viable, facilitating ongoing in-depth phenotypic studies. Such studies of these and other P. patens prenylation mutants can lead to insights into what mechanisms early plants used to adapt to land, the evolution of multicellularity, plant cell differentiation, and the mechanism of plant cell adhesion. Prenylation studies in plants have a variety of applications, including an agricultural role based on the ability of prenylation knockdowns to tolerate drought. These studies also have implications for the field of algae biofuel, as engineering algae to adhere to each other may lower the energy cost of harvesting.

Primary Poster Presenter: Susana Perez Martinez

Regulation of cell division in response to the metabolic status of cells in Chlamydomonas (0800-021)
Hall 2

Many microalgae accumulate oil in the form of triacylglycerol in response to nutrient deprivation, but also enter a state of quiescence, which impedes biomass production. Therefore, the regulation of cell division in response to the metabolic status of the cell is of interest for the optimization of microalgal feedstocks for biofuel and natural compound production. Using Chlamydomonas as our model, we have identified a protein (COMPROMISED HYDROLYSIS of TRIACYLGLCYEROL, CHT7), that represses cell cycle genes during N deprivation. This conclusion is

based on detailed cell biological analysis of the CHT7 loss-of-function mutant and global and targeted transcriptomic analysis of synchronized cultures of Chlamydomonas wild type and cht7 mutant grown under different conditions in bioreactors. The CHT7 protein belongs to a group of CXC domain proteins some of which are components of the DREAM complex, a large nuclear complex involved in the transcriptional co-regulation of extensive sets of genes. Several CXC domain proteins have been shown to bind DNA directly through their CXC domain. However, the CXC domain of CHT7 in Chlamydomonas is dispensable for function. Detailed deletion analysis of the CHT7 protein and testing of rescue of the cht7 mutant phenotypes by specific mutants identified a small, essential domain near the C-terminus, which is likely involved in protein-protein interactions affecting the abundance of the CHT7 protein complex. We are currently searching for other protein components of the complex and are studying the behavior of the complex during the cell cycle and in response to nutrient deprivation. Understanding how the CHT7 complex affects cell cycle gene activity in response to N deprivation will ultimately allow us to design novel strategies for the optimization of microalgal feedstocks.

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Primary Poster Presenter: Christoph Benning

The Effects of Isoprene Emissions on Senescence in Velvet Beans (0800-023)

Hall 2

Many plant species invest a portion of their fixed carbon into the synthesis of isoprene which affects air quality and reinforces global warming. Isoprene,synthesized in chloroplasts and released depending on temperature and light, is important in plant defense against environmental stressors,maintaining membrane integrity via its antioxidant activity.This study looked at if variations in isoprene emission rates, adjusted by the environment,affect plant development and senescence. Potted velvet bean (Mucuna pruriens),an isoprene emitter, was grown in greenhouse chambers at very high(39°C), high(35°C), intermediate(30°C)and low(25°C)temperatures, exposed to either 1100(high light)or 0400(low light) µmolm-2s-1 irradiances of photosynthetically active radiation(PAR), to change isoprene emission rates throughout the developmental cycle of plants. We found that plants with the highest isoprene emission rates(grown under high light and temperature conditions) showed a substantial delay in the onset of senescence(indicated by the absence of detectable reactive oxygen species and lipid peroxidation product accumulation or pigment degradation), as compared to plants grown under intermediate or low temperatures and lower irradiances, with suppressed emission rates.Plants with the highest isoprene emission rates also accumulated H2O2 at higher rates throughout their lifecycle than plants under the

other tested conditions,while higher H2O2 concentrations were not associated with membrane degradation.The endogenous antioxidant network of these plants was also upregulated when compared to low- or non-emitters. We attribute the detected delay in the onset of senescence in plants emitting isoprene at high rates to the possible antioxidant action of isoprene,capable of neutralizing reactive oxygen species during the onset of senescence, and the priming effect of the sustained higher H2O2 levels,upregulating the plants' endogenous antioxidant network and potentially,isoprene emission.

Primary Poster Presenter: Rachael Prawitz

The role of SWEET sugar transporters in phloem loading in maize and rice
(0800-006)
Hall 2

Carbon allocation from photosynthetically active leaves to seeds is a critical component determining crop yield. We characterize key components of phloem loading and seed filling. Our lab identified the first SUT and SWEET transporters as key players in phloem loading in dicots. In Arabidopsis, AtSWEET11 and 12 export sucrose from the phloem parenchyma, which is actively taken up by SUTs (SUC2) into the sieve element-companion cell complex for long distance translocation (Chen et al., 2012, Science). Subsequently, SWEETs are involved in seed filling in Arabidopsis (Chen et al., 2015, Plant Cell). To determine whether monocots such as maize, rice, wheat and barley use similar mechanisms, we analyze the role and function of SUTs and SWEETs in these crops. Knock-out mutations in AtSWEET11/12 homologs of several crop grasses, including ZmSWEET13a, b, and c in maize, and OsSWEET13 in rice were generated. The necessity of the sucrose proton symporter ZmSUT1 for sucrose import into companion cells for phloem loading in maize (Slewinski et al., 2009, J Exp. Bot.) suggested that sucrose is actively loaded into the phloem from the apoplasm. We show that the three closely related sucrose transporters ZmSWEET13a, b, and c are the primary means of sucrose efflux to the phloem-adjacent apoplasm, as zmsweet13abc plants display severely reduced growth and high accumulation of starch and soluble sugars in the leaves (Bezrutczyk et al., 2018, New Phytol.). However, it is unknown in which part of the vasculature the three ZmSWEET13's are expressed, or if each ZmSWEET13 plays a different role in sucrose transport in the leaf. By contrast, the rice single homolog OsSWEET13 appears inessential for phloem loading, implicating a different loading mechanism in this crop. We also analyze SWEET and SUT functions in seed filling, and found that OsSWEET11 and 15 together are essential for endosperm development (Yang et al., 2018, New Phytol.).

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Primary Poster Presenter: Margaret Bezrutczyk

The role of the circadian clock in the turnover of transitory carbohydrates in the wheat leaf (0800-002)
Hall 2

Plants accumulate a carbohydrate store in the leaf during the day which is then remobilised over the course of the night to fuel growth and respiration when it is dark. The transitory accumulation of starch in the Arabidopsis thaliana leaf and the circadian regulation of this process is well documented. Previous work has shown that the rate of starch accumulation and degradation can be tailored to suit the photoperiod and that mutations in key circadian clock genes disrupt the timing of accumulation and degradation. However, little is known about the turnover of transitory carbohydrate reserves in the leaves of crop plants such as wheat. It is hypothesised that correct control of the turnover of transitory carbohydrate reserves by the circadian clock is essential for optimal growth. Here, different wheat cultivars and wheat lines with compromised circadian clocks are investigated using a variety of circadian methods to try and elucidate the importance of the circadian clock for growth in wheat.

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Primary Poster Presenter: Laura Taylor

The role of THESEUS1 during cell division in Arabidopsis shoot apical meristems (0800-014)
Hall 2

While plant cells respond to local mechanical and chemical signals from cell walls, it’s unclear how they mediate these cues. THESEUS1 (THE1) is one receptor-like kinase predicted to function as a cell wall sensor that regulates cell expansion in response to altered cellulose content; however, its role in dividing cells remains largely uncharacterized. Thus, we asked if THE1 functions in coordination with cell walls in dividing cells of Arabidopsis shoot apical meristems (SAMs). We found that loss of the cytoplasmic domain of THE1 (THE1Cyto; the1-4) reduced the number of epidermal cells and cell divisions. Loss of function in its putative extracellular domain (the1-1) had no effect on SAMs. This occurred independently of CLV/WUS and cytokinin signaling and without affecting epidermal cell expansion. Instead, loss of THE1Cyto increased the number of cells expressing nuclear CYCB1;1-GFP, without affecting the number of cells with CYCB1;1-GFP localized at the metaphase plate. Moreover, BMF1-GFP and MAD1-GFP localized ectopically along spindles during prometaphase, suggesting that loss of THE1Cyto impedes metaphase by activating the spindle assembly checkpoint. THE1 is not a global cell wall sensor in dividing cells. Loss of xyloglucan rescued SAM size in the1-4 plants while also restoring the number of cells expressing nuclear CYCB1;1-GFP; however, loss of

β-1,4-galactan did not have the same effect in the1-4 SAMs. Mechanically increasing cell wall tension in SAMs lacking THE1Cyto repolarized PIN1-GFP and reoriented microtubules normally, further suggesting that THE1 is not a global cell wall mechanosensor. Together, these data suggest that THE1Cyto is required for dividing cells to properly progress to metaphase through specific coordination with xyloglucan in cell walls.

Primary Poster Presenter: Hanako Yashiro

The spindle assembly checkpoint in plants as a gateway to polyploidization (0800-024)
Hall 2

It is broadly believed that whole genome duplications (WGDs) are an important driving force in species diversification. WGD events commonly occur in plants, especially in angiosperms. At the same time, plant cells can be easily forced to have a duplicated genome, e.g. by application of spindle poisons. This procedure is often used in plant breeding, e.g. for the production of homozygous parents in hybrid breeding. However, the molecular mechanisms behind these doubling processes are still unclear. The spindle assembly checkpoint (SAC) is an M-phase surveillance mechanism that has been well studied in yeast and mammalian cell culture systems due to the implication in cancer. Under stress conditions, the SAC prevents the anaphase onset until all kinetochores are properly attached to spindle microtubules. Recently, we revealed that the SAC is also conserved in the model plant Arabidopsis although it acts under continues stress conditions differently. In animals, cells maintain an active SAC during continues stress conditions for long period, i.e. over 16 hr in human cells. In contrast, Arabidopsis cells shut off the SAC after one to two hours and rebuilt a single nuclear envelope without cell division. The resulting cells contain a duplicated genome since chromosomes were replicated during S phase. This observation implies that the specific SAC regulation facilitates WGD events in plants.

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Primary Poster Presenter: Shinichiro Komaki

Title: Subcellular distribution of nitrate reductases and their levels are controlled by ammonium an (0800-007)
Hall 2

Nitrate reductases (NRs) catalyze the reduction of nitrate to ammonium. Although NR activity is regulated by sumoylation through the E3 ligase activity of AtSIZ1, it is not clear how NRs interact with AtSIZ1 in the cell, or how nitrogen sources affect NR levels and their cellular localization. Here, we show that the subcellular

localization of NRs is modulated by the E3 SUMO(Small ubiquitin-related modifier) ligase AtSIZ1 and the NR protein levels are regulated by nitrogen sources. Transient expression analysis of GFP fusion proteins in onion epidermal cells showed that the NRs NIA1 and NIA2 localize to the cytoplasmic membrane and that AtSIZ1 localizes to the nucleoplasm when expressed separately, whereas NRs and AtSIZ1 localize to the nucleus when co-expressed. Nitrate did not change the localization of the NRs, while AtSIZ1 made some movements from nucleus to cytoplasm in response to nitrate treatment. In ammonium-treated cells, NRs were not detected, but AtSIZ1 continued to localize to the nucleus. NIA1 and NIA2 transcription levels increased in response to both nitrate and ammonium treatment. However, NR protein levels increased after nitrate treatment and decreased after ammonium treatment. In addition, NR protein levels increased after treated with the 26S proteasome inhibitor, MG-132, whereas the levels were much higher in cop1-4 and DN-COP1-Myc6 plants. Furthermore, the degradation rate of NR protein in cop1-4 plants was much slower than that in wild-type plants, although the NR proteins did not interact with COP1. Therefore, AtSiZ1 regulates the nuclear localization of NR proteins, and ammonium negatively affects their levels while nitrate does it the opposite way. The function and stability of NR proteins might be post-translationally modulated by ubiquitination.This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center no. PJ01327601), Rural Development Administration, Republic of Korea.

Primary Poster Presenter: Yuna Kang

TOR coordinates plant growth by dynamically regulating cell-cell transport
(0800-028)
Hall 2

The coordinated redistribution of sugars from mature “source” leaves to support the growth of developing “sink” leaves requires tight regulation of sugar transport between cells via plasmodesmata (PD). Although fundamental to plant physiology, the mechanisms that control PD transport and thereby support development of new leaves have remained elusive. From a forward genetic screen for altered PD transport, we discovered that PD transport is regulated by the conserved eukaryotic glucose-TOR (TARGET OF RAPAMYCIN) signaling hub. TOR is significantly more active in mature leaves photosynthesizing excess sugars than in young, growing leaves, and this shift in activity impacts rates of PD transport. Genetic, chemical, and physiological treatments promoting or disrupting TOR activity support the model that glucose-activated TOR controls PD transport in leaves. An established TOR effector in plants, PP2A, contributes to the control of PD transport during shoot development. We conclude that plant cells regulate PD trafficking in response to changing carbohydrate availability monitored by the TOR pathway.

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TOR kinase tunes autophagy and meristem activity for nutrient stress-induced developmental plasticit (0800-000)
Hall 2

Plants can respond to environmental challenges with comprehensive developmental transitions that allow them to cope with these stresses. It is shown that antagonistic activation of the Target of Rapamycin (TOR) kinase in the root and the shoot is essential for the nutrient deprivation-induced increase in root-to-shoot ratio. We demonstrate that sulfate limitation-induced downregulation of TOR in shoots activates autophagy resulting in carbon allocation to the root. This process is facilitated by specific upregulation of the sucrose-transporters SWEET11/12 in the shoots. SWEET11/12 activation is indispensable to enable sucrose to act as a carbon source for growth and a signal for tuning root apical meristem activity via glucose-TOR signaling. We show that the transcription factor and TOR-substrate, E2Fa, transduces this signal. The sugar-stimulated TOR activity in the root suppresses autophagy and maintains root apical meristem activity to support root growth for mining new sulfate resources in the soil. We expect that our findings not only contribute to the understanding of environment triggered organismal plasticity in plants in general, but enable new modification strategies towards crop plants with enhanced nutrient use efficiency.

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Primary Poster Presenter: Ruediger Hell

Variations in structure, localization, and protein folding activity of protein disulfide isomerases (0800-012)
Hall 2

The classical protein disulfide isomerase (PDI) of eukaryotes is an oxidoreductase that catalyzes the formation, reduction, and isomerization of disulfide bonds in nascent secretory proteins in the endoplasmic reticulum (ER). PDI properly folds target proteins into conformations necessary for stability, catalytic activity, trafficking and protein-protein interactions. PDI has six domains: a signal peptide, two thioredoxin catalytic sites (a, a'), two fold domains (b-b'), and a KDEL ER-retention signal with an acidic C-terminus. The Arabidopsis genome encodes 12 PDIs. Six of them (PDI1, PDI2, PDI3, PDI4, PDI5, PDI6) have the classical domain structure, whereas three closely related PDIs (PDI9, PDI10, PDI11) have lost the b-b' fold domains. A unique member (PDI7), lacks the b-type domains, possesses only one a-type domain, and contains N- and C-terminal transmembrane domains. Here, we examined the subcellular localization and protein folding activity of various PDIs. PDI subcellular localization was studied by transient co-expression of PDI-eGFP fusions with the ER-mCherry marker in Arabidopsis mesophyll

protoplasts. All PDIs co-localized with the ER marker. However, when GFP was positioned internally within PDI7, the fusion strongly colocalized with the cis-Golgi marker, mCherry-SYP31, and did not redistribute to the ER after Brefeldin A treatment. We propose a model where PDI7 functions as a cargo receptor, cycling between the Golgi and ER. Interestingly, PDI9-GFP and PDI10-GFP induced the formation of ER-protein bodies that co-localized with the seed storage albumin-1 (SESA1:mCherry). Several PDIs were found to complement the dsbA protein folding mutant of E. coli and restore alkaline phosphatase activity, which requires multiple disulfide bonds. Six ompA-PDI fusions restored alkaline phosphatase activity to wild type levels or higher, compared to controls. PDI1, PDI3 and PDI4, lacked protein folding activity.

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Primary Poster Presenter: Rina Carrillo

Multiple mechanisms determine subcellular dynamics and stress tolerance function of KORRIGAN 1 (0800-093 (Screen 7))
Hall 2

KORRIGAN 1 (KOR1) is a heavily N-glycosylated plant transmembrane β-1,4-endoglucanase which involves in cellulose synthesis. KOR1 cycles between the Trans Golgi Network (TGN) and the plasma membrane (PM) and is targeted to the developing cell plate during cell division. Our previous study showed that a point mutation in KOR1 (rsw2-1) or N-glycosylation defects of KOR1 promoted tonoplast (TP) targeting. In this study, we investigated the factors involve in targeting and trafficking routes of KOR1 to multiple organelles. The C-terminus of KOR1 contains a proline rich motif (P-motif), of which function is unknown. We observed GFP tagged KOR1 (GFP-KOR1) with deletion or replacement of amino acids in the P-motif in planta. These modifications promoted TP targeting of GFP-KOR1. It was considered that the P-motif was necessary for the cycling of KOR1. To understand details in the targeting mechanism of KOR1, we adapted a β-estradiol inducible promoter and a tandem fluorescent timer (tdFT) for in vivo observation of KOR1 and its variants. tdFT, which consist of sfGFP and mStrawberry is a reporter system to provide time information of proteins by difference in maturation time of fluorescent proteins. In addition, we studied a function of the dileucine motif at the N-terminus of KOR1 in its trafficking because the dileucine motif plays a function in endocytosis in other organisms. These observations revealed KOR1 was delivered to the TP after passing through the PM and the dileucine motif of KOR1 functions in endocytosis. Moreover, confocal microscope analysis showed GFP-KOR1 temporarily internalized under salt stress in order to confer salt tolerance to plants. This internalization, but not the regular cycling of KOR1 was inhibited by 1-butanol, an inhibitor of phospholipase D. Taken together, multiple mechanisms control subcellular distribution of KOR1 to maintain root growth under normal and stressed condition, especially salt stress.

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Primary E-Poster Presenter: Yukihiro Nagashima

Cell Biology: Plastids and Organelles

How plastidial retrograde signaling metabolite regulates adaptive responses? (0800-094 (Screen 10))
Hall 2

Interorganellar communication is an evolutionary necessity for maintenance of cellular homoeostasis in response to prevailing environment that, in part, is exquisitely controlled via retrograde-signaling pathways between organelle and nucleus. We have identified a novel stress-specific plastidial retrograde signaling metabolite, methylerythritol cyclodiphosphate (MEcPP), previously known solely as an intermediate in the isoprenoid biosynthetic pathway. The additional function of MEcPP as a stress sensor and a coordinator of transcriptional and post transcriptional regulation of key stress-responsive nuclear genes, has unraveled the central role of this metabolite in cellular functions in response to a wide range of environmental and developmental cues. To identify the underlying molecular mechanism of the MEcPP-mediated stress responses, we have performed a multi-omics approach. These studies have led to the identification of a transcriptional hub activated by MEcPP, and further established a previously unrecognized link between this plastidial retrograde signal and the transcriptional reprogramming of endoplasmic reticulum genes critical for readjustment of protein-folding capacity in stressed cells. Moreover, we have gained an insight into the molecular mechanism by which MEcPP alters subcellular structures and contributes to phytochemical diversity. In brief we have advanced our understanding of how plastidial retrograde signaling metabolite reprograms a repertoire of intricate networks crucial for coordinating the physiological and metabolic processes required for stress-induced developmental and adaptive responses.

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Primary E-Poster Presenter: Jin-Zheng Wang

Vesiculation of thylakoid membranes in chloroplasts of tomato flower pedicel and leaf petiole is a u (0800-095 (Screen 11))
Hall 2

Abscission of plant organs occurs specifically in the abscission zone tissue as a natural stage in plant development. A detailed transmission electron microscopy

study of cells in the abscission zone of tomato flower pedicels and leaf petioles after the induction of abscission showed degradation of the chloroplasts. This disruption was associated with thylakoid membrane vesiculation, which culminated in formation of numerous vesicles in the chloroplast periphery. Simultaneously, transcription of all of the main genes involved in all of the phases of photosynthesis was significantly down-regulated. Of note, formation of vesicles and degradation of thylakoids were prevented when the plants were pre-treated with an inhibitor of ethylene action, 1-methylcyclopropene. Similar accumulation of peripheral vesicles in plastids has been reported recently in mutants of proteins, suggested to have roles in ongoing, active and protein-mediated vesicle transport associated with thylakoid biogenesis: CV, CPRabA5e, CURT1A, CURT1 C, THF1 and SCO2. During abscission, the transcript levels of the gene that encodes CV was significantly higher compared to un-abscissed plants. On the other hand, the genes that encode CPRabA5e, CURT1A, CURT1 C, THF1 and SCO2 were down-regulated. However, only the transcription of CURT1A appears to be affected by ethylene.

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Mitophagy in Arabidopsis induced by a disruption in mitochondrial membrane potential (0800-097 (Screen 12))
Hall 2

Mitochondria are at risk from redox reactions in the electron transport chain and recycling of damaged mitochondria is mediated by mitophagy. Reduction in membrane potential is a sign of aberrant mitochondria and it activates the mitophagy machinery in mammalian cells. “Uncoupling” agent like 2,4-dinitrophenol (DNP) can shuttle protons across the inner membrane, ruining the membrane potential. To test whether DNP can induce mitophagy in plants, we incubated Arabidopsis roots in DNP-supplemented medium. DNP caused compromise in membrane potential, preferential degradation of mitochondrial proteins, ubiquitination of some mitochondrial membrane proteins. MG132 suppressed the breakdown of mitochondrial outer membrane proteins but not matrix proteins, providing evidence that turnover by proteasomes contributes to mitochondrial recycling. The mitochondrial protein degradation was inhibited in atg5 mutant roots. DNP-induced mitochondrial protein recycling accompanied ATG8 modification by phospholipid moieties and recruitment of ATG8-YFP to mitochondria. In time-lapse videos, ATG8-YFP fluorescence associated with a mitochondrion grew along its surface to enclose the mitochondrion completely within five minutes. Under TEM, mitochondria with collapsed cristae and damaged mitochondria sequestered in mitophagosomes were discerned. It could be concluded that mitochondria with

impaired proton gradient are selectively recycled by mitophagy in plants and assembly of ATG8-positive phagophores and proteasome-mediated turnover are involved in the recycling.

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Primary E-Poster Presenter: Byung-Ho Kang

OR-His physically interacts with plastid division factor ARC3 to regulate chromoplast division and c (0800-096 (Screen 7))
Hall 2

Chromoplasts are the colored plastids that synthesize and store massive amounts of carotenoids. Chromoplast number and size define the metabolic sink strength for carotenoid accumulation in plants. Yet nothing is known about the mechanism underlying chromoplast division. The OR-His protein represents a key regulator of chromoplast biogenesis to govern carotenoid accumulation in plants. However, it restricts chromoplast duplication with only one or two large chromoplasts per affected cell. We investigate how OR-His constrains chromoplast division. OR-His does not affect plastid division gene expression. Both in vitro and in vivo evidence demonstrate that OR-His specifically interacts with the MORN domain of ARC3, a crucial regulator of chloroplast division. In addition, OR-His was found to interfere with the interaction between ARC3 and PARC6, another key regulator of chloroplast division. These results show that OR-His acts as a competitor of PARC6 to hinder ARC3 and PARC6 interaction, causing restricted chromoplast division. Moreover, we found that over-expression or knockout of ARC3 significantly alters carotenoid levels. Furthermore, upregulation of another plastid division factor PDV1 greatly enhances carotenoid accumulation. These plastid division factors was discovered to affect carotenoid levels via altering chromoplast number and size. Taken together, our findings provide novel mechanistic insights into chromoplast division and document a new strategy by manipulating plastid division factors for carotenoid enrichment in crops. Broad Impact: Carotenoids are indispensable to plants and humans. However, nothing is known about the mechanism underlying chromoplast duplication and the effects of plastid division factors on carotenoid accumulation. This study not only establishes a hitherto unidentified mechanism of chromoplast division, but also provides a novel strategy to enrich carotenoids in crops with improved nutritional quality.

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Primary E-Poster Presenter: Li Li

Electrical conductivity of the thylakoid membrane in the presence of a constitutively assembled Tat (0800-099 (Screen 14))
Hall 2

The chloroplast Tat pathway mediates the transfer of a subset of proteins across the energy-transducing thylakoid membrane. This pathway is characterized by a translocation machinery composed of three subunits, Tha4, Hcf106 and cpTatC. During the resting state, Tha4 Hcf106 and cpTatC form a complex with a probable 1:1:1 stoichiometry. Upon energization of the membrane and in the presence of a transport substrate, Tha4 joins the resting complex in much higher abundance, and this active translocation complex then dissociates after the transport event. It has been reported that upon addition of a severely truncated cpTat substrate the transport machinery forms but does not dissociate, making a de facto constitutively assembled translocation complex. We report experiments designed to determine if this complex leaks ions, as would be expected from a stable proteinaceous pore. We applied a truncated version of the well-known prOE17 cpTat substrate, SpF20, to isolated thylakoids under transport conditions. We show that SpF20 engages the cpTat machinery but is not translocated into the thylakoid lumen. We further show that this causes Tha4 to remain in contact with the Hcf106/cpTatC complex, indicating that the translocation complex has not dissociated and remains intact. Thylakoids containing this stably assembled cpTat complex were then used to monitor the electrical conductivity of the thylakoids after imposition of a flash-induced electric field via monitoring of the well-characterized carotenoid electrochromic shift. We measured an identical decay rate of the electric field in the presence and absence of the constitutively assembled cpTat translocation machinery. This is not the behavior expected if proteins on the cpTat pathway cross the membrane through a proteinaceous pore. Instead, we proposed that this result is consistent with the cpTat pathway operating via transient and localized membrane defects through which substrates cross the membrane.

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Primary E-Poster Presenter: Steven Theg

Allotopic expression of mitochondrial nad7 in the nucleus restores complex I activity and plant grow (0800-049)
Hall 2

Plant pentatricopeptide repeat (PPR) proteins are mostly involved in chloroplast or mitochondrial RNA metabolism. Loss-of-function in the PPR proteins is often associated with strong phenotypes in plant growth and development. However, direct evidence that correction of the molecular defects in the organelles can restore the plant phenotypes has yet to be demonstrated in a ppr mutant. To study genes that are important for plant growth, we have isolated a collection of slow growth (slo) mutants in Arabidopsis. One of the slo mutants, slo3, is defective in a nuclear gene encoding a mitochondrion-localized PPR protein. Analysis of

mitochondrial RNA metabolism revealed that the slo3 mutant was impaired in the splicing of nad7 intron 2. This molecular defect may cause a reduction in complex I activity and eventually affect plant growth and development in the slo3 mutant. Since mitochondrial transformation is still a challenging technique in plants, we used an alternative approach to demonstrate that transformation of correctly spliced nad7 into the nuclear genome and targeting the Nad7 subunit into mitochondria can restore the complex I activity and the growth defects of the slo3 mutant. Together, these results provide direct evidence that the strong growth and developmental phenotypes of the slo3 mutant are caused by defects in mitochondrial nad7. Given that many ppr mutants have strong phenotypes, and the lack of an efficient mitochondrial transformation protocol, the technique developed here can be used to provide direct evidence for the function of a mitochondrial gene and may eventually be important for applications in agricultural biotechnology.

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Primary Poster Presenter: Ming-Hsiun Hsieh

Chaperone-assisted membrane protein trafficking in the chloroplast stroma (0800-041)
Hall 2

The chloroplast's thylakoid membrane houses photosynthetic light-harvesting. Thylakoid development depends upon coordinated synthesis, delivery, and assembly of nuclear- and chloroplast-encoded proteins. While cells often couple the membrane integration of transmembrane proteins to translation, all nuclear-encoded and half of chloroplast-encoded thylakoid proteins insert post-translationally. Chaperones likely facilitate transit of these proteins through the aqueous stroma. Recent data suggest that, for some proteins, this assistance may be provided by the chloroplast chaperonin (Cpn60), known for its essential role folding the large subunit of Rubisco. Nuclear-encoded Plastidic type I signal peptidase 1 (Plsp1) integrates into thylakoids via the cpSec1 translocon, which carries unfolded proteins. Plsp1 resides in a 0700-kDa complex during in vitro import assays. We identified Cpn60 α1 and β1 as stromal proteins which directly interact with Plsp1 by an in vitro pulldown assay. Plsp1 binds Cpn60 oligomers in vitro in a complex identical in size to Plsp1's stromal complex, and Cpn60-bound Plsp1 resists degradation by exogenous protease, a characteristic of chaperonin-bound proteins. Examination of the fate of Cpn60-bound Plsp1 in chloroplasts suggests Cpn60 interaction to be an intermediate prior to thylakoid integration. Increasing membrane integration correlated with decreasing association with Cpn60 in import-chase assays. Plsp1's release from Cpn60, assessed by increased protease susceptibility, required ATP hydrolysis; however, Cpn60 re-bound Plsp1 until occupied by other stromal proteins, like Rubisco. Transport assays into isolated thylakoids demonstrated that, when Plsp1 was released from a Cpn60 subsequently

occupied by another substrate, cpSec1 recognized Plsp1 and facilitated its integration. Understanding the mechanism by which Cpn60 exchanges Plsp1 with cpSec1 provides insight into how chloroplasts ensure delivery of proteins to thylakoid membranes.

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Primary Poster Presenter: Laura Klasek

Chloroplast helicase ISE2 reveals a link between PD-mediated trafficking and secondary metabolism (0800-039)
Hall 2

Plants have evolved plasmodesmata to communicate directly via the cytoplasm. Plasmodesmata traffic nutrients and signaling molecules including micro and macro-molecules, hormones, small RNAs, and proteins. The signaling networks that regulate the formation and function of plasmodesmata remain poorly understood. In Arabidopsis thaliana ise2 mutants and Nicotiana benthamiana ISE2-silenced leaves, there was increased intercellular trafficking. We found that in those Arabidopsis plants with decreased ISE2 expression, the expression of genes within the glucosinolate biosynthesis pathways and the first step of the methyl-D-erythritol 4-phosphate (MEP) pathway are affected. The metabolite methylerythritol cyclodiphosphosphate (MEcPP), an intermediate of the MEP pathway, is a known inducer of the glucosinolate pathway. This suggests a connection between glucosinolate metabolism and intercellular trafficking via plasmodesmata. We hypothesize that changes in ISE2 expression results in reprograming of nuclear gene expression, including the genes of the glucosinolate pathway, ultimately affecting plasmodesmata regulation. Here, we measured the levels of various glucosinolates in plants with varying levels of ISE2 expression through HPLC. In plants with decreased ISE2, there is an elimination of aliphatic glucosinolates derived from methionine while other glucosinolates are increased. The results from this analysis helps us identify the link ISE2 plays in glucosinolate metabolism and plasmodesmata trafficking.

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Primary Poster Presenter: Jessica Fernandez

Chloroplast Protein Homeostasis; proteolytic networks, protease substrates and the N-degron pathway (0800-037)
Hall 2

Intra-chloroplast maturation and proteolysis is essential in biogenesis, differentiation and protein homeostasis (proteostasis). However, determinants of chloroplast protein life-time and protease-substrate relationships are poorly understood, even if this is of critical importance for plant life. Protein N-termini are major determinants of protein stability in bacteria, eukaryotes, and perhaps also in

chloroplasts. To better understand chloroplast protein maturation and stability, and to provide a base line for protein degradation studies, we determined chloroplast protein N-termini using terminal amine isotopic labeling of substrates (TAILS) and mass spectrometry. This showed highly specific N-terminal patterns, suggesting a chloroplast N-end rule for protein stability. The Clp protease system is the most complex and abundant protease in chloroplasts, and consists of a protease core, several chaperones and adaptors. Structural and functional features of the plastid Clp system in Arabidopsis thaliana will be illustrated though reverse genetics analysis combined with biochemical analysis, X-ray crystallography, as well as large scale quantitative proteomics for loss-of-function mutants. Multiple substrates were identified based on their direct interaction with the ClpS1 adaptor (N-recognin), by in vivo trapping on affinity tagged AAA+ CLPC chaperone, and by screening of different loss-of-function protease mutants; we discuss the potential role of Clp in fine-tuning chloroplast metabolism.

Primary Poster Presenter: klaas van wijk

Direct and Indirect Effects of Cytokinin on Chloroplast Development and Function (0800-045)
Hall 2

The phytohormone cytokinin plays a pronounced role in both chloroplast development and function. To gain a deeper insight into the mechanism of cytokinin action, we used a ChIP-seq approach to identify gene targets of the type-B ARABIDOPSIS RESPONSE REGULATOR10 (ARR10), one of the primary cytokinin-response transcription factors (TFs). Among the ARR10 targets, several genes were identified that directly participate in chloroplast processes, including chlorophyll biosynthesis and photosynthesis (LHCB6, HEMA1, CHLM). Additionally, we found that ARR10 binds to promoters of genes belonging to two transcription factor families (GNC and GKL families) that function as “master controllers” of chloroplast biogenesis. We further explored how members of the GNC family stimulate chloroplast formation and clarified specific and overlapping roles of GNC and GLK families in the coordination of chloroplast development. DNA-binding motifs for GNC were identified through use of protein-binding microarrays, and their enrichment in transcriptome datasets indicated that GNC functions primarily as a transcription repressor. ChIP-seq analysis showed that GNC binds to promoters of PHYTOCHROME INTERACTING FACTORs (PIFs) and brassinosteroid activity genes, repression of which will facilitate chloroplast biogenesis. GNC also directly represses the expression of genes involved in ERECTA signaling and thereby facilitates stomatal development. Based on the findings, we propose a model in which cytokinin uses both direct and indirect pathways to influence chloroplast development and function.

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Elucidating organelle dynamics by characterizing pexophagy in Arabidopsis (0800-042)
Hall 2

Peroxisomes are organelles present in almost all eukaryotic cells, where they sequester reactions that generate harmful byproducts, such as hydrogen peroxide. In plants, these reactions include the beta-oxidation of fatty acids and hormone processing. Protein import and maintenance for these organelles are sustained by peroxin (PEX) proteins. However, the details of peroxisome homeostasis are not fully elucidated, and the mechanisms that target old, superfluous, or damaged peroxisomes for degradation remain enigmatic, especially in plants. Pexophagy is the selective autophagy of peroxisomes, where a double membrane engulfs a target and then fuses with the plant vacuole for recycling of its constituents. We are investigating pexophagy in Arabidopsis by monitoring peroxisomal protein levels under various autophagy-inducing conditions. Under starvation conditions, many organisms activate autophagy pathways, and in plants, one way to induce starvation is by deprivation of light, and therefore the ability to photosynthesize. We found that when seedlings were subjected to darkness, the levels of several peroxisomal proteins decreased, including peroxisomal malate dehydrogenase, peroxisomal hydroxypyruvate reductase, and PEX5. This decline was not observed in autophagy-defective mutants, and various pex mutations accelerated or impeded the degradation of these proteins, suggesting that perturbing PEX function can impede or accelerate pexophagy in addition to impacting peroxisome biogenesis. Illuminating peroxisomal regulation via pexophagy in Arabidopsis could allow modification of plant metabolism and stress response for agricultural benefit.(This research is supported by the NSF and the Welch Foundation.)

Co-author(s): Bonnie Bartel

Primary Poster Presenter: Kathryn Smith

Establishing and exploring a long-lived albino plant system from variegated 'Golden Pothos' for p (0800-036)
Hall 2

Albino plants can be used to study plastid development and apply to biological studies where chlorophyll effects have to be avoided. Although albino plants widely exist resulting from genetic mutations, such as natural mutation, chemical or physical mutagen induced mutation, tissue/cell culture, and genetic engineering, they are normally lethal or result in severe growth defects because of the lack of photosynthetic capacity. This lethal or severe growth defect limits their availability as sustainable materials for investigations. We took advantage of a long-lived,

vegetatively growing 'Pothos' (Epipremnum aureum) plants resulted from its non-flowering nature (Hung et al., 2016, DOI: 10.1038/srep28598), and utilized tissue culture techniques to create albino plants from color defective sectors of variegated 'Golden Pothos' leaves (Hung and Xie, 2009, DOI: 10.1007/s10535-009-0112-1). Regenerated albino plants were found to have impaired expression of EaZIP, encoding Mg-protoporphyrin IX monomethyl ester cyclase (Hung et al., 2010, DOI: 10.1093/jxb/erq020). These albino plants are viable and have been propagated and maintained on Murashige and Skoog medium with additional ascorbic acids since 2008. To explore its potential towards physiological studies, we first established its Agrobacterium-mediated transformation efficiency and found a transformation rate of ~58% for GFP, and then proved that both of their defective color and dysfunctional chloroplast development can be recovered by overexpressing Arabidopsis CHL27, a homolog of EaZIP. Regenerated albino plants maintained for years with their capacity to recover to green plants and established transformation system can be employed to study chloroplast development and physiological processes likely impacted by chlorophylls, which are difficult to achieve using green plants.

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Identifying regulators of organelle size using the tropical plant genus Peperomia (0800-044)
Hall 2

There is wide variation in the size of chloroplasts across different species and cell types, yet the mechanism by which the cell senses and controls plastid size is unknown. Severe alterations to plastid size can be detrimental, as demonstrated in A. thaliana chloroplast division mutants, some of which have dramatically large chloroplasts that in turn experience high rates of photodamage. We are taking advantage of the extreme natural variation in chloroplast size found within the tropical plant genus Peperomia to identify genes involved in the regulation of chloroplast size. Two species, P. metallica and P. pellucida, develop 2-5 extremely large chloroplasts specifically in their palisade mesophyll cells, whereas in the surrounding spongy mesophyll cells, and in all leaf cell types in other Peperomia species, chloroplasts are more similar in size and number to those of model plants. The reduced number of chloroplasts, coupled with their unusually large size, in the palisade mesophyll of P. metallica and P. pellucida indicates chloroplast division is drastically reduced in this cell layer. To identify candidate genes potentially involved in the development of enlarged chloroplasts in P. pellucida, we generated RNA sequencing data and performed de novo transcriptome assembly for P. pellucida and the small-chloroplast containing species P. dahlstedtii. We performed

differential gene expression analysis to identify genes of interest between expanding leaves and whole seedlings in P. pellucida, in addition to differential expression analyses between orthologous genes of the two species. Our data are valuable genomic resources, not only for understanding the regulation of chloroplast size, but also as no genome sequence is available for Peperomia, an early-diverging angiosperms known to synthesize compounds bioactive against lymphoma and Chagas disease. The findings from our work will answer basic questions on regulation of organelle size during development.

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Primary Poster Presenter: Emily Jennings

Interaction between POTRA domains of TOC75 and TIC22 protein during chloroplast protein import (0800-043)
Hall 2

The protein machineries located on the outer (TOC) and inner (TIC) membranes mediate preprotein import across the chloroplast envelope membranes. While a great deal of information is there regarding the events regulating the import process in the cytosol and the stromal compartments, not much is known about the components involved in the regulation of protein import at the intermembrane space of the chloroplast. Recent work indicate that the transport associated domains (POTRA) of TOC75, the TOC protein import channel, interact with the preproteins in the intermembrane space and possess chaperone like activity, thus facilitating the import process by preventing the misfolding or aggregation of the preproteins as they traverse the TOC and TIC machinery. The TOC75 POTRAs also interact with Tic22, a small chaperone protein in the intermembrane space. In the present work, we aim to understand the interaction between the POTRA domains and Tic22 in the intermembrane space and determine their relationship in facilitating protein import. Tic22 has two isoforms in Arabidopsis: TIC22-III and TIC22-IV, and it has been shown that the double mutant results in inefficient import of preproteins. Expression of POTRA1 deleted TOC75 (TOC75ΔP1) in the tic22-III mutant background resulted in a more severe phenotype than the individual mutants, indicating that the two proteins functionally interact in the intermembrane space. Moreover, using insulin aggregation assay we have demonstrated that Tic22-III also possess chaperone like activity and in vitro import experiments suggests that TOC75ΔP1:tic22-III plants are compromised in protein import. Therefore, we propose that the Toc75 POTRA domains and TIC22-III functionally interact to prevent the misfolding of the incoming preproteins (acting as chaperones) and facilitate the protein import process through the intermembrane space of the chloroplast.

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Primary Poster Presenter: rajneesh singhal

Membrane dynamics in plant autophagy and autophagosome biogenesis (0800-034)
Hall 2

Macroutophagy (hereafter as autophagy) is a conserved metabolic pathways in eukaryotic cells. Autophagy involves a set of Autophagy-related (ATG) genes, but the mechanisms for autophagosome formation are still not fully understood in plant. ATG9 is the only transmembrane protein among the core ATG machinery, and ATG9 vesicles have been long considered as a membrane source for autophagosome formation. Our dynamic and 3D electron tomography analysis demonstrated that, under stress conditions, deficiency of ATG9 leads to a drastic accumulation of autophagosomal tubules with direct connection to the endoplasmic reticulum (ER). Such defect is not detected in other atg mutants, implying that Arabidopsis ATG9 might play a distinct role for autophagosome outgrowth from the ER, in particularly under ER stress. Using a combination of fractionation, cellular and in vitro analysis, here we will present our recent findings on the molecular mechanism of ATG9 vesicle and its trafficking in plant autophagy and autophagosome biogenesis. Supported by grants from the Research Grants Council of Hong Kong (G-CUHK404/18, C4002-17G, R4005-18F, and AoE/M-05/12), and CUHK Research Committee, and the National Natural Science Foundation of China (31670179, and 91854201).

Primary Poster Presenter: Xiaohong Zhuang

Reduction of Differentiation and Greening Like by iCRISPRi Reveals a Novel Stress Signaling Route (0800-047)
Hall 2

To combat environmental stresses, plants rely on various defense mechanisms. The forefront of these processes is arguably attributed to chloroplast metabolism. Not only do chloroplasts produce a number of defensive chemicals, they are important organelles for plant hormone synthesis. Any physiological and developmental perturbation of chloroplasts can trigger a cohort of intracellular metabolic changes. In this project, we characterized the function of a nuclear-encoded chloroplast protein, called Differentiation and Greening Like (DAL), in a quantitatively controlled manner using a novel inducible CRISPR interference (iCRISPRi) approach. Gradually reducing DAL transcripts progressively inhibits early seedling growth of the mutants and eventually causes editing errors in multiple cytidine sites of their chloroplast mRNAs as happened in dal null mutants. To understand the underpinning mechanism, we analyzed the transcriptome changes at the early growth inhibition stage in dal-iCRISPRi mutants. We surprisingly found that the mutants significantly upregulated their responses to two defense hormones, salicylic acid and ethylene, and that the corresponding innate immune response was boosted while the chloroplast RNA editing machinery retained normal. In opposite, multiple sugar metabolic pathways were nearly shut down. To verify these results, in addition to the finding of highly elevated hydrogen peroxide in the dal-iCRISPRi mutants, we were able to apply different concentrations of sucrose to

partially rescue their growth inhibition. Consistent with the oxidative stress responses, we further identified by yeast two-hybrid library screen that DAL binds strongly to a flavonol synthase and a peptidemethionine sulfoxide reductase in addition to catalase and peroxidases that we identified previously. Collectively, our data suggest that DAL is a key element in multiple enzymatic complexes involved in oxidation reduction in addition to its role in chloroplast RNA-editing.

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Primary Poster Presenter: ZHIHUA HUA

Scanning electron tomography analysis of De-etiolation of Etioplasts into Chloroplasts in Arabidops (0800-038)
Hall 2

If a seed germinates under darkness, its proplastids develop into etioplast, which is characterized by the absence of chlorophylls and the presence of large crystalline structures known as the prolamellar body (PLB). Upon illumination, the etioplasts rapidly convert into chloroplasts through a greening photomorphogenesis. In this work, a combination of high-pressure freezing (HPF) and scanning transmission electron tomography (STET) were employed to monitor the transformation from etioplast to chloroplast in three dimensions at nanometer-level resolutions. Samples were collected at 0, 1, 2, 4, and 8 hours after illumination and intermediates of chloroplast biogenesis were examined using the IMOD suite and the image processing tools of MATLAB.Before the onset of de-etiolation, the etioplast was seen to consist of PLBs exhibiting parallelepiped arrangement and short strands of porous prothylakoid around PLBs. Within the first hour of illumination, a stochastic assortment of tubules wedged between the paracrystalline PLB were discerned and the prothylakoids elongated. The disorganized PLBs quickly displaced the paracrystalline architecture at the first hour of illumination onward. In the second hour samples, prothylakoids which proliferated was then observed to constitute bundles. After four hours of lighting, etioplasts displayed signs of stacking such as twists and folds over each other. By the eighth hour, the pro-granal folds enlarge and comprise more stacks while PLBs have disappeared almost completely. Key factors of the etioplast-to-chloroplast conversion in Arabidopsis will be identified with RNA-Seq, immunoblot, and immunogold labeling studies.

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Primary Poster Presenter: Zizhen Liang

Signal Transduction Pathways of Chloroplast Quality Control (0800-035)
Hall 2

Constant oxidative damage is the high cost of photosynthesis and energy production by chloroplasts. As such, a functioning photosynthetic cell must have quality control mechanisms that monitor the turnover and degradation of reactive oxygen species (ROS)-damaged chloroplasts and chloroplast components. We have recently described a conditionally lethal mutation in Arabidopsis that leads to the accumulation of excess protoporphyrin IX (Proto) in the chloroplast and the production of singlet oxygen (1O2). Damaged chloroplasts are subsequently ubiquitinated and selectively degraded. A genetic screen identified the Plant U-Box 4 (PUB4) E3-ligase as being necessary for this process and pub4-6 mutants have defects in stress adaptation and longevity. Together, these results describe a new class of chloroplast signal that leads to the targeted removal of ROS overproducing chloroplasts. To understand the mechanism behind this pathway, we are taking multiple approaches. 1) To identify new genes involved in this pathway, we are mapping several newly isolated mutants including one gain-of-function allele from an activation-tagging genetic screen. 2) We have recently begun identifying metabolite signatures generated during chloroplast 1O2 stress that may act as secondary messengers to initiate cellular degradation. 3) Furthermore, we are aiming to identify the chloroplast protein ubiquitination targets that may regulate selective chloroplast turnover. This has led to the identification of one candidate protein, whose accumulation affects chloroplast development and their ability to withstand severe oxidative stress. With these studies, we hope to understand a fundamental process that ensures productive energy capture and protects photosynthetic cells under dynamic environments. (This work has been generously supported by a Basic Energy Sciences grant from the Department of Energy)

Primary Poster Presenter: Jese Woodson

Suppression screens of peroxisome-defective mutants reveal novel roles for PEX3 in peroxisome f (0800-046)
Hall 2

Peroxisomes are vital eukaryotic organelles that support a diverse range of metabolic pathways critical to plant development, including steps in hormone production and lipid metabolism. Reactions hosted within the organelle are catalyzed by cytosolically-synthesized enzymes that are imported into the peroxisome matrix through the actions of several peroxin (PEX) proteins on the peroxisome membrane. While we have a general framework for understanding peroxin roles, we lack a complete molecular understanding of the activity and specific sequence of events through which peroxins function. To further dissect the import process, we screened for mutant suppressors that restored post-germinative growth to pex12-1 and pex6-1, which display severe growth defects stemming from disrupted matrix-protein import.PEX12, a ubiquitin-protein ligase, and PEX6, an AAA ATPase, are peroxins with critical roles in recycling the cargo receptor PEX5 that recruits matrix proteins for peroxisomal import. Both pex12-1 and pex6-1

inefficiently recycle the PEX5 receptor, resulting in partial mislocalization of the beta-oxidative enzymes necessary to utilize oil bodies that fuel pre-photosynthetic growth. Our independent pex12-1 and pex6-1 suppression screens recovered nearly identical nonsense mutations in PEX3B, providing the first pex3b mutants to emerge from forward-genetic screens. Paradoxically, both pex3b mutants markedly improved growth without restoring matrix-protein import, suggesting that previously unrecognized pex12-1 and pex6-1 defects contribute to their stunted growth. PEX3B is implicated in pre-peroxisome budding from the ER and peroxisomal membrane protein insertion. Elucidating these suppression mechanisms may reveal novel roles for PEX3B in oil body or peroxisome dynamics. (This research is supported by the NIH.)

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Primary Poster Presenter: Roxanna Llinas

Tic236 links the chloroplast outer and inner membrane translocons (0800-048)
Hall 2

The two-membraned envelope is a defining feature of chloroplasts. Chloroplasts evolved from a Gram-negative cyanobacterial endosymbiont. During evolution, genes of the endosymbiont were transferred to the host nuclear genome. Most chloroplast proteins are synthesized in the cytosol as higher molecular mass preproteins with an N-terminal transit peptide. Preproteins are transported into chloroplasts by the TOC and TIC (translocons at the outer and inner envelope membranes of chloroplasts, respectively) machineries, but how TOC and TIC are assembled together is unknown. Here we report the identification of a new TIC component Tic236, which is an integral inner-membrane protein projecting into the intermembrane space a 230-kD domain that directly binds the outer-membrane channel Toc75. TIC236 knockout mutation is embryonically lethal. In TIC236-knockdown mutants, less inner-membrane channel Tic20 was associated with Toc75 and the amount of TOC-TIC supercomplexes was reduced, resulting in a reduced import rate into the stroma, but outer-membrane protein insertion was unaffected. The size and the essentiality of Tic236 indicate that, unlike mitochondria in which the outer and inner membrane translocons exist as separate complexes and a supercomplex is only transiently assembled during preprotein translocation, a long and stable protein bridge in the intermembrane space is required for protein translocation into chloroplasts. Furthermore, Tic236 and Toc75 are homologs of bacterial inner membrane TamB5 and outer membrane BamA, respectively. Our evolutionary analyses show that, like Toc75, Tic236 is only preserved in plants and has co-evolved with Toc75 throughout the plant lineage, suggesting that the backbone of the chloroplast protein import machinery evolved from the bacterial TamB-BamA protein secretion system.

Primary Poster Presenter: Hsou-min Li

Development: Reproduction

Characterization of novel biological processes in fruit crops (0900-060 (Screen 11))
Hall 2

Ripening is a well-characterized process during fruit development. However, fruit crops represent several variations in this ubiquitous process. European pear (Pyrus communis L.), a climacteric fruit, represents one such anomaly where the fruit are harvested at maturity but in an unripe state. Ripening can only be achieved by incubating the fruit in a genetically pre-determined amount of cold during postharvest stages. Further, the use of an ethylene receptor inhibitor, 1-methylcyclopropene (1-MCP) on the unripe fruit results in permanent 'locking' of ripening. We identified that the alternate respiratory pathway is activated during pre-climacteric stages, unlike other climacteric fruit, as the fruit undergoes cold conditioning. Using this information, a chemical genomics approach was used to ripen 1-MCP treated fruit. We hypothesize that chemical activation of the alternative respiratory pathway activates the TCA cycle leading to the generation of ethylene. We have utilized this recently patented technology to enable the development of high quality fresh sliced pears. Sweet cherry (Prunus avium L.), taxonomic kin of pear in the Rosaceae family, is characterized as a non-climacteric fruit. However, exogenous application of ethylene induces the formation of an abscission zone at the fruit pedicel junction. We identified an ethylene-inducible variant of an ERF transcription factor in 'Bing' cultivar. Incorporation of this allele in breeding strategies or editing could facilitate mechanical harvesting of this important crop.

Primary E-Poster Presenter: Amit Dhingra

LORELEI and its most closely related paralog, LLG1, show evidence of regulatory subfunctionalization (0900-061 (Screen 15))
Hall 2

LORELEI (LRE), and its most closely related paralog LLG1 (LORELEI-LIKE GPI-Anchored Membrane Protein 1) arose from the most recent whole genome duplication (WGD) in Brassicaceae. lre and llg1 mutants in Arabidopsis have no overlapping phenotypes; hence, we hypothesized that LRE and LLG1 were maintained post gene duplication because the two genes split the functions of the ancestral single copy gene (subfunctionalization) found in Cleome violacea (a member of the Cleomaceae, a sister group to Brassicaceae). To test this hypothesis, we performed cross-complementation experiments with LRE and LLG1 and showed that each gene can complement the defects caused by the loss of the

other gene. Additionally, we used the single copy gene of LRE/LLG1 in Cleome violacea (CleviLRE//LLG1) to complement Arabidopsis thaliana lre and llg1 mutant phenotypes. Successful cross complementation results led us to propose another explanation for retention of both genes post duplication: the expression domains in the promoters of LRE and LLG1 diverged in a non-overlapping manner, allowing both genes to perform similar functions but in different tissues and cells (regulatory subfunctionalization hypothesis). Using promoter:GUS transcriptional fusions, we found that LRE and LLG1 have distinct expression in Arabidopsis. Additionally, we showed that CleviLRE/LLG1 is expressed in Cleome ovules and vegetative tissues. Additional diversification in the expression of these two genes have been reported, as LRE, but not LLG1, is a maternally-imprinted gene. These results strongly support the regulatory subfunctionalization hypothesis that post gene duplication, LORELEI and LLG1 maintained their molecular functions, but have divergent expression.

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The Role of CLV2/CRN in Floral Primordium Development in Arabidopsis thaliana (0900-062 (Screen 7))
Hall 2

Understanding the pathways that control plant development is critical in building a more complete account of how plants grow, especially with changing environmental conditions. This process still lacks mechanistic knowledge at the level of receptors and ligands. In the shoot, the CLE peptide CLAVATA3 (CLV3) limits stem cell production by signaling through receptor-like kinases such as CLAVATA1 (CLAVATA1) and the receptor complex of CLAVATA2 (CLV2) and CORYNE (CRN). Mutations in any of these genes cause an over-proliferation of stem cells and thus an excess of floral organs. We find that crn and clv2 mutants exhibit an additional phenotype that involves a pause in development along with a period of floral primordia termination. Interestingly, floral primordia termination is both temperature- and light-dependent which is often indicative of an imbalance of auxin, which we find to be disrupted in crn and clv2 backgrounds. Furthermore, we find that this pathway is CLV1-independent, which points to a novel pathway involving CLV2/CRN that is specific to floral primordia development and that likely relies on additional non-CLV3 CLE ligands. We show a unique function of the CLV2/CRN receptor complex in adapting to various environmental conditions for proper floral development. In this way, we provide a new mechanistic look at a pathway that can integrate external environmental signals and translate it into intercellular hormone signals to allow for proper floral development.

Primary E-Poster Presenter: Amala John

The subsequent story and function of MARIS and its orthologs in Arabidopsis pollen formation (0900-063 (Screen 15))
Hall 2

In flowering plants, male gametes are delivered to female gametes for double fertilization through pollen tubes. Therefore, pollen tube growth is crucial for double fertilization. Dispite its importance to sexual reproduction, genetic mechanisms of pollen tube growth remain poorly understood. In this study, we characterized the receptor-like cytoplasmic protein kinase (RLCK) gene, MARIS (MRI) that plays critical roles in pollen tube growth. MRI is preferentially expressed in pollen grains, pollen tubes and roots. Mutation in MRI by a Ds insertion led to burst of pollen tubes after pollen germination. Pollen-rescue assay by pollen and pollen tube-specific expression of MRI in the mri-4 mutant showed that loss of MRI function also severely affected root hair elongation. MRI protein interacted with the protein kinse OXIDATIVE SIGNAL INDUCIBLE1 (OXI1) in the in vitro and in vivo assays, which functions in plant defence and root hair development, and was phosphorylated by OXI1 in vitro. Our results suggest that MRI plays important roles in pollen tube growth and may function in root hair elongation through interaction with OXI1. Subsequently, all orthogues of MARIS genes in Arabidospsis were identified and found that ZDKs genes also play important roles in pollen grain formation and pollen tube growth. ZDKs have 5 genes and only zdk2 zdk4 zdk5 triple-mutant showed more phenotypes, such as abnormal pollen grain, pollen pre-germination in anther. We have obtained more and more enidence to prove MARIS and its orthogues involved in pollen development.

Primary E-Poster Presenter: Xueqin Zhang

A florigen paralog is required for short- day vernalization in a pooid grass (0900-018)
Hall 2

In many plant species, flowering occurs at a particular time of year in response to the sensing of seasonal cues such as changes in day-length and temperature. Many plants adapted to temperate climates have a biennial life history strategy. These plants become established in the fall, overwinter, and flower rapidly in the spring. Essential to this adaptive strategy is that flowering does not occur prior to winter, during which flowering would not lead to successful reproduction. Thus, plants have evolved ways to prevent fall flowering and sense the passing of winter to establish competence to flower. The block to flowering can be alleviated by exposure to prolonged cold or exposure to prolonged period of short-days (SD) which occurs during winter. The process by which flowering is promoted by SD followed by growth in longer photoperiods is known as SD vernalization. Some B. distachyon accessions exhibit SD vernalization whereas others do not. From crosses between

such accessions, we found that SD vernalization segregates as a single locus and the responsible gene is a paralog of FT ("florigen" mobile floral signal) referred to as FT-LIKE 9 (FTL9). Accessions with a functional allele of FTL9 have both the SD and cold responses, whereas accessions with loss-of-function alleles can only respond to cold. There is a striking geographic pattern to the allelic variation: active alleles are found in warmer climates perhaps because in such climates it is adaptive to be able to use SD as a reliable indicator of winter, whereas accessions from places with longer and variable winters have inactive alleles and cannot sense SD vernalization—perhaps because this might lead to premature flowering in conditions in which winter cold would damage delicate flowers. The cloning of FTL9 provides the first molecular insight into the SD vernalization phenomenon.

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Primary Poster Presenter: Daniel Woods

A role for Receptor Kinases in regulating compatible pollen responses in the Brassicaceae stigma (0900-027)
Hall 2

Brassicaceae flowers have evolved mechanisms to recognize their pollen grains as providing nutrients to the wrong mating partner would be disadvantageous. The dry stigmas lack surface secretion which normally would enable automatic pollen germination, and this allows the stigma to tightly regulate pollen acceptance following pollen-stigma contact. The cellular processes in the stigma that facilitate pollen acceptance are becoming more clearly defined, but the initial upstream signalling components are yet to be identified. Previous work in the Goring lab identified a set of receptor-like cytoplasmic kinases, BRASSIKINS (BKNs), as candidate stigmatic signalling proteins in this pathway. However, the BKNs are pseudokinases, which lack essential catalytic motifs, and are likely to function with other signalling proteins such as active kinases in the signalling complex. Thus, we hypothesize that the BKNs mediate signal transduction by forming a complex with membrane-bound receptor kinases to facilitate pollen acceptance and hydration. To address this hypothesis, the BKNs were used to screen for putative protein interactors by testing pairwise combinations with kinase domains from stigma-expressed receptor kinases in the yeast two-hybrid system. Further characterization of the putative interactors identified two distinct clusters of receptor kinases, and so far, the loss-of-function mutants show mild compatible pollen response defects. Current work is focused on creating additional mutant combinations and testing whether these receptor kinases from different Brassicaceae species can rescue the mutant stigma phenotype. Overall, we aim to better understand how these stigma-expressed receptor kinases facilitate the early stages of compatible pollen acceptance and whether they represent a conserved signalling module across the Brassicaceae.

Co-author(s): Daphne Goring

Primary Poster Presenter: Hyun Kyung Lee

A Silk-Expressed Pectin Methylesterase Confers Cross-Incompatibility Between Wild and Domesticat (0900-023)
Hall 2

A central problem in speciation is the origin and mechanisms of reproductive barriers that block gene flow between sympatric populations. In sexually reproducing plants, reproductive barriers exist at different stages during reproduction, including pre-pollination, post-pollination and post-fertilization. Post-pollination barriers depend on interaction between the male gametophyte (pollen) and the cells of the female reproductive organs (stigma, style, and ovule). In Zea mays, three haplotypes, Gametophyte factor1-s (Ga1-s), Gametophyte factor2-s (Ga2-s), and Teosinte crossing barrier1-s (Tcb1-s) at three different loci confer Unilateral Cross-Incompatibility by arresting none-self growing pollen tubes. While Ga1-s and Ga2-s are widespread in domesticated maize, Tcb1-s is almost exclusively found in wild teosinte populations. Despite being members of the same species, some strains of wild teosinte maintain themselves as a distinct breeding population by blocking fertilization by pollen from neighboring maize plants. These teosinte strains may be in the process of evolving into a separate species, since formation of reproductive barriers is a critical step in speciation. These teosinte strains typically carry the Tcb1-s haplotype. Tcb1-s contains a female barrier gene that blocks non-self-type pollen and a male function that enables self-type pollen to overcome that block.With genetic and genomic approaches, here we show that the Tcb1-female barrier gene encodes a Pectin Methylesterase38 homolog, implying that pollen cell wall modification is a key cellular mechanism by which these teostine females reject foreign but closely related pollen. Cloning of this female barrier gene in Zea mays represent a major advance in speciation research and opens up exciting working hypotheses to test. Agriculturally, this work may also help to facilitate breeding effort to manage specialty crop populations and enrich crop germplasm by backcrossing to their ancestors.

Primary Poster Presenter: Yongxian Lu

Abscission Zone Anatomy of Grasses Changes Rapidly in Evolutionary Time

Seed shattering is an economically important agricultural trait. It is the process by which the fruit or seed falls off the plant. Excess or non-uniform shattering reduces crop yield, which makes it the driving force for crop domestication and thousands of years of breeding. Shattering occurs in the abscission zone (AZ) of the plant, which is often described as one or a few layers of small, cytoplasmic dense and non-lignified cells located at the junction of plant organs. So far, a few genes that are attributed to the loss of shattering of domesticated rice, wheat, barley, and maize have been identified. However, whether a common genetic pathway underlies AZ development in different grass species is unclear. Our previous study showed that the anatomy and gene network of the AZ is different in distantly related grass species, including rice, Brachypodium, and Setaria. To further look for conservation and divergence of AZ development across the grass family (Poaceae), we

performed extensive histological analyses of the AZ, using a total of fourteen different species from seven different subfamilies of Poaceae. We hypothesized that the AZ of grass species within the same tribe and/or subfamily would have similar patterns of cell morphology, cell wall composition, and location. To our surprise, safranin O and fast green staining showed no such pattern. Histological characteristics do not correlate with the phylogenetic relationship of the species. Most species exhibit at least one of the typical AZ characteristics, including small cell size and differential lignification, with a few exceptions. These results suggest that the anatomy of the AZ is subject to rapid change during evolution.

Chair and Concurrent Symposium Speaker: Elizabeth A. Kellogg, Yunqing Yu

Primary Poster Presenter: Patricia Leyva

Analysis of stunter2 and stunter3, Maize Maternal Effect Mutants with Reduced Kernel Size (0900-008)
Hall 2

Regulation of growth and development of seeds in plants is largely controlled by the haploid female gametophyte through gene expression following meiosis. stunter2 (stt2) and stunter3 (stt3) are novel maize mutants that disrupt proper development of the female gametophyte, which ultimately affects seed development post fertilization. These two mutants phenocopy stunter1 (stt1), a previously characterized maize mutant with viable but reduced embryos and endosperms and small female gametophytes. stt2 and stt3 embryo sacs are smaller, with smaller central cells and fewer antipodal cells than wild type. Additionally, both mutants exhibit reduced transmission through the male gametophyte. Like stt1, stt2 and stt3 pollen grains are smaller, and the stt2 mutation negatively affects pollen tube germination. Post-fertilization, both embryo and endosperm development is delayed, with stt2 and stt3 exhibiting disruptions in the development of the basal endosperm transfer layer, which facilitates nutrient transport to the developing seed. Whereas stt2 may be allelic to stt1, stt3 is unlinked and represents a unique lesion. These mutants will help elucidate mechanisms for maternal control of seed development in maize.

Co-author(s): Matthew Wendt, Jacob Brandt, Jack Bechtel, Jake Anderson, Elyse Vischulis, Antony Chettoor, Harrison Fleming, Matt Evans

Primary Poster Presenter: Allison Phillips

Anther and pollen viability at high growing temperature rely on effective export of sucrose from lea (0900-002)
Hall 2

Many crops are sensitive to high temperatures and rising global temperatures reduce crop productivity. Plants at the reproductive stage are more susceptible to heat stress due to damage of male (anthers) and female (ovary) reproductive tissues; anthers are more susceptible than the ovaries. Damage includes early tapetal cell degradation, anther indehiscence, pollen grain deformation, and loss of pollen viability. Further, it has been concluded that pollen inviability is mainly due to early tapetum cell degeneration. However, it is not entirely clear why and how tapetum cells undergo accelerated cell death at high temperatures. We hypothesize that transport of sucrose from source leaves is critical to tapetum cell integrity at high temperatures and that it helps confer thermotolerance in plants. To test this, we used a combination of phenotypic, biochemical, and physiological approaches. Analysis of heat-susceptible and heat-tolerant common beans (Phaseolus vulgaris L.) showed that both cultivars have reduced pollen viability and pollen count at high temperature. However, at high temperature, the tolerant bean variety formed significantly more pollen grains compared to the susceptible cultivar. Carbon partitioning analysis further revealed no change in leaf carbon export in the heat-stressed tolerant beans, but a significant decrease was observed in susceptible plants. Moreover, expression analysis of a putative bean phloem sucrose transporter showed significantly reduced expression at high temperature regardless of genotype. Sink tissue metabolite analysis further support the leaf sucrose export data. Reduced carbon status of pollen was hypothesized to be due to increased respiration rate. However, anther respiration measurements revealed significant decrease at high temperature for both genotypes. Our results suggest that sucrose transport from leaf to anthers contribute to heat tolerance of beans and anther respiration does not play a role in pollen "starvation".

Co-author(s): Thomas D Sharkey

Primary Poster Presenter: James Patrick Santiago

Comprehensive characterization of a floral mutant reveals the mechanism of hooked petal morphogenes (0900-016)
Hall 2

The diversity of form of the chrysanthemum flower makes this species an ideal model for studying petal morphogenesis, but as yet, the molecular mechanisms underlying petal shape development remain largely unexplored. Here, a floral mutant, which arose as a bud sport in a plant of the variety 'Anastasia Dark Green', and formed straight, rather than hooked petals, was subjected to both comparative morphological analysis and transcriptome profiling. The hooked petals only became discernable during a late stage of flower development. At the late stage of 'Anastasia Dark Green', genes related to chloroplast, hormone metabolism, cell wall and microtubules were active, as were cell division-promoting factors. Auxin concentration was significantly reduced, and a positive regulator of cell expansion

was down-regulated. Two types of critical candidates, boundary genes and adaxial–abaxial regulators, were identified from 7937 differentially expressed genes in pairwise comparisons, which were up-regulated at the late stage in 'Anastasia Dark Green' and another two hooked varieties. Ectopic expression of a candidate abaxial gene, CmYAB1, in chrysanthemum led to changes in petal curvature and inflorescence morphology. Our findings provide new insights into the regulatory networks underlying chrysanthemum petal morphogenesis.

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Primary Poster Presenter: Lian Ding

Correlation and constraint of self and interspecific incompatibility across the range of Texas Phlox (0900-017)
Hall 2

Many plant species have genetic recognition systems between pollen and pistils that identify and reject inappropriate pollen. Two of the most important systems involve self- and interspecific-pollen recognition. Important outstanding questions are if and how these two recognition systems are mechanistically pleiotropic and if this pleiotropy could constrain the evolution of incompatibility. The hypothesized mechanistic pleiotropy is motivated by the observed correlation across plant species between self and interspecific pollen-pistil incompatibility. We demonstrate a within-species correlation of self and interspecific incompatibility and provide strong evidence that interspecific incompatibility imposes a constraint on the evolution of self-compatibility. Our study characterizes variation in self and interspecific incompatibility in the native Texas wildflower Phlox drummondii. This species has heritable, quantitative variation in self-incompatibility ranging from complete incompatibility to complete compatibility. This variation in self-incapability is significantly correlated with variation in incompatibility with its close congener P. cuspidata. Furthermore, both self and heterospecific incompatibility is due to pollen recognition and rejection at the stigmatic surface. Finally, variation in incompatibility is geographically distributed suggesting the evolution of self-compatibility is constrained by selection favoring interspecific -incompatibility. The two Phlox co-occur and hybridize in a broad area of sympatry in eastern Texas. The resulting hybrids are largely sterile indicating selection could favor increased interspecific -incompatibility in sympatry. As predicted, sympatric populations have significantly higher incompatibility than allopatric populations.

Primary Poster Presenter: Robin Hopkins

Discovery and functional validation of a periodic anthocyanin patterning regulator in Mimulus gutta (0900-021)
Hall 2

Floral color and pigmentation patterning vary greatly among flowering plants, as differences in these traits often alter pollinator behavior and affect floral interactions with the abiotic environment in ways to foster adaptation and speciation. Although much is known how petal hue is specified and varies, far less is known about how pigments are painted into complex patterns during floral development and how these patterns diversify. The ample diversity in floral coloration and preponderant genomic resources for the monkeyflower genus Mimulus make it an ideal group for studying how floral pigmentation patterning develops and evolves. A striking feature of flowers of the common monkeyflower (M.guttatus) is the red anthocyanin spots forming a nectar guide on the ventral lobe of the yellow corolla. By bulked segregant analysis and fine-mapping, we identified independent polymorphisms in the R3-MYB RTO (RED TONGUE) as the genetic bases of blotchy spot variants in multiple wild populations. To validate that these natural loss of function variants cause the variant phenotype, we successfully developed and deployed genome-editing methods for the first time in M. guttatus. Abolishing RTO function with CRISPR/Cas9-introduced frameshifts recapitulated the expanded spot phenotype. Notably, loss of RTO protein function increased RTO expression and expression of the R2R3 MYB transcription factor NEGAN, an activator of anthocyanin production in M.lewisii. RNAi mediated knockdown of NEGAN in M. guttatus confirmed that it promotes petal spot formation and also activates RTO expression. Together, our results from genetic mapping and from newly applying tools for manipulating gene function in M. guttatus indicate that NEGAN and RTO comprise the molecular basis for a reaction-diffusion patterning mechanism. Our data support a model where NEGAN locally activates pigment production and RTO expression, then RTO diffuses to neighboring cells to repress NEGAN and restrict spot expansion.

Co-author(s): Benjamin Blackman,
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Primary Poster Presenter: Srinidhi Holalu

Expressing Putative Invertase Inhibitor in Cellularized Endosperm Delayed Arabidopsis Embryo Growth (0900-019)
Hall 2

Proper seed development requires coordinated growth among the three genetically distinct components, the embryo, the endosperm, and the seed coat. In Arabidopsis, embryo growth rate accelerates after endosperm cellularization, which requires a chromatin-remodeling complex, the FIS2-Polycomb Repressive Complex 2 (PRC2). After cellularization, the endosperm ceases to grow and is eventually absorbed by the embryo. This sequential growth pattern displayed by the

endosperm and the embryo suggests a possibility that the supply of sugar might be shifted from the endosperm to the embryo upon endosperm cellularization. Since invertases and invertase inhibitors play an important role in sugar partition, we investigated their expression pattern during early stages of seed development in Arabidopsis. Two putative invertase inhibitors (InvINH1 and InvINH2) were identified as being preferentially expressed in the micropylar endosperm that surrounds the embryo. After endosperm cellularization, InvINH1 and InvINH2 were down-regulated in a FIS2-dependent manner. We hypothesized that FIS2-PRC2 complex either directly or indirectly represses InvINH1 and InvINH2 to increase invertase activity around the embryo, making more hexose available to support the accelerated embryo growth after endosperm cellularization. In support of our hypothesis, embryo growth was delayed in transgenic lines that ectopically expressed InvINH1 in the cellularized endosperm. Our data suggested a novel mechanism for the FIS2-PRC2 complex to control embryo growth rate via the regulation of invertase activity in the endosperm.

Primary Poster Presenter: Dongfang Wang

Genetic Regulation of Ethylene Dosage for Cucumber Fruit Elongation (0900-064 (Screen 4))
Hall 2

Plant organ growth and development are determined by a subtle balance between growth stimulation and inhibition. Fruit size and shape are important quality traits influencing yield and market value; however, the underlying mechanism regulating the balance of fruit growth to achieve final size and shape is not well understood. Here, we report a mechanistic model that governs cucumber (Cucumis sativus) fruit elongation through fine-tuning of ethylene homeostasis. We identified a cucumber mutant that bears short fruits owing to repressed cell division. SF1 (Short Fruit 1) encodes a cucurbit-specific RING-type E3 ligase, and the mutation resulted in its enhanced self-ubiquitination and degradation, but accumulation of ACS2 (1-aminocyclopropane-1-carboxylate synthase 2), a rate-limiting enzyme for ethylene biosynthesis. The overproduction of ethylene contributes to the short-fruit phenotype of sf1. Dysfunction of ACS2 resulted in reduced ethylene production, but still repressed cell division and shorter fruit, suggesting that ethylene is still required for basal fruit elongation. SF1 ubiquitinates and degrades both itself and ACS2 to control ethylene synthesis for dose-dependent effect on cell division and fruit elongation. Our findings reveal the mechanism by which ethylene dosage is regulated for the control of cell division in developing fruit.

Presenters: xueyong yang

Co-author(s): TONGXU XIN, ZHEN ZHANG

GEX2-JANUS1/2 interactions regulate MGU assembly during fertilization in Arabidopsis thaliana (0900-010)

Hall 2

Flowering plants have evolved a unique method of sexual reproduction in which two male gametes (sperm cells) and two female gametes (egg and central cell) fuse to form a seed. The adhesion contact between the two sperm cells within a pollen grain maintain the male germ unit (MGU) organization during pollen tube growth and is essential to assure the simultaneous delivery of both sperm cells to the embryo sac. Although sperm-cell-expressed proteins are known to contribute to female-male gamete adhesion and fusion, the factor(s) that maintain sperm-sperm cell adhesion remain elusive as well as its function in double fertilization. Recent findings identified two sperm-expressed membrane proteins, JANUS1/JANUS2 as negative regulators of sperm-sperm cell adhesion. Sperm cells from the janus1/2 present an extended sperm-sperm interface adhesion domain and affect male-female gamete targeting during double fertilization. In this study, we explored the hypothesis that GEX2, a single-pass, transmembrane protein expressed in sperm cells, could function as an adhesion factor between sperm cells. We examined a GEX2 T-DNA insertion mutant in Arabidopsis thaliana and analyzed GEX2-GFP expression in janus1/2 mutants. Our results indicate that GEX2-GFP accumulates at the sperm-sperm cell interface during pollen tube growth, partially rescuing the extended adhesion phenotype observed in janus1/2 mutants. Taken together, we propose that GEX2 contributes to the maintenance of the MGU organization during in vitro pollen tube growth.

Co-author(s): Leonor C. Boavida

Primary Poster Presenter: Ryan Hockemeyer

GhAAI66 triggers a phase transition to induce early flowering (0900-028)
Hall 2

Plants undergo a phase transition from vegetative to reproductive development that triggers floral induction. Genes containing an AAI (alpha amylase inhibitor) domain form a large gene family, but there have been no comprehensive analyses of this gene family in any plant species. Here, we identified 336 AAI genes from nine plant species including122 AAI genes in cotton (Gossypium hirsutum). The AAI gene family has evolutionarily conserved amino acid residues throughout the plant kingdom. Phylogenetic analysis classified AAI genes into five major clades with significant polyploidization and showing effects of genome duplication. Our study identified 42 paralogous and 216 orthologous gene pairs resulting from segmental and whole genome duplication, respectively, demonstrating significant contributions of gene duplication in expansion of the cotton AAI gene family. Further, GhAAI66 was preferentially expressed in flower tissue and responses to phytohormone treatments. Ectopic expression in Arabidopsis and silencing of GhAAI66revealed that GhAAI66 triggers a phase transition to induce early flowering. Further, GO and KEGG analysis of RNA-seq data and qRT-PCR analysis indicated that GhAAI66 integrates multiple flower signaling pathways including GA, JA, and floral integrators to trigger an early flowering cascade in Arabidopsis. Therefore,

characterization of AAI family provides invaluable insights for improving cotton breeding.

Co-author(s): Ghulam Qanmber, Fuguang Li

Primary Poster Presenter: Zuoren Yang

Inducing apomeiosis and disrupting meiotic reduction in gametes of sexual Antennaria (Asteraceae) an (0900-029)
Hall 2

Sexual reproduction is formation of genetically reduced (haploid) gametes (e.g., eggs and sperm) that fuse, during fertilization, to restore diploidy and to initiate a new generation. In many plants, unreduced eggs form (apomeiosis) that produce embryos without fertilization (parthenogenesis). This is called apomixis, and it causes plants to clone themselves through their own seed. If apomixis could be induced in crops, hybrid seed production costs for hybrid crops like corn could be reduced and commercial quantities of superior yielding hybrid seed of inbred crops like wheat and rice could be developed for the first time. Based on gene profiling and subsequent experimentation, our lab recently identified pharmacological treatments that induce, at high frequencies, apomeiosis in sexual Boechera stricta, Arabidopsis thaliana and Vigna unguiculata (cowpea). These treatments target the SnRK-TOR pathway that regulates various antioxidant and bioenergetic behaviors required for growth responses to environmental factors. Abscisic acid (ABA) is a hormonal signal that triggers the stress response behavior of the SnRK-TOR transduction pathway. We exposed A. thaliana pistils to Floridone (ABA inhibitor) and ABA in vitro to determine their effects on meiosis. To determine if the SnRK-TOR pathway might also influence microsporogenesis, these pharmacological treatments were also applied through a stem culture technique to male-only plants of sexually dioecious Antennaria. Without damaging the buds, these procedures induced apomeiosis in both pistils and anthers. Verification of apomeiosis versus meiosis was accomplished cytologically. Our results provide strong evidence that our pharmacological treatments influence the SnRK-TOR pathway and induce apomeiosis by disrupting meiotic divisions of both female and male gametes.

Co-author(s): John Carman

Primary Poster Presenter: Bo Price

Investigating a Genetic Suppressor of nortia Pollen Tube Overgrowth (0900-009)
Hall 2

Extensive intercellular communication between female and male plant tissues is required for successful pollination of flowering plants. First, compatible pollen germinates on a stigma and produces a tip-growing pollen tube which gains entry into a receptive pistil. The pollen tube is guided toward an ovule, invades the

female gametophyte, and lastly ruptures inside a synergid cell to release two sperm cells and achieve double fertilization in a process called pollen tube reception. In Arabidopsis thaliana, a mutation in NORTIA (NTA), one member of the MILDEW RESISTANCE LOCUS O (MLO) family of 7-transmembrane domain proteins and an identified component of the pollen tube reception pathway, results in pollen tube overgrowth in around thirty-percent of ovules (Kessler, 2010). This means that some pollen tubes fail to arrest their growth inside a synergid cell and do not rupture to release the sperm cells, leading to unsuccessful fertilization of ovules, and consequently, less seed. An EMS mutagenesis genetic screen has revealed that the loss of a specific kinase suppresses the pollen tube overgrowth phenotype present in a nta mutant, and successful fertilization occurs at a higher rate in the mutants due to the rescue of pollen tube bursting. Further characterization will be performed to study this genetic interaction. Gaining more insight into the process of pollination is valuable for understanding how plants perceive beneficial invasions such as a pollen tube's entrance to an ovule and prevent detrimental ones such as pathogen invasions. By studying the existing links between fertility and disease in plants, we may be able to uncover important implications in potential trade-offs with fertility when breeding for pathogen resistance. In addition, by further elucidating the molecular processes of plant reproduction, scientists can better understand how to breed plants which have increased reproductive success under changing environmental conditions.

Primary Poster Presenter: Rachel Flynn

Investigating the intersection of Ca2+ and GTPase signaling in the polar growth of pollen tubes (0900-031)
Hall 2

Pollen represents the male gametophyte in all seed plants and plays the critical role of producing sperm to be delivered to the egg to facilitate fertilization. After landing on the stigma of a flower, a compatible pollen grain will germinate and a tube will protrude and begin penetrating the floral tissue growing towards the egg-containing ovules. The pollen tube grows by rapid extension of the tube apex in a form of highly polarized growth, known as tip growth. Tip growth is conserved among eukaryotes and can be observed in budding yeast and fungal hyphae and even other plant cells like root hairs. However the signaling pathways required to fine-tune polar growth remain elusive. Our lab previously identified and characterized a Ca2+-dependent protein kinase (CDPK1) expressed in pollen and required for polar growth. When transiently over-expressed in pollen, tubes lost their polarity and took on a "sock-like" phenotype. Preliminary data suggests CDPK1 interacts with and phosphorylates a GTPase-regulating protein, GDI. GTPases are highly conserved signal molecules involved in an array of developmental processes. Using stable transgenic approaches and plant tissue culture we have begun to confirm the interaction between CDPK and GDI in vivo by rescuing the CDPK over-expression phenotype through co-expression of GDI in Tobacco. We will further support the interaction between CDPK1 and GDI using microscopy techniques, pull-down assays

and phosphorylation assays. We are also interested in investigating CDPK1 effect on vegetative tissues. We have engineered Arabidopsis over-expressing CDPK1 on a constitutive promoter and begun obtaining preliminary results. Ca2+-mediated and GTPase signaling are two major pathways in plants, and this works aims to connect the two while unraveling the specific mechanisms maintaining polar growth. This work will provide a framework for understanding not only polar growth but also other important developmental events in eukaryotes.

Primary Poster Presenter: Nolan Scheible

MAS integrates ovular signals and exocytosis to guide pollen tube (0900-026)
Hall 2

Plants use Ca2+ signaling to trigger universal cellular signaling pathways in development and response to the environment. But how the extracellular signals are translated to trigger the Ca2+ flux is poorly understood. The pollen tube as an invasive growing cell is beaconed by diverse female signals and transduces these signals into the intracellular growth machineries, such as the Ca2+ signaling, for the navigation into the embryo sac. How the pollen tube realizes this molecular integration from outside to the inside Ca2+ dynamics for the guided growth is unknown and important to understand the reproduction and adaption strategies of plant cells. Here we report a mechanism for the directional exocytosis of cargos in pollen tube response to the female signals. Mutants of MALE SENSOR (MAS), which encodes a plasma membrane protein, show abnormal pollen tube response to the secreted ovular cues in Arabidopsis thaliana. Protein affinity-based mass spectrometry showed that MAS forms a physical complex with a cysteine-rich peptide and a receptor-like kinase that regulate pollen tube guidance. Molecular and biochemical studies reveal that MAS selectively tether Ca2+-related cargos to the plasma membrane where the extracellular signals are perceived through the SNARE proteins in a trans mode. These results reveal a new mechanism of molecular integration of extracellular cues and selective exocytosis, and will shed light on the general regulation of cell response to the environment.

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Primary Poster Presenter: Hong-Ju Li

Mechanosensitive ion channels, MSL7 and MSL8, play multiple roles in pollen hydration, germination a (0900-005)
Hall 2

It’s critical to understand the molecular mechanisms by which plants sense and perceive mechanical force, including osmotic pressure. We recently found that MscS-Like (MSL) 8 functions as a mechanosensitive channel and is required to

protect Arabidopsis pollen from osmotic challenges during in vitro pollen rehydration, germination and tube growth (Hamilton et al., 2015). Further investigation into MSL8 and its closest homolog MSL7 revealed intriguing new phenotypes in msl7 msl8 knock down (via amiRNA) and knock out (by CRISPR/Cas9) mutants. As previously reported, msl8 mutant pollen burst more frequently than the wild type. Furthermore, time lapse imaging and viability dye staining indicated that some msl8 mutant burst pollen grains were able to germinate a growing pollen tube, and that not all burst grains, alone or with a tube, were dead. These data suggest that the pollen membrane system is able to rapidly respond to damage to maintain cell integrity and viability, at least in the msl8 mutant background. In addition, we found that pollen tubes in msl8 mutants grew faster than the wild type in vitro. However, in vivo, pollen tubes from msl7 msl8 mutants grew more slowly than the wild type at early stages, though they caught up to the wild type later on. Transmission electron microscopy images indicated defective membrane and cell wall structures at the germination pole in the msl7 msl8 mutant. Taken together, our data show that MSL8 (and in some cases, MSL7) is required for normal pollen grain and pollen tube integrity, growth rate, and germination pole structure. We speculate that MSL8 functions as an osmotic safety valve during hydration, germination, and tube growth—but plays an additional, more subtle role during assembly of the germination pole, and in viability and recovery after bursting events. Current data implicate MSL7 only in tube growth in vivo, implying that it may function in the female tissues. Additional data to test these ideas will be presented.

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Primary Poster Presenter: Yanbing Wang

Moderate heat stress reveals a function for PIRL7 in Arabidopsis pollen development (0900-020)
Hall 2

Pollen development is crucial for plant reproduction. The initial stages of pollen development comprise mitotic events that produce a spatially-organized male germ unit (MGU) consisting of two sperm cells and a vegetative nucleus. At least 3 members of the Arabidopsis Plant Intracellular Ras-group LRR (PIRL) gene family are known to be important in the formation and organization of the MGU. Transcriptome data and RT-PCR suggest another, PIRL7, is expressed during pollen development. We investigated a possible role for PIRL7 in this process through the characterization of pirl7 T-DNA insertion mutants. Three candidate knockout (KO) alleles were characterized and one, pirl7-3, appeared to be a bona fide KO based on RT-PCR expression analysis. Pirl7-3 homozygotes did not initially display any obvious developmental phenotype. Functional redundancy with the closely related PIRL6 gene seemed an unlikely explanation, because RNAi knockdown of PIRL6 alone results in highly-penetrant male- and female- gametophytic defects. Therefore, we attempted to tease out potential weak developmental phenotypes by

subjecting pirl7 homozygotes and wild-type (WT) controls to a moderate heat stress. Screening pollen by rapid viability staining, we identified conditions that triggered an increased frequency of abnormal pollen in pirl7 plants, while having only minimal impact on WT pollen. Mutant pollen were frequently small, morphologically aberrant, or inviable. Confocal microscopy of DAPI-stained mutant pollen identified abnormal MGU configurations such as dispersed sperm cells and diffuse, "wispy" vegetative nuclei. These results suggest a function for PIRL7 in pollen cell organization and configuration of the MGU. The moderate stress treatment we employed here may be useful as a means to identify otherwise elusive developmental defects in other gametophytic mutants. // Supported by Whitman College Summer Research Awards; NSF-0616166; NSF-MRI-103995

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Primary Poster Presenter: Daniel M. Vernon

Mutation of Arabidopsis thaliana O-fucosyltransferase 1 (AtOFT1) results in in vivo pollen tube defe (0900-004)
Hall 2

Double-fertilization in angiosperms results in the production of most of the world's food crops and represents a coordinated effort between the male gametophyte (pollen), the female tissues (pistil), and the female gamete (ovule). During this process, the pollen grain hydrates and produces a pollen tube, which acts as a vehicle to penetrate through the pistil tissues and deliver its two sperm nuclei to an ovule. The mechanism of pollen tube penetration through the pistil has been anatomically well described, however, the genetic basis for this process remains poorly understood. We have identified a novel Arabidopsis (Arabidopsis thaliana) gene, O-FUCOSYLTRANSFERASE1 (AtOFT1), which plays a key role in pollen tube penetration through the initial female reproductive tissues, specifically the stigma and style. Mutant oft1 pollen germinated and elongated normally in vitro. However, oft1 pollen exhibited a reduced ability to penetrate the stigma and style under semi in-vivo conditions, resulting in a nearly 2,000-fold decrease in oft1 pollen transmission efficiency and a 5- to 10-fold decreased seed set. Phylogenetic analysis of the AtOFT1 amino acid sequence demonstrated that AtOFT1 is a member of a novel clade of 39 putative protein O-fucosylltransferase genes in Arabidopsis based on sequence similarity with known metazoan protein O-fucosyltransferase 1's (POFT1). Further analysis revealed AtOFT1 requires conservation of the same key catalytic residues important for metazoan POFT1 function, suggesting a conserved enzymatic function. To investigate other genes involved in functionally regulating AtOFT1, an EMS screen was conducted, which yielded a unique mutant that suppressed the reproductive phenotype of oft1 mutants. Resequencing of these lines indicated the suppressor phenotype was due to a missense mutation of a second Arabidopsis putative POFT family member, At1g11990, which we refer to as SUPPRESSOR OF OFT1 (SOFT1).

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Primary Poster Presenter: Devin Smith

Nuclear Import of LIKE HETERPCHROMATIN PROTEIN1 is essential for Flowering Regulation in Arabidopsis (0900-011)
Hall 2

LIKE HETEROCHROMATIN PROTEIN1(LHP1) encodes the only plant homologueof metazoan HETEROCHROMATIN PROTEIN1 (HP1) protein family. LHP1 isknown as transcriptional repressor of flowering related genes such asFLOWER LOCUS T (FT), FLOWERING LOCUS C (FLC), AGAMOUS (AG) and APETALA3 (AP3). We recently found that LHP1 interacts withImportin a-1(IMPa-1), Importin a-2 (IMPa-2) and Importin a-3 (IMP a-3) invitro and in vivo. Genetic approach revealed that triple mutant plantsof imp a-1, imp a-2 and imp a-3 show severe growth defect and rapidflowering phenotypes similar to that of lhp1-3 mutant plants due toup-regulation of FT expression in long day and short day conditions.When the targeting of LHP1-GFP was investigated in planta, nucleartargeting of LHP1-GFP was impaired in the triple mutant plants,resulting in derepressing several LHP1 target genes such AG, AP3, SHP1as well as FT. Chromatin immunoprecipitation (ChIP) assay revealedthat LHP1-GFP association on chromatin of FT and AG was severelydecreased in triple mutant plants. These data suggest that nuclearimporting of LHP1 by IMPa-1, IMPa-2 and IMPa-3 is required forrepressing and activating its several target genes.

Co-author(s): Chong Chen

Primary Poster Presenter: JAEBOK HEO

Pollen tube responses to maternal genotype, stigma and transmitting tract factors and abiotic stress (0900-001)
Hall 2

Nicotiana tabacum is an excellent model for researching plant reproduction with large flowers that facilitate pollination, established methods for measuring in vivo and in vitro pollen tube growth, diverse reproductive interactions, and self-pollination, providing in-bred lines. In vitro pollen tube growth conditions are easily manipulated providing an efficient experimental method to measure growth rates without pistil contributions under varied conditions. Pollen undergoes critical processes on the stigma and in vitro including hydration, germination, and pollen tube growth, which affects its ability to travel through the pistil and fertilize ovules. These post-pollination and prezygotic-steps are critical to seed set making them significant for seed and production and important in evolution. We used genetically diverse N. tabacum genotypes as a reproductive model to test whether genotype affects pollen-pistil interactions, and pollen tube growth rates in situ and in vitro. Pollen tube growth rates were tested under super- and sub-optimal temperature conditions, increased sodium chloride, removal of the stigma, and genetic ablation

of the transmitting tissue. The male and female genotypes each contributed differentially to the regulation of pollen tubes, showing large differences in growth rates. We then compared relative growth rates under varying conditions and in situ vs in vitro. Data from these experiments show differences among N. tabacum genotypes in the spatial and temporal regulation of in situ and in vitro pollen tube growth and provide a system to understand pollen-pistil interactions and its tolerance to abiotic stress.

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Primary Poster Presenter: Alan Smith

Rice MADS78 and MADS79 regulate endosperm cellularization and concurrent loss of function for bot (0900-013)
Hall 2

MADS-box transcription factors (TFs) are well-known to be involved in all major aspects of a plant's life. Here, we report two type-I MADS-box TFs, MADS78 and MADS79, critical for regulating early seed development in rice. The two TFs are preferentially expressed during the transition of endosperm from syncytium to cellularization stage, confirmed by RT-qPCR analysis and in-situ hybridization assay. The expression of MADS78 and MADS79 is negatively correlated with FIE1, indicating their potential regulation by polycomb repressor complex2. Further, to functionally characterize the genes, over-expression (OE) transgenic mutants were generated, which resulted in delayed cellularization thereby leading to increase in seed size. Moreover, high spikelet sterility was observed in the OE mutants that is attributed to abortion of seeds during the phase of syncytium – cellularization transition. On the other hand, CIRSPR-Cas9 edited single knock-out mutants show precocious cellularization. Moreover, double knock-out mutants for MADS78 and MADS79 is lethal, suggesting their indispensable role during early seed development. Also, MADS78 and MADS79 partner with MADS89, another type-I MADS-box TF, which enhances their specificity to localize in the nucleus. Interestingly, mis-regulation of MADS78 and MADS79, either by OE or knock-out mutants, implicates source-sink communication, thereby altering mature grain quality. To summarize, we have characterized the role of two type-I MADS-box TFs from physiology, molecular and epigenetic point of view, thereby providing possible clues to the regulatory mechanism controlling seed development in rice.

Primary Poster Presenter: Puneet Paul

Sculpting an imperfect flower: The study of KNUCKLES in primordia regulation (0900-015)
Hall 2

The evolution of sex determination in plants is a central problem in plant evolutionary biology. Currently, there have been limited studies in which the sex determination genes are identified yet we do not know most of the alternative downstream pathways that lead to developmental differences in plants that exhibit sexual dimorphism. Addressing this gap in knowledge is important as it will give insight into the genetic regulation of developmental processes in unisexual flowers and in angiosperm flowers in general. The investigation into the link between the differential expression patters of genetic pathways and the differential expression in floral development involves the differential expression of AG, WUS, and the proposed transcription repressor gene KNUCKLES (KNU) as they relate to the differential formation of floral organ primordia in male and female Spinacia oleracea flowers. Our central hypothesis is that the AG-KNU-WUS pathway regulates the differential morphogenesis of organ primordia between male and female flowers leading to sexual dimorphism in spinach. To test this hypothesis, molecular genetics tools are utilized to quantify KNUCKLES temporal and spatial expression patterns, along with functional testing. Preliminary studies have begun that include characterizing KNU-like gene expression and the phenotypes of KNU-like knockdowns in S. oleracea. Preliminary results show strong phenotypes in the vegetative tissue that are related to the regulation of organ primordia and meristem maintenance.

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Primary Poster Presenter: Asia Hightower

SlSWEET transporters are required for fruit and seed development in tomato (0900-003)
Hall 2

Tomato is an important economic fruit crop in the world and requires sugars for fruit development and quality. Studies indicate that SWEET (Sugars Will Eventually be Exported Transporters) expression is closely linked with fructose/glucose ratio in tomato fruits. How SWEETs physiologically function in fruit development deserves further investigations. Here, by using quantitative PCR, we have discovered that SlSWEET-X and SlSWEET-U were specifically highly expressed in the 21- and 35-day-old fruits, respectively, and little expressed in other organs, such as leaves and flowers. Localization of SlSWEET-X/U-GFP to vacuolar membrane suggested their putative functions in vacuolar sugar accumulation that largely contributes to fruit sweetness and seed filling. Tissue-specific expression of SlSWEET-X/U-GUS fusion proteins in transgenic tomato plants will be examined shortly to pinpoint their physiological roles. Moreover, radio-tracer uptake assay showed that SlSWEET-X/U exhibited weak but specific transport activity to sucrose. When SlSWEET-X function was knockout via the CRISPR/cas9 system, average sizes and weights of fruits in mutant tomato plants were significantly decreased. Moreover, seed filling and viability was almost inhibited. Sugar composition of developing mutant fruits will be examined. These results imply that SlSWEET-X may function to mediate vacuolar

sugar accumulation in young fruits that is required for early fruit development and seed filling.

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Temporal and spatial profiling of micro RNAs during soybean seed development (0900-024)
Hall 2

Soybean seeds are utilized widely for agricultural and commercial purposes because of its high oil and protein content. Understanding the molecular mechanisms that underlie soybean seed development will be useful to increase crop yield. Seeds consist of three regions, embryo, endosperm and seed coat, and each seed region is further differentiated into subregions. Each subregion undergoes unique temporal and spatial developmental programs that are mediated by changes in gene expression controlled at the transcriptional and post-transcriptional level. Micro RNAs (miRNAs) are a key regulator of gene expression. In recent years, plant miRNAs have been extensively studied. However, little is known about miRNA accumulation at the tissue level during seed development. We profiled miRNAs from 37 different subregions of soybean seeds at four different developmental stages by using Laser Capture Microdissection coupled with RNA sequencing. We identified 264 miRNAs during soybean seed development using stringent miRNA evaluation criteria based on stem-loop formation, unique characteristics of miRNA biogenesis, and miRNA abundance. The majority of miRNAs accumulate in one specific subregion or stage, in many instances, at a significantly high level, suggesting that miRNAs may play important roles in regulating developmental processes in soybean seeds. To identify the mRNAs that are cleaved by miRNAs, we analyzed 14 publicly available parallel analysis of RNA ends (PARE) datasets. Based on our analyses, miRNAs are predicted to regulate a wide range of biological processes including those related to development, hormone, and defense. Understanding the functions of miRNAs at the tissue level at different developmental stages will provide new insights into the molecular mechanisms controlling soybean seed development.

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Primary Poster Presenter: Rie Uzawa

The Arabidopsis MAP Kinase Kinase Kinase 19, 20 and 21 are involved in pollen fertilization. (0900-007)

Hall 2

Mitogen-activated protein kinases (MAPKs) cascades are important mediators of signal transduction during plant reproduction. However, very few studies demonstrated the necessity of MAPKs superfamily during pollen tube growth. Here we show that using the U0126 compound, a highly selective inhibitor of MAP kinase kinases (MEKs) with little effect on MAP kinase kinase kinases (MEKKs), inhibition of all MAPK Kinases (MEKs), showed a crucial role of MAPK signalization during elongation of pollen tube. Our search for MAPKs involved in the formation of the male gamete and during the elongation of the pollen tube in A. thaliana started with three Mitogen-Activated Protein Kinase Kinase Kinase (AtMAPKKK19, 20 and 21) since they were orthologous to the ScFRK1, ScFRK2 and ScFRK3 from Solanum chacoense that were shown to be crucial for the formation of male and female gametophyte. Using GUS reporter genes, we showed that AtMAPKKK19, 20 and 21 are highly expressed during pollen development as well as during pollen tube elongation. Single knockout mutants showed no obvious phenotypes during gametogenesis, suggesting a possible functional redundancy from these three MAP3Ks. However, we observed a decrease in germination in the double mutant Atmap3k20;21 as well as a decrease in growth rates for the entire single mutant in vitro and in vivo. These results demonstrate the importance of the MAP Kinase superfamily during the growth of the pollen tube and that the AtMAPKKK19, 20 and 21 are involved during the germination and elongation of the pollen tube.

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Primary Poster Presenter: Mazin Benjamin

The Arabidopsis transcription factor AINTEGUMENTA orchestrates hormone signaling pathways an (0900-014)
Hall 2

Understanding how flowers form is an important problem in plant biology, as the human food supply depends upon the production of flowers and seeds. Flower development also provides an excellent model for understanding how cell division, expansion and differentiation are coordinated during organogenesis. AINTEGUMENTA (ANT) and AINTEGUMENTA-LIKE6 (AIL6), two members of the Arabidopsis AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family, regulate several aspects of floral organogenesis including floral organ initiation, growth, identity specification and patterning. RNA-Seq analysis of ant ail6 mutant flowers identified thousands of genes that depend on ANT and/or AIL6 for expression, but few direct downstream targets of these transcription factors during floral organ development are known. To identify direct targets of ANT regulation, we performed RNA-Seq on 35S:ANT-GR inflorescences treated with steroid to induce ANT activity. Gene Ontology analysis on the set of differentially expressed genes associates ANT activity with multiple hormone signaling pathways and developmental processes such as polarity specification, meristem maintenance and stamen development. Additional expression studies using 35S:ANT-GR and

ANT:ANT-GR ant inflorescences identified genes whose differential expression after ANT induction is independent of protein synthesis. Furthermore, chromatin immunoprecipitation (ChIP) experiments demonstrate that ANT binds to genomic regions near or within several of the differentially expressed genes. Results from a ChIP-Seq experiment identifying genome-wide ANT binding sites in stage 6/7 flowers will also be presented. Our study identifies both novel and previously characterized genes as direct targets of ANT regulation in flowers.

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Primary Poster Presenter: Beth Krizek

The role of IDA-like gene expression in tomato flower abscission (0900-030)
Hall 2

Precise and timely regulation of organ separation from the main body of the plant (abscission) is crucial to improvement of crop productivity, as it influences both the timing of harvest and fruit quality. Abscission behaviors differ in many plant species and the separation processes are complex and varied in different abscission systems (e.g., leaf, flower, fruit). The proposed essential role of a small signaling peptide INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) in Arabidopsis floral organ abscission and the conserved expression of IDA-like (IDL) genes in abscission of many other plants have raised a question as to their role in diverse abscission systems. To understand the role of IDL genes in tomato, we generated tomato (Solanum lycopersicum) RNAi lines that target AZ-specific expression of SlIDA1 (SlIDA1 RNAi) and examined the effects of suppression of SlIDA1. Alternatively, we investigated expression for SlIDL genes (SlIDA1 to SlIDA5) in the pedicel AZ from a variety of natural abscission variants in tomato (i.e., jointless, functionally impaired jointless (knuckle-like AZ), normal joint). Whereas the suppression of SlIDA1 expression did not correlate with the expected delay in pedicel abscission of SlIDA1 RNAi, transcript profiles of SlIDL genes in the natural tomato variants indicated that expression of SlIDL genes, to some extent, is associated with the formation of AZ. RNA-seq data of the pedicel AZ of SlIDA1 RNAi further revealed that the role of SlIDA1 may be linked to the stress (defense) related gene expression in the tomato flower abscission. Treatment of ethylene action inhibitor, 1-MCP, in detached fruits and leaves of SlIDA1 RNAi blocked senescence processes, which substantiates the previous finding that ethylene is essential in tomato senescence processes, and the function of SlIDA1 expression is ethylene-dependent in tomato.

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Primary Poster Presenter: Joonyup Kim

The ScFRK2 and ScFRK3 MAP Kinase Kinase Kinase are involved in ovule development in Solanum chacoens (0900-006)
Hall 2

The Fertilization-related kinases (FRK) class belongs to the mitogen-activated protein kinase kinase kinase (MAP3K) subfamily in plants. Studies on the wild potato Solanum chacoense have shown that three ScFRKs are directly involved in female gametophyte development. Decreasing the expression of ScFRK1 by RNA interference lead to embryonic sac development arrest at the functional megaspore stage while ScFRK2 overexpression lead to a drastic decrease in seed numbers, presumably caused by a conversion of the ovule into a carpel-like structure. Here we show that in ScFRK2 overexpression lines, carpel-like structures from the ovule did not fully explain the drastic decrease of seeds. Although these carpelloid structures filled most of the locular space due to their size and length, ovules number were like WT ovaries. Instead, most ovules were arrested at the functional megaspore stage, like in ScFRK1. We also show that another fertilization-related kinase, ScFRK3, is expressed early on during female gametogenesis, reaching its highest level immediately after meiosis and during the mitosis steps. ScFRK3 transgenic plants mRNAs lead to the production of small fruits with severely reduced seed set, due to severe number of degenerate ovules like ScFRK1. Like ScFRK2 overexpression lines and ScFRK1 RNA interference lines, most ovules of ScFRK3 RNA interference lines are arrested at the functional megaspore stage. These studies strongly suggest the importance of the FRK family during early stages of ovule development in Solanum chacoense embryo sac.

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Primary Poster Presenter: Mazin Benjamin

Using CRISPR/Cas9 to study S-locus F-box protein-containing SCF complexes in self-incompatibility (0900-022)
Hall 2

Self-incompatibility (SI) is an intraspecific reproductive barrier, by which pistils reject self-pollen, but accept non-self pollen. SI possessed by Petunia involves multiple polymorphic S-locus F-box (SLF) genes and a single polymorphic S-RNase gene. 17 SLF genes (SLF1 to SLF17) have been identified in both S2- and S3-haplotypes of P. inflata, and all their encoded proteins are assembled into similar SCF (Skp1–Cullin1–F-box) E3 ubiquitin ligase complexes, which contain pollen-specific Skp1-like protein (PiSSK1) and Cullin1 (PiCUL1-P), and a conventional RBX1 (PiRBX1). To address the role of SCFSLF complexes in SI, we used CRISPR/Cas9 to separately knockout PiSSK1, PiCUL1-P and S2-SLF1, and generated two frame-shift indel alleles for each gene. In the absence of PiSSK1, S2 pollen was incompatible with pistils of seven normally compatible S-genotypes, but compatible

with pistils of an S3S3 transgenic plant in which production of S3-RNase was suppressed by an antisense S3-RNase gene. Surprisingly, S2 and S3 pollen carrying either indel allele of PiCUL1-P remained compatible with S7S7 and S6aS12 pistils. As we found that another pollen-expressed CUL1, PiCUL1-B, also interacts with PiSSK1, we designed another guide RNA to generate frame-shift alleles of both PiCUL1-P and PiCUL1-B, and found that S2 pollen was rejected by S7S7, S5S5, S6aS12 and S3S13 pistils only when both PiCUL1-P and PiCUL1-B are absent. S2 pollen carrying either indel allele of S2-SLF1 was incompatible with S3S3 pistils, but compatible with S7S7 and S12S12 pistils. Consistent with these results, we found that among the 17 S2-SLFs, S2-SLF1 is the only one that can interact with and detoxify S3-RNase, whereas, in addition to S2-SLF1, S2-SLF2 and S2-SLF5 can interact with S7-RNase and S12-RNase, respectively. All these results suggest that PiSSK1 and SLF proteins function specifically in SI and are essential for cross-compatibility, whereas PiCUL1-P functions redundantly with PiCUL1-B.

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Primary Poster Presenter: Linhan Sun

WUSCHEL and Cytokinin Singaling Function Independently To Regulate Shoot Apical Meristem Function (0900-025)
Hall 2

The above-ground tissues and organs of plants are generated by the activity of the shoot apical meristem (SAM). An unresolved question in plant developmental biology is how the SAM is specified and maintained. The current model states that a regulatory network involving the CLAVATA (CLV) signaling pathway and a mobile homeodomain transcription factor WUSCHEL (WUS) function as a negative feedback loop to maintain a stable pool of stem cells. This requires cell-cell signaling between domains of different cellular activity within the SAM. WUS produced from a collection of corpus cells known as the organizing center (OC) migrates into the overlying central zone (CZ) cells. The CZ, which is comprised of the pluripotent stem cells, secretes the CLV3 signaling peptide, the ligand for the CLV1 receptor-like kinase expressed in the cells of the OC. CLV signaling output in the OC limits the stem-cell promoting function of WUS from these cells. However, this model does not address how WUS expression is established or maintained in the OC. Currently, the cytokinin class of phytohormones is thought to play a direct role in the specification and maintenance of WUS expression. As a test of this hypothesis we have performed an extensive genetic analysis with loss of function mutants in the cytokinin metabolic and signaling pathways and find WUS expression is not impacted by loss of cytokinin signaling. These results indicate cytokinin signaling is

not required for the specification or maintenance of WUS in the SAM as currently proposed. However, our analysis points to an emerging model whereby cytokinin and WUS function to balance cell division and cell differentiation in the SAM. Cytokinin signaling output is required for radial growth of the SAM while WUS functions to largely inhibit differentiation by limiting organ initiation on the flanks of the SAM. We also present data that suggests the subepidermal L2 layer of the SAM may serve an essential role in its maintenance.

Primary Poster Presenter: Paul Tarr

Development: Vegetative

Big lessons from a small plant: The lack of a negative growth regulator, CHIQUITA1, results in dwarf (0900-065 (Screen 8))
Hall 2

Organ size control is fundamental in biology. However, the mechanisms that determine final organ size in multicellular organisms are not fully understood. We found and characterized a novel gene, CHIQUITA1 (CHIQ1), which might be a key to elucidating organ size control mechanisms. Mature leaves of plants harboring the chiq1-1 null allele are smaller than wild type with fewer and smaller cells. Cell cycle marker studies indicated that cell proliferation ends prematurely in chiq1-1 leaves; and most chiq1-1 pavement cells do not enter endoreduplication after exiting the mitotic cell cycle. In addition, chiq1-1 pavement cells stop expanding prematurely. Surprisingly, 4D imaging studies on leaves in the proliferating phase indicated that meristematic cells divide and expand faster in chiq1-1 leaves and proliferating leaves are bigger. We hypothesize that an early onset of differentiation is triggered in chiq1-1 leaves because of a defect in attenuating division and/or growth during proliferation, which results in smaller adult plants. This work uncovers a genetic basis that connects cell proliferation, differentiation, and organ size in plants.

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Primary E-Poster Presenter: Flavia Bossi

Identification of rhizome-specific hormone accumulation and gene expression by various tissue analys (0900-066 (Screen 11))
Hall 2

Rhizomes are horizontal, underground elongating shoots. Rhizomes have advantages for energy storage, vegetative reproduction and vitality under harsh condition. Despite these features, little is known on the molecular mechanism of

rhizome development. In this study, we revealed that the specific hormone accumulating pattern and related gene expression profile depends on developmental stage of rhizome using Oryza longistaminata, a wild rice species, originally derived from Africa. Firstly, we defined the developmental stages to understand the physiological traits of rhizome. According to our chronological observation there are three steps in rhizome development; (1) Initiation stage: the bud becomes onion-like shape and rotates 90 degrees to abaxial side, (2) Elongation stage: the rhizome bud starts internode elongation horizontally, (3) Shoot-shifting stage: the rhizome changes its character to aerial shoot and growth toward the soil surface. To examine what phytohormones regulates each step, we measured phytohormones in various tissues. Active type of gibberellic acid (GA), especially GA4 is accumulating in elongation step rhizome compared to aerial shoot whereas GA4 is rarely detected in the vegetative stage of O. sativa. RNAseq data indicated GA20ox2 is highly expressed in rhizome. GA20ox2 in O. longistaminata showed higher enzymatic activity than GA20ox2 in O. sativa. Further, the amount of jasmonate acid (JA) is 40-times higher in aerial shoot than rhizome. As JA repress internode elongation in O. sativa (Minami et al. 2018), less accumulation of JA in rhizome could be important for its elongation. Hormone treatment suggested that auxin appears to act as a negative regulator, while GA acts as the activator in rhizome development. The development of the rhizomatous traits in O. longistaminata is controlled by very complex gene networks involving several plant hormones and its regulatory genes indicating tissue specificity and their regulated pathways.

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Primary E-Poster Presenter: Kanako Bessho

The function of ABA and ROP GTPases in root patterning and development
(0900-067 (Screen 10))
Hall 2

In response to salt and drought, levels of abscisic acid (ABA) increase rapidly in the root. I will present data showing that osmotic stress via core ABA signaling in meristematic endodermal cells induces differentiation of protoxylem (PX) in radial and longitudinal axes in association with increased VND7 expression. ABA increased expression of microRNAs miR165a/166b, which non-cell autonomously and post-transcriptionally reduced the levels of all five HD-ZIPIII proteins in the stele. Furthermore, ABA also reduce the levels of the miR165/166 negative regulator AGO10/ZWILLE in the stele. The effects of ABA were compromised in phb1-d miR165/166 resistant mutants. In lateral root initials, ABA induced increase in miR165a levels in endodermal precursors and inhibited their reduction in the future

quiescent center specifically at pre-emergence stage. This suggests that ABA-induced inhibition of lateral root development depends on reduction of HD-ZIPIII level. ABA and ROP11 signaling are mutually antagonistic. In the PX ROP11 activation status regulate secondary wall patterning while in the endodermis suppression of ROP11 by ABA is required for integrity of the Casparian strip. Together, our results highlight the function of ABA in pattern formation via miR165/166 and its crosstalk with ROP signaling.

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Primary E-Poster Presenter: Shaul Yalovsky

A cross-species analysis reveals limited translatome conservation at cell type resolution

Plant roots are comprised of different cell types that undergo spatiotemporal changes from meristematic tissues to fully differentiated and specialized cell types. Several transcriptional networks that tightly regulate the developmental trajectories of specific cell types within the root have been elucidated (e.g. trichoblast, endodermis, and xylem cells). Yet, our understanding of the evolutionary conservation of these processes is scarce. To this end, we developed and exploited a set of cell type- and tissue-specific promoters in Solanum lycopersicum (tomato), Oryza sativa (rice) and Arabidopsis thaliana that enable TRAP (Translating Ribosome Affinity Purification). Using these, we have analyzed the transcriptional landscape of four comparable cell types across these divergent species. In some tissue, gene expression data is more conserved within comparable cell types than between species. We next focused on specific sets of genes that show enriched expression in each cell type. The identity of the cell type-enriched genes (i.e. orthologs), were only partially overlapping across species. Furthermore, enriched biological processes of these gene sets showed limited commonalities within comparable cell types. These analyses indicate that at the level of the translatome, cell type-enriched genes are for the most part species-specific with partial functional conservation across species.

Chair and Concurrent Symposium Speaker: Siobhan Brady

Primary Poster Presenter: Lidor Shaar-Moshe

A multi-step morphogenetic process governs pavement cell shaping in the leaf epidermis (0900-038)
Hall 2

The generation of simple plant cell geometries such as cylindrical shoot epidermal cells is known to be regulated by the extensibility pattern of the primary cell wall, thought to be largely determined by cellulose microfibrils. However, the mechanism leading to more complex shapes such as the interdigitated, jigsaw puzzle-like patterns in the epidermis of eudicotyledon leaves is poorly understood. We

investigated how the cell wall regulates the morphogenetic process in these cells and which initial steps lead to the characteristic undulations in the cell circumference. Brillouin microscopy and polarized fluorescence imaging allowed us to untangle the respective roles of cellulose and homogalacturonan pectin during lobe formation in the epidermal pavement cells of the cotyledons of Arabidopsis thaliana. We show that non-uniform distribution of cellulose microfibrils and demethylated pectin correlate with spatial differences in cell wall stiffness but intervene at different developmental stages. Challenging the widely accepted paradigm of cellulose as a crucial morphogenetic agent, we discovered that lobe initiation involves a modulation of cell wall stiffness through the local enrichment in demethylated pectin, whereas only the subsequent increase in lobe amplitude is mediated by the stress-induced deposition of aligned cellulose microfibrils. Finite element simulations lead us to propose that both steps are preceded by a turgor-driven mechanical buckling event that serves as the initial trigger for the multi-step morphogenetic process.

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Primary Poster Presenter: Anja Geitmann

AtGRXS8 regulates nitrate response and root system architecture in Arabidopsis thaliana (0900-048)
Hall 2

Glutaredoxins (GRXs) are small oxidoreductase enzymes that can reduce disulfide bonds in target proteins. Arabidopsis thaliana has >30 GRX genes, but the biological functions of most of these GRXs is unknown. We previously found that several A.thaliana GRX genes are transcriptionally activated by nitrate, and that some of these GRXs may act as negative regulators of primary root growth. Here, we present a focused study of two of the nitrate-regulated glutaredoxins, AtGRXS5 and AtGRXS8. Analyses of transcriptional fusions (promoter-GUS) and translational fusions (coding sequence-YFP) demonstrated that AtGRXS5 and AtGRXS8 are expressed primarily in phloem, and that the corresponding proteins are localized to the cell nucleus and cytosol. Constitutive overexpression of AtGRXS5 resulted in lethality, while overexpression of AtGRXS8 produced plants with a dwarf shoot system and highly altered root system architecture. Specifically, AtGRXS8-overexpressing lines displayed normal primary root growth, but lateral roots were almost completely absent. To better understand the molecular perturbations caused by ectopic expression of AtGRXS8, we performed RNA-sequencing on the roots of wild-type and transgenic lines. AtGRXS8-overexpressing lines displayed a coordinated downregulation of high affinity nitrate transporters and increased expression of several CEP proteins that are associated with nitrogen starvation signaling. Collectively, phenotypes such as decreased lateral root growth, decreased expression of nitrate transporters, and increased expression of CEP genes suggest that AtGRXS8 inhibits typical plant responses to nitrate, despite the fact that AtGRXS8 itself is transcriptionally activated by nitrate. Thus, it is possible that the

primary role of AtGRXS8 is to dampen or inhibit initial primary nitrogen responses of A. thaliana roots in the soil.

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Primary Poster Presenter: Matthew Escobar

BASL scaffolds BPP phosphatases to regulate stomatal asymmetric cell division (0900-043)
Hall 2

Asymmetric cell division (ACD), a fundamental process that produces two daughter cells with distinct cell fates, underlies developmental progress and patterning in multicellular organisms. The polarity protein BASL(BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGEA) regulates stomatal ACD in Arabidopsis. Our previous work showed that BASL is phosphorylated and activated by MAPK 3 and 6 (MPK3/6) and becomes polarized to the cell cortex, where it recruits the MAPKK Kinase YODA and MPK3/6 to inhibit stomatal differentiation in one of the two daughter cells. Recent work showed that, prior to a stomatal ACD, the polarity complex employs POLAR to recruit the GSK3-like kinase BIN2 that releases the suppression of YODA on stomatal differentiation, therefore stomatal ACD is promoted. Therefore, the stomatal polarity complex by scaffolding different signaling molecules could promote the division potential before an ACD and suppress the division potential after an ACD. However, how the transition of these two seemingly opposing procedures can be achieved by the same polarity complex remained a major challenge towards understanding stomatal ACD. Here, by using immunoprecipitation combined with mass spectrometry (IP-MS), we identify a family of protein Ser/Thr phosphatases, BPPs (BASL phosphatase partners), as BSAL-interacting proteins. Genetic analysis places BPPs upstream of the YDA MAP kinase cascade and downstream of the plasma membrane receptors. In addition, the founding member BPP-1 colocalizes with BASL in a polarized manner at the cell periphery. Interestingly, the recruitment of the BPP phosphatases in the polarity module confers a negative role to BIN2 complex but a positive role to the YDA MAPK module. Thus, our study reveals a crucial function of the BPP phosphatases in bridging the two opposing protein functional modules to control the balance of cell division potential and cell fate determination in plant ACDs.

Co-author(s): Juan Dong

Primary Poster Presenter: Xiaoyu Guo

Cell Re-programming Illuminates Fate Acquisition in the Arabidopsis Root (0900-068 (Screen 6))
Hall 2

The organs of all multicellular organisms originate from stem cells, yet many steps in the journey from pluripotency to terminal differentiation remain unknown. A growing body of evidence suggests that gene regulatory networks (GRNs) play an important role in orchestrating cell maturation. The Arabidopsis root, with its simple structure and organized stem cell niche, is a tractable model for studying the regulatory dynamics that govern cell fate and tissue patterning. Over two decades of work have outlined the GRN that controls cell proliferation and specification of the endodermis, a tissue analogous to the mammalian epithelium. In contrast, regulators of downstream events contributing to endodermal differentiation remain mostly unknown. Recently, our lab successfully primed young cells from a non-native lineage to acquire endodermal identity in the context of living roots ('cell reprogramming'; Drapek et al., 2018). We hypothesized that in re-programmed tissue, ectopic endodermal structures are less canalized, less developmentally stable, and therefore constitute a sensitized genetic background in which to uncover new regulators of native endodermal fate stabilization and differentiation. To test this hypothesis, we performed a forward genetic screen and have identified several mutants unable to stabilize re-programmed endodermal identity. In parallel, we are using single cell RNA-sequencing to ask how reprogrammed endodermal cells regulate their maturation. Cascades of differentially expressed genes along the developmental trajectory contain candidate regulators of endodermal fate. Together, these complementary classical and state-of-the-art experiments in a sensitized genetic background constitute a promising approach to illuminate how endodermal cells traverse the pathway to differentiation.

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Primary E-Poster Presenter: Rachel Shahan

Chemical enhancement of seedling root growth in legumes (0900-045)
Hall 2

Legumes (Leguminosae) represent the second most economically important family of crop plants. Grain legumes account for 27% of world crop production and provide 33% of the dietary protein consumed by humans. Furthermore, forage legumes are a vital component of animal feed. Yield of legumes whether it be for grains or forages depends on robust seedling establishment, a process that is in large part dependent on roots that are able to grow rapidly after the seed germinates. In our study, we utilize a small synthetic molecule that can significantly promote root growth in legume crops such as soybean, alfalfa, cowpea and the model legume Medicago truncatula. Roots of those legume seedlings treated with this chemical were 85% longer than untreated plants on average. We also noted a significant reduction of root thickness upon exposure of seedlings to the chemical. Longer and thinner roots could lead to more efficient resource acquisition in deeper soil layers enabling plants to be more productive under marginal environments. Transcriptomic analysis of M. truncatula roots exposed to the chemical uncovered key genetic regulators involving cell wall organization that govern root growth in legumes. A

possible novel molecular mechanisms underlying crosstalk between root growth and defense also emerged from this study. Genes within these networks could serve as potential targets for improving root vigor of legume crops without compromising plant immunity, through transgenic approaches and/or genome editing.

Primary Poster Presenter: Chenglin Chai

Cloning of wi1, Wi2, Wi3 and Wi4 mutants of maize via bulk-segregant whole genome re-sequencing (0900-050)
Hall 2

The vascular system is not only an integral part of the transport system in plants but it also plays roles in plant growth, development and adaptive responses to the environment. Wilty2 (Wi2), Wi3 and Wi4 are class of non-allelic EMS-induced dominant mutants of maize isolated by Gerry Neuffer in the 1980s. Wilty mutants manifest severe wilting under well-watered conditions, but are unaffected in ABA biosynthesis/response. Wilty1 is a single nuclear gene spontaneous recessive mutant described by Oliver Nelson in 1950s. Wilty mutants show varying degrees of pleiotropy with Wi4 showing a highly reduced growth habit followed by wi1, Wi2, and Wi3. The phenotypes manifest at 3 to 5 leaf stage and the mutants have smaller, yet more abundant vascular bundles per unit stem/leaf area as compared to their WT sibs. Histochemical staining of the fresh internode and biophysical analysis of extracted VBs by FTIR-ATR suggests changes in cellulose and lignin contents in the mutant VBs. We identified a sole top candidate causal single nucleotide variant for each Wilty mutant by bulk-segregant whole genome re-sequencing of pools comprising of homozygous/heterozygous mutant sibs. The workflow entailed NovaSeq library sequencing to ~80xgenome depth coverage and parent–offspring trio package COBASI to identify the de-novo child variant alleles. To provide independent tests of our claims of gene identification, we are currently performing transcriptome analysis and an immunoblot for the candidate Wi2 protein. We hypothesize that these transcriptome studies can further substantiate our top candidate genes as causal effectors of the wilty phenotype. Going forward, transgene phenotype complementation experiments with mutant alleles will provide independent evidence for cloning the Wilty genes. These studies may reveal new insights into the underlying molecular mechanisms of vascular development and adaptive stress response pathways of particular importance to engineering cellulosic biofuels.

Co-author(s): Chris Rock

Primary Poster Presenter: Anuradha Dhingra

Do roots undergo developmental phase change? (0900-046)
Hall 2

The plant life cycle progresses through a series of predictable phases: from seed to seedling, from vegetative leaf production to reproductive flower formation, and

from fruit set to senescence. Each of these phases is defined by a particular set of attributes, such as leaf shape, that is controlled by the hormone levels and gene expression profiles that occur together at that stage. While several phases, and the transitions between them, are well understood in the shoot, studies of root development typically treat the root as if it were always the same. We hypothesized that roots undergo phase change. If this were the case, there must be changes in root development that have a tendency to co-occur as a result of a developmental program, rather than being primarily driven by environmental influences. To test this hypothesis, we first investigated whether Arabidopsis thaliana roots grown on agar plates undergo changes in development during the first week after germination. We observed numerous changes in rates of development and morphology, several of which had a transition point around 4 days post germination. For example, during this period, cortical cell length increased, rates of lateral root emergence increased, and hormone profiles changed. Following this transition period, auxin response in the root tip increased as did the rates of root growth and lateral root initiation. The extent to which hormonal profiles influence the timing of these changes is currently under investigation; however, taken together, these findings provide support for the hypothesis that developmental phase changes occur in the root.

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Primary Poster Presenter: Marta Laskowski

Embryogenesis and regeneration of Moringa oleifera (0900-052)
Hall 2

Moringa oleifera is a nutritious perennial native to parts of Africa and Asia. The seeds are known for their antibacterial properties and role in water purification. Moringa oleifera seeds are easily propagated by seed and by grafting. However, regeneration using tissue culture would allow for mass production of plant tissue without having a seed and offer a time efficient alternative to traditional propagation. Attempts at calli formation from leaves and stems has proven successful, however, regeneration only occurred through embryos. Induction of calli was a result of exposing plant material to media containing 2,4-Dichlorophenoxyacetic acid. Preliminary results show regeneration of embryos through the transfer of calli to media containing indole-3-acetic acid and kinetin. Calli were incubated in the dark at 25 °C for two to six weeks. The regenerated plants were subsequently exposed to 16-h light incubation at 25 °C for several

weeks. Further work is needed to repeat the tissue culture protocol to ensure adequate regeneration.

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Primary Poster Presenter: Jennifer Noseworthy

Functional anisotropy: does it ever pay to be slow growing and fat? (0900-037)
Hall 2

Anisotropic growth, more in one direction over another, is a feature of plant growth at many stages and in many organs. Here we examine how the young seedling grows anisotropically to emerge successfully from the soil. This directional growth of the seedling in Arabidopsis is due mainly to cell expansion. We will present our new model of anisotropic growth control by the cell wall which includes multiple tissues. Furthermore, we will examine a case where anisotropy is reduced - meaning the seedling grows out of the soil slower and is fatter - and examine the cell biology and signalling mechanisms behind this phenomenon. We also explore the functional consequences of reduced anisotropy for seedling emergence. Come and find out if it ever pays to be slow and fat! Within this work, we will also describe new insights into cell wall mechanics related to viscoelasticity and cell wall pectin biochemistry. This will include the introduction of a new technique: nano-creep! This miniaturization of a classical viscoelastic testing method can be applied to single cells and single cell walls in vivo.

Co-author(s): Firas Bou Daher,
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Primary Poster Presenter: Siobhan Braybrook

High Level Sugar Inhibits BR Signaling in Light-grown Arabidopsis dependent on BIN2 and SPY (0900-051)
Hall 2

Sugar is essential for plant growth, not only as carbon supply but also as a regulatory signal. Recent studies showed that sugar promotes hypocotyl elongation by activating the Brassinosteroid (BR) signaling pathway in Arabidopsis seedlings shifted from light to extended darkness. However, it is reported that high sugar levels inhibit seedling growth, repress photosynthetic gene expression and induce genes of storage metabolism such as those of starch biosynthesis in light. Here, we show that high concentrations of sugar inhibit BR signaling in Arabidopsis seedlings grown under light. BR induced much more dramatic hypocotyl elongation in seedlings grown on sugar-free medium than those on high sucrose medium. The

reduced growth response is correlated with a decreased effect of BR on the dephosphorylation of BZR1, the master transcription factor of the BR signaling pathway, suggesting that sugar inhibits BR signaling. This sugar effect is independent of the sugar sensors Hexokinase 1 (HXK1) and Target of Rapamycin (TOR), but requires the GSK3-like kinase Brassinosteroid-Insensitive 2 (BIN2). We further found loss-of-function of the O-fucosyltransferase SPINDLY(SPY) abolished the sugar suppression on BR signaling in light. Our study uncovers an intimate relationship between carbon supply and BR that is highly modulated by light – a mechanism that apparently contributes to smart responses to light-dark conditions according to availability of photosynthate.

Co-author(s): Zhiyong Wang

Primary Poster Presenter: Zhenzhen Zhang

Interactions between the parasitic plant Cuscuta and its tomato host (0900-040)
Hall 2

Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. Cuscuta species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Many Cuscuta species are listed in the Federal or State Noxious Weed lists, including Cuscuta pentagona (C. pentagona). Reports have shown a 50–72% reduction in tomato yield due to Cuscuta. Because of the intimate physiological connection between host plants and parasites, most traditional herbicides and control methods have not been effective or are too costly. We used transcriptomics to identify genes upregulated in Cuscuta upon attachment to host. Expression of key upregulated genes was reduced using host-induced-gene-silencing and haustorium formation monitored. Reduction in expression of some of the identified genes attenuated parasitism. While most tomato cultivars can be parasitized by C. pentagona, we obtained some Heinz hybrid cultivars, which exhibited resistance to dodders. Local lignification in the stem cortex upon dodder attachment led to resistance to haustorium penetration in the resistant cultivars. Key resistance genes included an AP2-like transcription factor, a MYB transcription factor and an NBS-LRR (a gene encoding a nucleotide-binding site leucine-rich repeat protein). The function of these genes was deciphered using virus based gene expression. The results of this study may help develop a parasite-resistant system in crops to reduce economic losses in agriculture.

Primary Poster Presenter: Neelima Sinha

Light regulates stomatal development by modulating paracrine signaling from inner tissues (0900-033)
Hall 2

Developmental outcomes are shaped by the interplay between intrinsic and external factors. The production of stomata—essential pores for gas exchange in plants—is extremely plastic and offers an excellent system to study this interplay at the cell lineage level. For plants, light is a key external cue, and it promotes stomatal development and the accumulation of the master regulator SPEECHLESS (SPCH), which initiates the stomatal lineage. However, how light signals are relayed to influence SPCH remains unknown. Here, we show that the light- regulated transcription factor ELONGATED HYPOCOTYL 5 (HY5), a critical regulator for photomorphogenic growth, is present in inner mesophylls and directly binds and activates STOMAGEN. STOMAGEN, the mesophyll-derived secreted peptide, in turn stabilizes SPCH on the epidermis, leading to enhanced stomatal production. Our work identifies a molecular link between light signaling and stomatal development that spans two tissue layers and highlights how an environmental signaling factor may coordinate growth across tissue types.

Primary Poster Presenter: On Sun Lau

Mechanical cues regulate differential growth by shaping cortical microtubule/ cellulose network i (0900-039)
Hall 2

Being sessile, the developmental processes of plants are subjected to various environmental stimuli. A prominent example is the apical hook, which in the natural context is formed and maintained under constant mechanical cues from the surroundings- as the seedling grows upwards through the soil post-germination. However, formation of the hook has evolved as a de-novo developmental process that can occur in the absence of the usual impedance from the surroundings. It is therefore difficult to assess the impact of mechanical cues on the paradigm of differential growth associated with hook formation. bot1, a mutant deficient in the microtubule-severing protein katanin has been shown to have a disorganized microtubule network and is impaired in realigning the microtubules in response to external cues. We found that the bot1 mutant has a strong defect in the de-novo hook formation–which makes it an ideal candidate to investigate the modulation of this process by implementing external mechanical stimuli. The polar localization of several auxin efflux carriers (PINs) are altered in the bot1 mutant. Chemical and genetic perturbation of cellulose organization also resulted in similar defects, suggesting a novel and crucial role of cortical microtubule-guided cellulose arrays in determining the proper PIN polarity and establishing the auxin asymmetry necessary for de-novo hook formation. External mechanical cues partially rescued the hook formation in bot1 by reshaping the microtubules in a katanin-independent manner. We also found that such mechanical reshaping of microtubules needs to be reinforced by cellulose for restoring the PIN polarity and to reestablish the auxin asymmetry. Our observations suggest that in addition to prolonging hook maintenance, mechanical cues have a promoting effect on hook formation by reshaping the microtubule-cellulose network.

Co-author(s): Olivier Hamant, Rishikesh Bhalerao, Stephane Verger

Primary Poster Presenter: Anirban Baral

Molecular and genetic mechanisms that govern the formation of a suberized exodermis (0900-032)
Hall 2

Ensuring a dynamic interface between the roots and the soil is essential for plant survival. Plant cell walls sit at the cornerstone of this interface, acting as the contact point through which communication and exchange with the environment occurs. The matrix that comprises the plant cell wall varies not only across cell types but also through the developmental progress of each cell. Some cell layers form additional diffusion barriers via the deposition of polymers such as suberin. A well-studied example is the endodermis, which forms such barriers to control the entry to the plant vasculature. However, many plant species also contain an additional "sister cell type" right underneath the outer epidermis known as the exodermis. Exodermal differentiation and its ability to dynamically adapt to external conditions have not been formally characterized, nor the genetic and molecular mechanisms that govern them. Deposition of suberin is a complex process coordinated by several transcription factors and biosynthetic enzymes. While hundreds of genes potentially associated with biosynthesis and polymerization of suberin have been annotated, only subsets of these will participate in cell types that form barriers. In order to identify potential candidates, we leveraged cross-species root cell type-resolution transcriptomic, phylogenetic, and gene network analyses. We then coupled the ability of Agrobacterium rhizogenes to induce stable transgenic ("hairy") roots in tomato (S. lycopersicum), with the efficiency of CRISPR-Cas9 technology to rapidly generate mutants of these candidates. Finally, we used histochemical and transcriptional analyses to functionally validate the genes that form the exodermal diffusion barrier. Understanding how these processes are regulated is critical to a plant's ability to adapt to changes in water or nutrient availability. Ultimately, determining barrier-relevant genes will enable the breeding of crops with higher resistance to abiotic stresses.

Co-author(s): Siobhan Brady

Primary Poster Presenter: Alex Canto Pastor

Novel small molecule compounds affecting shoot regeneration (0900-035)
Hall 2

Plant organogenesis is strongly responsive to environmental cues due to the postembryonic activity of stem cell niches in apically positioned meristems. This adaptive mode of development is not only driven by the activity level of existing meristems; new stem cell populations can also be formed de novo under adverse circumstances such as wounding. This process is exploited in tissue-culture based

plant transformation protocols, where regeneration of plants from single cell is required. The plant hormone cytokinin plays a central role in shoot meristem regeneration, however there is evidence for additional small molecule signals involved. Reactivation of the shoot stem cell identity marker WUS in root explants of Arabidopsis is driven by an interplay of cytokinin-controlled B-type ARR proteins and HD-ZIP III transcription factors. HD-ZIP III proteins contain putative ligand binding domains for unknown small molecule regulators. In a chemical screen we identified compounds, which activate the expression of an HD-ZIP III direct target gene. Application of these chemicals causes ectopic shoot stem cell marker expression in intact plants. A subset of these chemicals also promotes shoot formation responses in Arabidopsis. Ongoing research indicates that they act partially independent of auxin and cytokinin. We will present our progress in characterizing how these compounds impact HD-ZIP III activity. Furthermore, we will show the effect of these chemicals on the shoot regeneration response of tissue culture-recalcitrant species such as sunflower.

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Primary Poster Presenter: Tobias Sieberer

O-glycosylated AGP21 acts on Brassinosteroid pathway to modulate root hair cell fate in Arabidopsis (0900-042)
Hall 2

Root hairs (RH) are single cells that develop from a group of specialized epidermal cells referred to as trichoblasts while cells that lack them are called atrichoblasts. RH cell fate is regulated by a complex of transcription factors that promotes the expression of the homeodomain protein GLABRA 2 (GL2), which ultimately blocks the RH pathway by inhibiting ROOT HAIR DEFECTIVE 6 (RHD6). The suppression of GL2 expression triggers epidermal cells to enter into the RH cell fate program by the concomitant activation of RHD6 and a downstream series of TFs including ROOT HAIR DEFECTIVE 6 LIKE-4 (RSL4) and downstream target genes. Cell fate in the root epidermis is influenced by phytohormones like auxins and Brassinosteroids (BR). It has been shown that in the absence of BR, phosphorylated BIN2 (a Type-II GSK3-like kinase) promotes the inhibition of a protein complex leading to the down-regulation of the main RH repressor GL2. In this work, an arabinogalactan protein (AGP) mutant agp21 as well as β-Glucosyl Yariv (β-Glc-Y) treatment (that disturbs AGPs) and several mutants deficient in AGP modifications, all trigger an abnormal RH cell fate phenotype reminiscent of mutants with deficient BR responses. We have found that an O-glycosylated AGP21-peptide positively regulated by BZR1, impacts on RH cell fate by disturbing GL2 expression in a BIN2 dependent manner. Together, these results show that disruption of cell surface AGPs, and in particular AGP21, interfere in a specific manner with BR perception and BIN2 mediated responses on the RH repressor GL2 in root epidermal cells in Arabidopsis thaliana.

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Primary Poster Presenter: Cecilia Borassi

Persistent water stress inhibits stomatal development in Betula nigra and Cercis canadensis, but not (0900-034)
Hall 2

Plant stomata are critical for regulation of transpirational water loss to the environment and modulation of stomatal of development may be an adaptive trait to respond to reduced water availability. However, the plasticity of stomatal traits has been assessed in only a very few perennial species. Furthermore, most studies have not focused on stomatal pore area, thus presenting an incomplete picture of stomatal plasticity. We quantified changes in stomatal and vein development in response to water deficit stress in river birch (Betula nigra L.), eastern redbud (Cercis canadensis L.), and red maple (Acer rubrum L.) by measuring stomatal index (SI), density (SD), size (SS), and pore index (SPI), as well as vein density (VD) and other leaf functional traits. Birch and redbud stomatal development under water deficit conditions was highly plastic towards the same anatomical goal: reducing SPI. In both mild and severe water deficit conditions, both species had reduced SS and/or SI to reduce SPI. Maple leaf stomata were less plastic, although SS was reduced under severe water deficit. In birch and redbud, stomatal plasticity was correlated with relative water content and leaf water potential, respectively. VD and SD were positively correlated in well-watered leaves, but this relationship was mostly lost in water-stressed leaves. SI and SS were positively correlated with net CO2 assimilation and stomatal conductance rates in birch and redbud. However, gas exchange rates were comparable to well-watered plants following the resumption of water sufficient conditions. Our study demonstrates that in certain tree species, stomatal development is downregulated in response to water deficit conditions, that stomatal plasticity varies depending on the intensity of stress, and that water-deficit-induced plasticity in stomatal and vein development is species specific, likely due to species adaptation to ecological niches.

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Primary Poster Presenter: Noel Mano

PLK1, a transmembrane receptor kinase, links lateral cell polarity with radial tissue patterning dur (0900-036)
Hall 2

The nearly invariant organization of cells and tissues in the Arabidopsis root is maintained by stringent control of the timing and orientation of cell division. Cell polarity and directional signaling are frequently proposed to have a key role in these processes; yet, very few proteins with polar localization, beyond transport proteins, have been characterized. We have identified a transmembrane receptor

kinase POLARLY LOCALIZED KINASE 1 (PLK1) that is required to control cell divisions oriented parallel to the root's growth axis and, thus, maintain radial organization. plk1 mutants have ectopic endodermal cell divisions resulting more cells in the radial axis and, unexpectedly, we also observe increased vascular area (width). Both endodermal proliferation and increased vascular area in plk1 mutants are rescued by PLK1 expression specifically in endodermal cells, suggesting both abnormal phenotypes can be attributed to endodermal proliferation. We find that the PLK1-GFP fusion protein is polarly localized to the outer plasma membrane domain of endodermal cells; furthermore, PLK1-GFP localization varies in distinct cell types. Differences in PLK1-GFP localization in specific cell types coincide with defective cell divisions observed in plk1 mutants, suggesting that specific localization of PLK1 to different regions of the plasma membrane is functionally important. We propose that PLK1 functions in a directional signaling pathway that inhibits certain cell divisions, which restricts endodermal proliferation in the radial axis.

Primary Poster Presenter: Jaimie Van Norman

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Primary Poster Presenter: Jaimie Van Norman

PLK3: A receptor-like kinase with lateral polar localization in root

epidermal cells (0900-041)
Hall 2

In plants directional signaling and cell-cell communication are proposed to play a critical role in the coordination of developmental events among cell types. However, few proteins with polar localization, beyond those involved in transport, have been characterized. We have identified an Arabidopsis transmembrane receptor-like kinase, POLARLY LOCALIZED KINASE3 (PLK3), with lateral polar localization in specific root cell types. Under its own promoter, PLK3-GFP accumulates the in outermost lateral root cap cell layer where it localizes to the inner polar domain. In the elongation and differentiation zones, PLK3-GFP localizes to the inner polar domain of epidermal cells and accumulates in root hair tips (RHs) during the initiation and elongation stages. When misexpressed by various cell type-specific promoters, PLK3-GFP localizes to the inner polar domain regardless of cell type. However, upon removal of the intracellular domains, PLK3Δ-GFP localizes to the outer polar domain of epidermal cells and is absent from tips of emerging RHs. This suggests that PLK3 polar localization is not dependent upon cell identity, but is a feature of the protein itself and indicates that the intracellular domains of PLK3 are important for polar orientation. Additionally, we observe that roots expressing pPLK3-PLK3-GFP are hypersensitive to mechanical force and/or osmotic changes. This phenotype can be recovered by maintaining stable osmotic conditions or exposing roots to phosphate-deficient media, which rigidifies root cell walls. We propose PLK3 localization is linked to it's function; given that PLK3-GFP is polarly localized in hair cells and in tips of forming hairs, PLK3 may function to modulate cell wall integrity during epidermal cell differentiation.

Co-author(s): Jaimie Van Norman

Primary Poster Presenter: Jessica Toth

Quantitative morphological phenomics of rice G protein mutants portend autoimmunity disease (0900-055)
Hall 2

The heterotrimeric G protein complex, composed of Gα, Gβ, and Gγ subunits, plays some role in structural development in both rice and Arabidopsis. In this study, we comprehensively profiled the root and shoot structural traits of rice Gα-null and viable Gβ-RNAi "knockdown" mutants, and found anomalous morphologies caused by Gβ-RNAi that are distinct from the Arabidopsis orthologue. The rice Gβ-RNAi mutant exhibited reduced radial growth of aerial parts as well as a more compact root architecture, among which smaller root mass seems mainly due to increased necrosis when grown on soil. In addition, three dimensional analyses of rice root system architecture revealed that the smaller root architecture of Gβ-RNAi plant is also due to both reduced root elongation and adventitious root formation. While this contrasts to the Arabidopsis Gβ-null mutant that promotes cell proliferation, we found the presence of elevated cell senescence activity during root formation through meta-analysis of the Arabidopsis root transcriptome. Through comprehensive and quantitative phenotypic comparisons across two angiosperm

models Arabidopsis and rice, we propose that the morphological phenotypes of rice Gβ-RNAi plants are predominantly associated to the mediation of various stresses and cell senescence. This study reveals that Gβ pathways during the course of plant growth regulate both cell death and proliferation activities in rice and Arabidopsis, however to different extents rendering the opposing morphological changes in orthologous Gβ mutants. We also elaborate our working hypothesis that cell division is a type of stress and as such due to impairment in responding to stress in the G protein mutants, manifests as altered morphology but not an altered body plan in plants.

Primary Poster Presenter: Daisuke Urano

Regulation of cotton fiber development by seven-in-absentia (SINA) E3 ligase (0900-054)
Hall 2

Cotton fibers are single specialized, highly elongated cells that grow from the seed integument. Although phytohormones are important in controlling cotton fiber development, little is known about the mechanism by which auxin mediates fiber cell initiation and elongation. In this study, we report that members of seven-in-absentia E3 ligase family in cotton play a critical role in fiber development, in part by regulating the auxin signaling pathway. Members of the SINA protein family contain RING-finger domains and function as specific ubiquitin ligases in animals and plants. The fiber specific cotton SINA is closely related to SINAT5 from Arabidopsis (>78% amino acid sequence identity) which has been shown to catalyze the ubiquitination of NAC1, an auxin-dependent transcription factor in roots. Ubiquitination assays show that GhSINA1 has E3 ligase activity. Further, yeast-two hybrid analysis indicates that GhSINA1 interacts with several transcriptional factors including NAC1 orthologue (GhNAC1). GhSINA1 is expressed in developing fibers and its expression is highly induced by auxin. The main roots of GhSINA1overexpressing transgenic Arabidopsis were significantly longer than those of the wild-type. Whereas the plants that over-express a dominant negative form of GhSINA1 show an opposite phenotype. These observations are in accordance with the role of the Arabidopsis SINAT5, which attenuates auxin signaling during root development.Transgenic cotton plants that over-express GhSINA1 and its dominant negative form under the control of fiber specific promoter (Gh10) were constructed and their phenotypes were analyzed. These transgenic lines displayed drastic changes during the initiation and elongation phases of fiber development. Based on these results, we hypothesize that GhSINA1 may modulate the role of GhNAC1 and other transcriptional factors during cotton fiber development through the ubiquitination pathway.

Co-author(s): Marjan Behzadirad,
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Primary Poster Presenter: Ruwanthi Wettasinghe

Root phenotyping by ground penetrating radar gives good correlations with root biomass in cassava gr (0900-056)
Hall 2

Great advances in the field of rapid non-destructive plant phenotyping have been made recently for aerial plant parts. However, few options exist for rapid non-destructive monitoring root growth and their phenotypic characteristics under control environments or in the field. We tested the application of ground penetrating radar (GPR) to non-destructively monitor the growth of cassava storage roots. The objective of the study was to test if GPR has sufficient to estimate growing storage root biomass. Because we tested the GPR in the Cassava by Free Air CO2 Enrichment experiment (CassFACE), we expected to see differences in root biomass due to the elevated CO2 concentration treatment. In total, we screened the roots of three African cassava cultivars during 2017 and 2018 field seasons. In both years, we collected data at three different root developmental stages. Improvements in the methodology a near doubling of data collection in 2018 compared to 2017. In 2018 the above-ground portion of the plant was harvested before the GPR screening allowing the GPR antennae to be run directly over the center of plant thereby improving resolution. Destructive harvests of the storage roots were performed directly after GPR scans to calibrate the GPR determinations. Fresh and dry weight were determined, digital pictures collected, and the average density of the roots determined. Preliminary analysis, show that the GPR can detect maturing storage roots of cassava and 3D reconstructions of the roots can be rendered. Preliminary results showed strong correlations between the estimations of the weight biomass calculated with the GPR data and with the destructive harvest data. The results of this research support the application of the GPR as a useful tool to non-destructively phenotype storage roots and tubers in the field.

Primary Poster Presenter: Ursula Ruiz Vera

The SHR-SCR Regulatory Networks in the Arabidopsis Shoot (0900-047)
Hall 2

The GRAS transcription factor SHORT-ROOT (SHR), mostly together with its direct target SCARECROW (SCR), plays key roles in root development, including ground tissue patterning, stem cell maintenance, and vascular development in Arabidopsis. In addition, it is shown that the two GRAS transcription factors act as positive regulators in proliferative cell divisions in the leaf and that SHR and SCR, along with SCARECROW-LIKE 23 (SCL23), the close homolog of SCR, regulate specification of the leaf bundle sheath (known as equivalent to the endodermis). Compared to what is known in the root, the SHR-SCR regulatory networks in the shoot, however, are largely unknown. Here we demonstrate that SHR directly regulates the expression of cell-wall modifying enzyme genes (xyloglucan endotransglucosylase/hydrolase; XTHs) under dark-grown conditions. Further analysis revealed that overexpression of some of SHR-regulated XTH genes could suppress, at least partially, the short-

hypocotyl phenotype of the etiolated shr mutants. Interestingly, we found that the restriction of SHR movement in the vascular tissues was still able to rescue the short hypocotyl length of the shr seedlings under dark-grown conditions. In addition, the loss of SHR function causes switching from indeterminate to determinate shoot growth, through reduced proliferation and aberrant differentiation in the shoot vascular stem cells. Similar to its role in the root, we found that SHR controls the growth and development of the Arabidopsis shoot via stem cell maintenance in the vascular cambium. Taken together, we propose that SHR plays key roles in the shoot, including hypocotyl cell elongation and vascular stem cell development.

Co-author(s): Souvik Dhar

Primary Poster Presenter: Jun Lim

ULTRAPETALA1 FUNCTION ON THE ARABIDOPSIS ROOT STEM CELL NICHE MAINTENANCE (0900-049)
Hall 2

Meristem homeostasis depends of a complex regulatory network constituted by different factors and hormone signaling that regulate gene expression to coordinate the correct balance between cell proliferation and differentiation. ULTRAPETALA1 (ULT1), a protein described as Arabidopsis TrxG factor, participates in the regulation of gene expression of the shoot and floral meristems, acting as a cofactor of the histone methyltransferase ATX1 for the deposition of H3K4me3 mark.In order to determinate the role of ULT1 in the root apical meristem (RAM), we described the ULT1 expression pattern in root tissues and characterized the root phenotype of ULT1 loss of function mutants. In addition, we analyzed the genetic interaction between ULT1 and ATX1 in the RAM. Our results indicate important roles of ULT1 in the maintenance of root stem cell niche, which will be discussed.

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Primary Poster Presenter: Diego Arturo Ornelas Ayala

Uncovering the Role of Hormones in Brace Root Initiation in Maize (0900-053)
Hall 2

Brace roots are aerial nodal roots found in maize and sorghum that are proposed to function in late-stage nutrient uptake and lodging resistance. While the developmental pathways of the underground root system are well studied, much less is known about the development of the aboveground brace roots. Brace roots

are a type of post-embryonic root, and their development has been suggested to parallel that of other post-embryonic roots such as lateral roots. Lateral roots are initiated from the pericycle in the parent root; however, a pericycle-like cell is not known to exist in the monocot stem. Thus, it remains unclear how stem-borne brace roots are initiated. One hypothesis for the initiation signal is the well-studied hormone auxin. My research aims to determine if auxin is necessary and sufficient for brace root initiation. My analysis of the DR5::erRFP and PIN1::PIN1-YFP auxin reporter lines show these reporters are not expressed in brace root primordia. To determine if auxin is present, but not transcriptionally active I plan to analyze another reporter line, pUbiquitin::DII-VENUS. To guide my future studies, I have obtained RNA sequencing data from the first three above-ground nodes at vegetative stage V6 of the maize B73 genotype. The V6 stage was chosen because the lowest node shows fully developed, mature primordia, the middle node shows immature primordia, and the uppermost node very rarely contains primordia at all; essentially capturing all stages of brace root initiation. I will focus my analysis of this data on the differential expression of auxin-related genes and on other hormones that may be involved in this process. Together my project aims to uncover the role of hormone signaling pathways in maize brace root initiation.

Co-author(s): Erin Sparks

Primary Poster Presenter: Sarah Blizard

1:30 PM - 3:00 PM

Using mathematical modeling and natural variation to investigate root growth behavior and responses (1100-001)

Gravity is a constant force that guides plant organs' growth, allowing roots to take up water and nutrients from the soil, and shoots to grow above ground where they can access light for photosynthesis. In this study, we took advantage of the natural variation existing between accessions of Brachypodium distachyon, a monocot model, to investigate root-growth behavior in response to gravistimulation. When Brachypodium seedlings are reoriented within the gravity field, their roots display a biphasic response. The root tip initially shows a strong downward gravitropic curvature, followed by a slower downward response that is accompanied by tip oscillations. Curvatures associated with both phases occur at the distal elongation zone. To quantify features related to both phases, we developed a mathematical model that simulates the kinetics of root-tip angle, using (1) a sigmoid function to represent the rapid bending phase, and (2) a sinusoidal function that recapitulates the oscillatory component of the second phase along with a linear trend that simulates the progressive bending toward gravity. Fast-Fourier Transform analysis was used to evaluate the periodicity (P) of root-tip oscillations at the end of a gravitropic response. Equation fitting led to determination of quantitative parameters that explain distinct characteristics of the behavior, including: speed of initial curvature (MRBR), transition angle between response phases (TA), and amplitude (A) of oscillations. For each of these traits, average value and standard deviation were included as separate parameters in genome-wide association studies to identify associated polymorphisms likely to contribute to the behavior. In our

results, three of eight parameters showed association peaks, including amplitude, standard deviation of amplitude, and MRBR. Multiple candidate genes were identified using this approach, whose molecular characterization will be discussed. This work is supported by a grant from NASA.

Co-author(s): Richard Barker,
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Primary Poster Presenter: Shih-Heng Su

Vascular patterns in grape berries (0900-044)
Hall 2

Vascular bundles are the main transport channels of water and nutrients into grape berries, and play a very important role in fruit development and quality. During the late ripening stages, water flow through the xylem declines significantly. Phloem transport takes over in supplying water for further growth and water replacement to transpirational loss. Xylem may serve as the channel for water backflow and recycling of excess phloem water. The balance of water dynamics dictates yield and fruit quality. How the vascular bundles connect from the pedicel into the grape berry at brush zone remains unclear. We used fluorescent microscopy to document the morphology and anatomy of brush zone vascular bundles of four grape cultivars. Fresh and methanol stored berries of Shiraz (BVRC 12 & 1654), Sauvignon Blanc, Ruby Seedless and Flame Seedless were hand sectioned and stained with Aniline Blue Fluorochrome and Acridine Orange respectively. A distinctive change in vascular arrangement from the pedicel into the grape brush zone was apparent in all examined cultivars. At the junction of the receptacle (stem) and the berry pericarp, the vascular bundles exhibited the arrangement where xylem was surrounded by phloem. In the brush zone, the vascular bundles reoriented so that the phloem was situated on the inner side of the vascular bundles. This is the first study to characterise the vascular patterns of the grape berry brush zone of four commercial cultivars.

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Primary Poster Presenter: Zeyu Xiao

LONG AFTER LONG DAY 1 is a positive regulator of early photomorphogenesis in Arabidopsis thaliana (0900-069 (Screen 8))

Hall 2

Light is an essential developmental stimulus in plants, yet we still have an incomplete understanding of the transcription factors involved in various light-activated pathways. Using EMS-based forward genetic screening and next-generation sequencing, we identified LONG AFTER LONG DAY 1 (LAL1), an R2R3 MYB transcription factor, as a putative positive regulator of photomorphogenesis. In silico analyses and experimental validation suggests that LAL1 has diurnal expression patterns under short-day, and cloning of the gene identified at least five alternative splice variants. Interestingly, only one LAL1 splice variant contains the C-terminal activation/repression domain that typifies canonical R2R3 MYB proteins. Seedlings over-expressing full length LAL1 protein have short hypocotyls in wild type, phyb-9, hy5, and nf-yc3/4/9 hy5 backgrounds. However, nf-yc3/4/9seedlings overexpressing LAL1 have similar hypocotyl length compared to parental genotype. We hypothesize that hyperaccumulation of a specific alternate splice variant in an EMS-induced mutant caused a dominant negative long hypocotyl phenotype under various light regimes and enhanced accumulation of PIF4/5 transcripts under short-days. Moreover, the ems mutant had significantly reduced expression of LUX and LHY during their peak expression time under short days. This suggests that truncated LAL1 exerts transcriptional control on these genes by some unknown mechanism. Characterization of knock out CRISPR lines and molecular analyses of the splice variants are currently underway and will lead to a better understanding of the role of LAL1 during early seedling development.

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Primary E-Poster Presenter: Swadhin Swain

Education and Outreach

Chlamydomonas reinhardtii: A "Rock Star" Green Biology Teaching Tool
(0100-018 (Screen 8))
Hall 2

Chlamydomonas reinhardtii is a micro-green alga that retains many of the features of the green plant and of the common ancestor of plants and animals. It is an elegant experimental model system for conducting plant biology, biomedical and bioenergy research. Chlamydomonas is currently an under-utilized teaching tool and has immense potential to be developed into a powerful popular teaching tool. I have designed simple, inexpensive, hands-on-activities based on published Chlamydomonas research for active learning in K-16 classrooms. These activities are part of my awarded 2018 Plant BLOOME grant and have been designed for students ranging from 4th graders to college undergraduates in financially disadvantaged schools/universities. Some of the Biology topics in the K-16

curriculum that can be taught using Chlamydomonas are sexual reproduction, cell division, genetics, structure and function of eukaryotic flagella and eye spot, Optogenetics, eukaryotic photosynthesis, high light stress responses, generation of ROS and its detoxification via anti-oxidants in plant cells, photosynthetic pigment metabolism and, biomass and bioenergy production. Labs are designed using simple plant physiology, molecular, biochemistry and bioinformatics tools and art and crafts supply. I will present four sets of designed hands-on-activities. These topics are: photosynthesis/photosynthetic pigment biosynthesis, eyespot and flagella. I will show how each activity topic can be customized for students at different grade levels and, demonstrate how concepts from the labs on photosynthesis, pigment biosynthesis, eye and flagella can be linked together for NGSS Biology core concept mapping. These teaching strategies will show the intra- and inter- disciplinary nature of Biology and will help to generate enthusiasm and respect for a "pond scum" among 21st century Biology students, especially to those who have the notion that allied health disciplines have no connection with plant biology.

Primary E-Poster Presenter: Mautusi Mitra

Undergraduate Students Report Cognitive Gains and Scientific Career Interests after Screening for N- (0100-019 (Screen 3))
Hall 2

Universities have been transforming STEM education by exposing undergraduates to the process of research for decades. One way this has been done is through Course-based Undergraduate Research Experiences (CUREs) where students work on a research project during a semester-long course. At the University of North Texas (UNT), students have been participating in an advanced research course focused on the areas of Molecular and Cell Biology and Biochemistry for several years. BIOL 3900, Advanced Research in Life Sciences, provides a research experience for up to 16 students, at one of the nation's largest and most diverse universities where 42 % of undergraduates are first-generation students. During this course, students read scientific literature, conduct experiments, maintain a lab notebook, and evaluate and interpret their results. At the end of the semester, students are required to write a scientific paper describing their results in the submission format of a suitable scientific journal and to deliver an oral presentation summarizing their project and results. Recently, this course focused on identifying protein interactors of N-Acylethanolamines (NAEs), a class of fatty acid derivatives that play a role in plant growth and development. Students cloned coding sequences for proteins previously identified in a NAE-interactor screen and conducted yeast three-hybrid assays. In order to assess the benefits of the course, students were asked to take pre- and post-course surveys about their experience. After participating in this CURE, students at UNT reported gains in scientific skills and a clarification in career path that is similar or higher than students participating in CUREs across the country. In all, this CURE program provided an effective and

efficient opportunity to broaden participation in life science research at an institution where demand for these experiences outpaces the supply. Supported in part by National Science Foundation grant- NSF-IOS 1656263.

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Concurrent Symposium Speaker: Ashley Cannon

Soil Microbiome Project at Contra Costa Community College : Authentic research in a course structure (0100-020 (Screen 2))
Hall 2

The Soil Microbiome Project at Contra Costa Community College (CCC) mimics the typical environment a student experiences a lab internship, in a format that is accessible to a large number of community college students. The majority of CCC students are from populations traditionally underrepresented in the science fields. Students participating in the project are in their first or second year of college, and are typically biotechnology, biology, and chemistry students, many who intend to transfer to a 4-year institution, but include some who seek technical training and plan to join the biotechnology workforce soon after earning a certificate. In two separate courses, students learn and employ the techniques of microbiology, plant care, phenotyping, and sampling, molecular biology, bioinformatics, and good laboratory practice. While student participation in the work is semester-by-semester, the project itself is a multi-year analysis of soil development at a nearby site where Urban Tilth, a non-profit organization, employs permaculture and other farming methods to improve 3 acres of land at the North Richmond Farm, an urban location which will serve as a food and community hub in what is currently a "food desert". This presentation will discuss the innovative pedagogy and course design that allows relatively inexperienced students to contribute to an ongoing scientific investigation by contextualizing technical training to authentic research, and builds both awareness and practice of work skills and habits. Application of concepts and competencies common in the field of biotechnology operations and supply chain management to this multi-year, team-based investigation will be discussed, along with learning outcome results. Student-generated data showing how the soil microbiome at the North Richmond Farm has changed and affected plant growth during the first two years of Urban Tilth's soil-building will also be presented.

Primary E-Poster Presenter: Katie Krolikowski

Antimicrobial effects of essential oils: incorporating undergraduate interest, ability, and time (0100-001)
Hall 2

Undergraduate research at Primarily Undergraduate Institutions (PUIs) has been shown to increase retention in STEM majors and provides training for the next generation of scientists. PUI faculty typically have heavy teaching loads, an expectation to mentor undergraduate research, and inadequate funding and resources. Additionally, it is difficult to balance the interest, ability, and availability of student researchers with the faculty member's area of expertise, particularly at schools where the majority of majors focus on pre-health careers. Eight students over five years have investigated the antimicrobial potential of five essential oils on a range of plant and human bacterial and fungal pathogens. Medically-oriented students gravitate towards this project as it ties to public health issues around multi-drug resistant pathogens with few novel compounds coming up the pipeline. Kirby-Bauer assays have demonstrated the potential of the various essential oils to limit growth of the majority of pathogens tested as well as or better than conventionally used antibiotics. Students with more time on the project (ie. multiple semesters) expanded the analysis to minimum inhibitory and bactericidal concentrations of the oils and helped mentor less experienced lab members. Limitations have been a lack of willingness to finalize results for publication as undergraduates prefer to test novel essential oils rather than complete what is perceived as someone else's project. The project has the potential to involve local collaborators in chemistry and pharmacy to create a more comprehensive look at the effectiveness and mechanism of action of plant-derived products in medicine.

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Primary Poster Presenter: Maryann Herman

Calderwood Seminars: Teaching Students the Art of Communicating Science to the Public (0100-012)
Hall 2

A group of faculty members at Wellesley College developed Calderwood Seminars in Public Writing in disciplines ranging from Economics to Biology and Math, with the goal of honing the communication skills of our students. The feedback from students is overwhelmingly positive. Many students find the seminars to be the most important class taken in their undergraduate years. We will lay out how these courses work and how to adapt the organizational structure to run similar classes with a focus of your choosing: The courses use a common text eg a primary literature article, lecture or book on a different topic each week. Rather than being narrow in focus, these courses, aimed at juniors and seniors, allow students to revisit a wide range of topics in their area of study. The common text is used as the starting point for writing an 800-word article for the general public with a specific audience in mind. Students are 'forced' to truly understand the material before

deciding which aspects are important. They learn to effectively explain complex ideas in a clear yet sophisticated manner, and how to make the ideas relevant to their audience by putting them into a larger context. Lectures are used to clarify concepts that were confusing as evidenced by vague or jargon-laced writing in students' first drafts. Other essential parts of each course are peer editing and workshopping of the papers, which allow students to see how others have tackled the same material and helps them to become constructive editors. Each student writes at least 3 versions of each paper, but the instructor sees only the first and last version. Calderwood seminars lead to vastly improved writing skills over the course of the semester, but even more excitingly, the students gain a sense of ownership of their writing, think about issues in a larger context and realize that their points of view count.

Co-author(s): David Lindauer

Primary Poster Presenter: Martina Koniger

Closing the Gap: Engaging the Public in Phenology-based Citizen Science (0100-010)
Hall 2

We have developed a local citizen science program where volunteers monitor plant phenology on nature trails in Duluth, MN. One of the goals of our program was to empower the volunteers to be scientists and formulate their own questions. We started by hosting focus groups where we discussed what people wanted to know about their local environment. The volunteers came up with three key questions they wanted to answer: (1) What is long-term trajectory of declining species like paper birch, Betula papyrifera and green ash, Fraxinus pennsylvanica? (2) How is phenology impacted by Lake Superior? (3) How is phenology impacted by changes in the climate? Based on these questions, we established three phenology trails that have six common focal species and are placed in three different mesoclimates around Lake Superior. Currently, we have thirty volunteers involved in the program. Starting this spring, one to two volunteers are visiting each site per week and entering phenology data into Nature's Notebook (an application developed by the National Phenology Network). In the coming year, we plan to expand this program, recruit more volunteers and work with local teachers to establish additional sites. We have also developed K-12 outreach activities associated with our trails that will be used in a teacher professional training workshop in the late summer. Our hope is that this program can serve as a model for future citizen science programs designed from the bottom up. This project was support by a BLOOME grant.

Co-author(s): Ryan Hueffmeier

Primary Poster Presenter: Jessica Savage

Coz I'm Appy! Linking lectures with the real world using a mobile treasure hunt app. (0100-013)

Hall 2

Despite the importance of plants to society, people are increasingly blind to local plants. Additionally, students regularly report a lack of connection between the lectures and practical experience. To begin to address these issues in two first year undergraduate courses, I used the ActionBound mobile app with three stages to get students thinking about plants in the environment in a fun engaging way. The three stages: 1) designing their own plant key using material collected and placed in the lab; 2) using their key on plants numbered outside in situ; 3) finding plant groups or traits in the real world that had been covered during lectures. We evaluated how plant blind our students are, engagement levels, and student experiences as well as whether the students increased their awareness of lecture-taught materials. We found there were differences in Plant Blindness with the more diverse 'Life on earth' students demonstrating more difficulty in identifying plant families than the more focuses 'Plant Science' students. Engagement levels were high and most students enjoyed the activity and reported that it increased links to lecture materials.

Primary Poster Presenter: Amanda Rasmussen

CRISPR and Computational Molecular Modeling: The CURE for Undergraduate Genetics Students (0100-004)
Hall 2

Course-based Undergraduate Research Experiences (CUREs) broaden participation in undergraduate research by bringing research into the courses students are required to take. Numerous studies have shown that students engaged in CUREs gain confidence in their own abilities and are more likely to pursue advanced STEM degrees. Radford University has two large initiatives aimed at scaffolding undergraduate research into Biology, Chemistry, and Physics courses. As a recipient of a Howard Hughes Medical Institute Inclusive Excellence award, Radford is working to support faculty and students as we create a more inclusive learning environment by embedding project-based learning into the classroom. As a recipient of a Council on Undergraduate Research award we are redesigning our curriculum to scaffold undergraduate research throughout so that all students have a meaningful research experience. Radford University's sophomore-level genetics course has been fully redesigned so that students engage in authentic research and gain a deep understanding of how mutations at the DNA level modify the structure and function of proteins to cause a mutant phenotype. Through a twelve week series of activities the students: 1) learn the basics of gene regulation and plant development, 2) computationally model the three-dimensional structure of regulatory proteins required for plant development, 3) design and carry out CRISPR experiments, and 4) write a lab report or present a poster describing their results. The students reflect on their semester long research experience by responding to e-portfolio prompts where they describe the laboratory skills learned and how those skills will be used in their future careers. At the end of the semester, students can successfully describe how mutations in DNA cause phenotypes by altering protein

structure and function. Finally, students report they enjoy performing real research and are more confident in their ability to collect and analyze data.

Primary Poster Presenter: Tara Phelps-Durr

Developing natural scientists through the DIVAS computational training pipeline to enable a large-sc (0100-002)
Hall 2

Large-scale phenotyping experiments are conducted by collaborative teams containing individuals with knowledge and skills that sit at points along a continuum between the biological system under study and complementary disciplines offering approaches useful for studying it. Common gaps in this continuum represent places where the training pipeline can be improved. The DIVAS project (Digital Imaging and Vision Applications in Science) is an NSF-funded effort to build an 'on-ramp' for preparing natural scientists to engage in computational tasks, which are essential to the success of phenotyping studies. Using image data as a hook, preliminary results indicate that program interventions which include a week-long coding workshop in Python using OpenCV libraries, two-week pair programming projects, and one-credit professional development and special topics seminars are effective in improving self efficacy toward computing as well as increasing interest in pursuing additional computational skills within the participants' careers. DIVAS program elements have been utilized by individuals ranging from high school students through faculty members. The majority of participants are undergraduate natural science majors. This training pipeline has facilitated development of a phenotyping platform used to characterize spatial variation in root exudate production in corn. Surface compounds of seedling roots are absorbed onto polyethersulfone (PES) sheets. A chemical indicator printed onto the PES surface reacts with specific adsorbed compounds to produce an observable color change. Printed standards are detected and analyzed using automated image processing routines in Python using OpenCV libraries and their intensity values used to construct a standard curve for each sheet. This standard curve is used to quantify adsorbed compounds. Images of developed PES sheets are overlaid onto seedling images to localize signals to specific root structures.

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Primary Poster Presenter: Tessa Brooks

Embracing transparencies: developing an undergraduate mentoring toolkit

(0100-008)
Hall 2

An effective research program can act as a conduit to empower undergraduate researchers (URs) to become autonomous learners, critical thinkers, and inclusive problem solvers. During my postdoc, I have mentored URs to carry out a forward genetic screen to uncover novel regulators of plant stem cell maintenance. Using a sensitized genetic background, my team has performed a forward mutagenesis screen isolating candidate suppressors of POLTERGEIST, called ghostbuster mutants. The academic relationship between a researcher and their mentor is a core feature of scientific research training; yet few tools or resources are available to aid us from transitioning from mentees to mentors. I have developed a mentoring toolkit to guide young scientists (graduate students, postdocs, and early career PIs) through the process of mentoring URs. This model consists of 4 successive steps: goal setting, UR input, mentor feedback, and an evaluative assessment. This semester-based framework can be applied for a single academic term or it can be adapted for use over several repeated terms for advanced URs. My toolkit provides a positive model for formal evaluation that encourages URs to take ownership of their work, drive their own formative evaluation process, support self-awareness, and ultimately elevate every students' potential to succeed in the STEM workforce.

Primary Poster Presenter: Caroline Sjogren

Individual and team scores, personal strengths, and peer evaluation in team-based intro biology (0100-006)
Hall 2

We examined the relationships of team and individual scores and whether personal strengths affect either in a team-based introductory biology course for majors in which semester-long teams collaborate daily on in-class activities, take weekly quizzes, and work on a semester long project. To encourage development of team skills and personal accountability, every week student roles on the team rotate and team members note each other's contributions. At the end of the semester students peer evaluate teammates' contributions. We collected data from 357 students in three course sections. To achieve a high degree of diversity in team makeup, we formed teams based on various demographic and personal criteria including personal strengths that we matched to the categories or "guilds" (Administrator, Artist, Communicator, or Expeditor) laid out in Wright and Boggs 2003. At the end of the semester, team members indicated who exhibited traits characteristic of each personal strength category.•Individual and team scores were not correlated.•23% of students earned a team score that negatively affected their final course grade percentage; only 1% of students earned a team score that negatively affected their final letter grade.•Men and women had no significant difference in scores.•With the exception of Artist, there were no score differences among personal strength categories.•Peer-evaluation score and team score were not correlated.•Peer-evaluation score and individual score were positively correlated.•Peer-evaluation

scores were not different between men and women.•All teams identified individuals who contributed to teamwork according to each personal strength category.•Self-identified personal strength and team-identified personal strength often did not correspond. We conclude that wide variety of students can be successful in this course, which emphasizes personal accountability and team skill development.

Primary Poster Presenter: Sue Wick

Inspiring Evidence-Based Teaching Innovations with the Journal CourseSource (0100-005)
Hall 2

Changes in the way colleges teach biology are seen nationwide in the form of initiatives dedicated to advancing evidence-based science education practices, including active learning. One stumbling block in this transformation is the time and energy commitment needed to produce the learning materials. In response to this need, the journal CourseSource was created. CourseSource publishes undergraduate biology teaching materials that implement approaches already shown to be effective. CourseSource fills the previously unmet need for a peer-reviewed journal that captures and shares high quality, evidence-based teaching methods. This format means that adopters of active learning have a place they can go to obtain teaching materials that have been vetted by experts. One can think of CourseSource as a methods journal for teaching, with articles that provide specific field-tested implementations of evidence-based practice in a variety of undergraduate classroom contexts. CourseSource contributors develop and implement evidence-based approaches to meet specific learning objectives established by scientific societies. The opportunity to publish in CourseSource incentivizes instructors to share their materials publicly, promoting scholarly teaching in a format that intentionally supports use by others. Recently there have been several movements to rethink evaluation of teaching effectiveness. There are generally agreed upon metrics for evaluating research success, but evaluating teaching expertise using only student evaluations can be biased and not necessarily tied to student learning. Publishing activities in peer-reviewed journals like CourseSource can be used as evidence for effective teaching. By publishing in CourseSource, authors can amplify the impact of their work, promote course transformation, and help students learn—all at once.

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Primary Poster Presenter: Sue Wick

Isolation and characterization of bacteria from the plant rhizosphere (0100-003)
Hall 2

Plant Growth Promoting Rhizobacteria (PGPR) are bacteria that reside in the rhizosphere of plants and have a beneficial effect on plant growth. The bacteria do

this either by directly promoting plant growth by making nutrients available, producing phytohormones, etc., or indirectly by controlling plant pathogens. There has been much interest in the use of PGPR as a biofertilizer, as it would reduce the use of chemical fertilizers and promote more sustainable agricultural practices. The isolation and characterization of potential PGPR is the focus of the second semester of the General Biology Laboratory at LMU. Students learn and use basic tools and techniques of traditional and molecular microbiology, record and analyze their data for meaning and importance, prepare data in effective figures and tables, practice various forms of scientific communication, and have opportunities for free inquiry. Overall, the goal is to provide students an "authentic" research experience with the opportunity to think, work, and present their data as biologists and to cultivate in students an engagement in the process of science.Students isolate bacteria from the rhizosphere of plants, screen selected isolates biochemically to determine whether they have properties associated with PGPR, and inoculate plants to determine whether their isolates promote plant growth. Along with the phenotypic characterization of their bacterial isolates, students do molecular analysis of the 16S rDNA to identity their isolates by comparison to the database and derive phylogenetic relationships between their organisms and those of their classmates. Hundreds of isolates have been characterized, with species of Bacillus, Streptomyces, Flavobacterium, and Microbacterium the most numerous. Trends have been seen over the years in the biochemical makeup of the organisms that students have characterized, with about 39% producing auxin, 59% showing cellulase production, 34% solubilizing phosphate, 35% showing

Co-author(s): Tatiana Kuzmenko

Primary Poster Presenter: Michelle Lum

Plant Tracer: a new app to track and quantify plant movement (0100-009)
Hall 2

One of the most fascinating characteristics of plants is that they display sophisticated movements, particularly in growing tissues. Creating time-lapse movies from plants has recently become inexpensive and easy with the proliferation of smart phones. To better understand plant movement, we have developed Plant Tracer, an NSF funded App designed to enable the quantification of gravitropism (movement towards or away from gravity) and circumnutation (the periodic regular swaying found in plant organs). As part of a crowd sourced method, Plant Tracer is being used by both students and researchers to detect mutant genes in Arabidopsis that are impaired in plant movement. Plant Tracer represents a new approach to draw young scientists into the field of plant biology through research and inquiry using technology

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Primary Poster Presenter: Eric Brenner

1:30 PM - 2:00 PM

Strategic Recommendations for Plant Science Research and Workforce Development over the Next Decade (0100-021 (Screen 14))
Hall 2

Our planet and species are facing an interrelated set of profound challenges with limited time and diminishing opportunity to solve. The future health of the planet and its inhabitants centers on the role plants play in the environment and our knowledge of them. We must think and operate across entire domains of discovery that span scales from global to molecular to leverage the power of plants, rather than approaching challenges from the perspective of individual disciplines or collections thereof. To address these challenges, the Plant Science Research Network (PSRN) was formed in 2015, bringing together plant scientists to develop an innovative Decadal Vision (DV) for plant research. The DV priority areas are 1.) Workforce Development – Building inclusive capacity and interest to enter plant science in its relation to other disciplines; Create an adaptive, resilient workforce; and Support transdisciplinary and convergent research; 2.) Systems and Support – Agile and mobile plant science; Intentional design of data assets; Foster disruptive technologies; Resilient plants in natural and controlled systems; and Innovative funding models; and 3.) The Plant Science Research Agenda – four goals identified as priority areas for investment over the next ten years.

The Digital Imaging and Vision Applications in Science (DIVAS) Project: Student Experiences (0100-011)
Hall 2

The DIVAS (Digital Imaging and Vision Applications in Science) Project aims to build computational self-efficacy, inform careers, and support the computational thinking skills of undergraduate natural science majors using image data and analysis to attract students. This NSF-funded project is a collaboration between faculty representing biology, chemistry and computer science disciplines at Doane University in Crete, Nebraska and St. Edward's University in Austin, Texas and involves recruitment of six 'DIVAS scholars' each year. DIVAS scholars participate in two one-credit seminars, a coding workshop and 4-6 weeks of pair programming and summer research. Project participation begins with a seminar during the spring semester, recruiting primarily first-year students, who learn about image basics, images as data, and Python basics. The project continues into the summer with a week-long coding workshop that teaches basic bash commands, version control using git, and basic image analysis using Python/OpenCV. Pair programming projects, including a morphometrics challenge and colorimetric challenge, were

given to the group to complete in a period of two weeks. Scholars also worked on independent projects that focused on problems including detection and quantification of free amines secreted by maize roots, detection of the number of plaques on a media plate, counting viability of c. elegans in different treatment conditions, and tracking the progress of a chemical reaction. During the spring semester of the following academic year, students take another one-credit seminar in which they review and clean up the code repository they contributed to over the summer, learned the basics of cluster computing and parallelization, and learned to utilize Doane's Onyx supercomputer. The DIVAS project provides natural science majors an essential foundation in computing, via image processing, in a relatively short span of time.

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Primary Poster Presenter: Austin Sembera

Writing children's stories improves STEM student communication with non-specialists (0100-007)
Hall 2

As STEM majors, students often become the "science expert" in their social circles. In today's world of misinformation and misunderstanding, it is essential for our students to develop proficiency in non-specialist communication. As an attempt to develop this skill, junior electrical engineering and introductory botany students were asked to write children's stories explaining a complex topic in their field. We hypothesized student non-specialist communication would improve after writing and receiving feedback on these stories. To first explore this idea, pairs of electrical engineering students wrote a story explaining diodes, were provided qualitative feedback from non-engineers, and then wrote a second story explaining amplifiers. In the second iteration, students increased their reviewer scores by 28.3% (n=3) demonstrating an improvement in their ability to communicate technical information to non-specialists. This was marked by a decrease in jargon and reduced sentence complexity. To determine if this assignment improved general

non-specialist communication, the project was further developed for introductory botany students. As a baseline, non-specialist communication was assessed on the unit exam (energy & metabolism) before the project was assigned. Students then wrote children's books targeted to 6 to 9 year olds explaining gene expression. These students received qualitative feedback from non-biologists and revised their stories. On the next unit exam (evolution) students' non-specialist communication was re-assessed. After controlling for content mastery, a significant increase of 14% (n=21) in non-specialist communication was observed. These results provide encouraging evidence that children's stories have the potential to improve STEM majors' communication to non-specialist audiences.

Co-author(s): Benjamin McPheron

Primary Poster Presenter: Katelyn Butler

Genes & Genomes: Bioinformatics

TGMI: A high efficient algorithm for identifying biological pathway and process regulators using con (0300-145 (Screen 8))
Hall 2

Despite their important roles, the regulators that control most biological pathways and processes remain elusive. Presently, the novel methods for identifying biological pathways and processes regulators are desperately sought after. We developed a novel algorithm called triple-gene mutual interaction (TGMI) for identifying these regulators using high-throughput gene expression data. It first calculated the regulatory interactions among the combined triple gene blocks that include two pathway genes and one transcription factor (TF)) using conditional mutual information, and then identifies significantly interacted triple genes using a newly identified novel mutual interaction measure (MIM), which was substantiated to reflect the strengths of regulatory interactions within each triple gene block. The TGMI calculated the MIM for each triple gene block and then examined its statistical significance using bootstrap. Finally, the frequencies of all TFs present in all significantly interacted triple gene blocks were calculated and ranked. We showed that the TFs with higher frequencies were usually genuine pathway regulators after evaluating multiple pathways in plants, animals and yeast. Comparison of TGMI with several other algorithms demonstrated its higher accuracy. Therefore, TGMI will be a valuable tool that can help biologists identify regulators of biological pathways and processes.

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PhyloGenes, a new online phylogenetics resource for plant gene function inference (0300-146 (Screen 8))
Hall 2

Accurately and efficiently transferring knowledge gained from model organisms to other species, and inferring the function of a gene of interest, are continuing challenges in plant biology. Individual gene duplications, allopolyploidy, and autopolyploidy are common in all plant lineages, and result in complex evolutionary relationships between related genes. Such genes can have similar sequences but highly divergent functions. Therefore, function inference often requires analysis and integration of multiple types of information beyond sequence similarity, such as phylogenetic relationships, expression data, and phenotypes. We have developed a new online resource (phylogenes.org) that presents phylogenetic trees of gene families along with available gene function information, experimental evidence, and publications for each gene in the family. By assembling information in a way that visually preserves phylogenetic relationships, PhyloGenes enables more efficient inference of gene function. By making annotation evidence and sources and other metadata clearly evident and traceable, PhyloGenes will increase the accuracy of inferred gene functions. PhyloGenes' first public release (version 1.0) includes 29 plant genomes and 10 non-plant model organisms represented in over 8,000 gene families. The gene families and trees were constructed by using the PANTHER pipeline (pantherdb.org). Molecular functions of genes that were supported by experimental evidence were extracted from QuickGO (https://www.ebi.ac.uk/QuickGO/). Gene function information is displayed in data rows that are aligned with the corresponding gene node in a gene tree. Features planned for subsequent releases include the addition of datasets (e.g. expression pattern, protein domains), grafting a new sequence to a gene tree and user interface improvements.

Primary E-Poster Presenter: Peifen Zhang

Genome Sequence Annotation Server (GenSAS): A online platform for structural and functional annota (0300-147 (Screen 3))
Hall 2

The Genome Sequence Annotation Server v6.0 (GenSAS, www.gensas.org) is a secure, online annotation platform that combines several common annotation tools in one easy-to-use resource. Features include assessment of assembly and annotation quality, sequence size filtering, repeat masking, gene and other structural feature prediction, functional annotation and publication ready output files. The guided annotation process flows through user-friendly interfaces with embedded instructions and supports annotation of model and non-model plants. It allows users to upload Illumina RNA-Seq reads (or specify datasets from the NCBI

SRA database), align the reads to the genome using HISAT2 or TopHat2, and use aligned data to train the gene model prediction programs AUGUSTUS and BRAKER2, allowing for more accurate gene models. Integration of JBrowse and Apollo allow for structural annotation to be easily viewed, manually curated, and shared with other users for project or community annotation. Functional annotation tools assign protein functions and identify functional domains for the official gene set. As a final step the GenSAS pipeline generates the required files for publication and allows the user to run BUSCO on the predicted proteins to assess the completeness of the annotation.

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A bioinformatics approach (PIPE) in functional genomics of soybean and soybean-cross species interac (0300-002)
Hall 2

Soybean (Glycine max) is one of the largest sources of vegetable oil and protein in the world, and also an important legume crop to the Canadian economy. In order to expand soybean further north and west in Canada, the identification and characterization of genes involved in time of flowering and maturity as well as genes conferring resistance to the Soybean Cyst Nematode (Heterodera glycines) are crucial. The genes underlying many QTLs for time of flowering and maturity and for resistance to SCN are yet to be identified. Protein-Protein Interactions (PPIs) are essential molecular interactions that define the biology of a cell, its development and its responses to various stimuli. Theoretically, if a gene interacts with groups of genes involved in one specific pathway, that gene might also be involved in that specific pathway (i.e. “guilt by association”). The soybean Protein-protein Interaction Prediction Engine (Soybean-PIPE) is a computational tool capable of predicting genome-wide PPI in soybean.The latest version of PIPE (v.4) was used to predict the soybean and soybean-SCN comprehensive interactomes (genome-wide). As an example, the E8 maturity locus which was previously identified in our lab using classical breeding practices and a genome-wide SSR marker analysis, revealing a large region on chromosome 4 has been investigated by PIPE. Soybean-PIPE gene ontology, loss of function analysis, DNA sequencing, and expression analysis etc. were used in combination to identify the potential candidate gene for this maturity locus.Identification of candidate genes for economically important loci may have a far-reaching agricultural and economic impact for diverse goals including marker assisted selection.

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A microRNA biogenesis-like pathway for producing phased small interfering RNA from a long non-coding (0300-148 (Screen 3))
Hall 2

Phased small interfering RNAs (phasiRNAs) are a class of non-coding RNAs that perform essential functions in plants. Unlike microRNA biogenesis from a hairpin structure, the production of phasiRNAs usually requires a phase initiator and an RNA-dependent RNA polymerase (RDR) to form double-strand RNAs. By using full-length rice cDNA (KL-cDNA) to identify phasiRNA loci, we found that a putative non-coding sequence with a long hairpin structure generates the phasiRNAs, which we name Long Hairpin-structure containing non-coding RNA (LHR). The biogenesis of LHR-derived phasiRNAs was dependent on rice DCL4, but not on RDR2/6, DCL1, or DCL3. Since all of the LHR-phasiRNAs (-5p from the forward strand and -3p from the reverse strand of the dsRNAs) are mapped to the forward strand of LHR, LHR-phasiRNAs should be derived from its hairpin structure, similar to a microRNA precursor. A degradome-based validation suggested that several thylakoid-related genes were targeted by LHR-phasiRNAs. In addition, the production of LHR-phasiRNAs was completely abolished in the lhr mutant, which also exhibited decreased plant height, leaf size, and grain weight, probably through the regulation of photosynthesis. Based on our results, we propose a microRNA biogenesis-like pathway for producing phased siRNAs that expands our understanding of the current model of phased siRNA biogenesis in plants.

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A potent high-resolution DNA variant filtering improves in silico detection accuracy (0300-008)
Hall 2

The accurate detection of DNA mutations is a very critical step for gene function discovery studies. Current next-generation sequencing (NGS) technologies have rapidly accelerated the pace of discoveries in molecular genetics, but are characterized by an inherently higher sequencing error rates, and the concomitant adverse effects of false-positive in silico mutation detections. Various algorithmic techniques had been proposed for filtering false-positives. Two undesirable consequences of standard hard-filtering techniques are false-negative predictions, where bona fide mutations are erroneously discarded, and also the potential for low-concordance of predicted mutations among the leading DNA mutation detection

algorithms. We describe a general high-resolution computational method for uncovering false-negative induced DNA mutations in NGS sequencing data for mutagenized populations. Regardless of the variant-calling algorithm utilized, the method uses a binning approach by assigning predicted mutations with the same variant-call quality-score in the same category bin, and then empirically determining the quality-score threshold at which the exactitude of the predicted mutations in the NGS sequencing data mirrors the experimentally described DNA mutation spectrum of the mutation inducing agent. To evaluate the proposed technique, we applied the method to a previously described EMS-mutagenized sorghum population. Using the SAMtools variant-calling algorithm and a variant quality-score threshold of 12, almost 96% (3,141,908) of the 3,274,606 SNPs were GC□AT mutations. Alternatively, using the GATK algorithm and a quality-score threshold of 28, 93% (3,211,794) of the 3,435,789 SNPs were GC□AT mutations. This preliminary result represents an 87% (1,521,203 likely false-negatives) increase over the previously predicted 1,753,403 EMS-induced SNPs. The result also shows a high 94% (3,075,884 SNPs) concordance between the SAMtools and GATK variant-calling algorithms.

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AgBioData: Genomic, Genetic and Breeding Databases working together to ensure standards and best pra (0300-003)
Hall 2

Biological research is becoming increasingly data-driven. Data from funded research, when made FAIR (Findable, Accessible, Interoperable, Reusable), often becomes invaluable for further research. Making data from all publicly funded research available, however, requires authentic, detailed, accurate and explicit communication between all parties involved in generating and delivering the scientific data. In addition, making data FAIR requires development of effective methods, tools and resources, a goal made more achievable when biological database resources collaborate. The AgBioData consortium (https://www.agbiodata.org) formed in 2015 consists of over 150 database scientists from 30 plus genomic, genetic and breeding (GGB) databases and allied resources. Collectively, the AgBioData member databases served 27 million pages and 950,000 users in 2017, and between 2012-2017, they were cited in over 24,000 publications. The databases cover an extensive range of crops, livestock and model organisms, including arabidopsis, corn, wheat, legumes, fruits and nuts, vegetables, insects, cattle, chicken, fish, horses, pigs, and sheep. To move closer to making every piece of biological data available to researchers through organized,

easy-to-find and use resources, we need to continue to work together to adopt a common set of metadata, and associate more data with ontologies; make it easy to share data; share curation practices; and provide solutions for long-term funding for all genomic, genetic and breeding databases. AgBioData is a model for how databases can work together to be more resource-efficient and use a collective voice to lead efforts for better data management and database resource availability.

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De novo sequencing and analysis of Salvia hispanica leaf and root transcriptome (0300-007)
Hall 2

Salvia hispanica L. (commonly known as chia) is gaining popularity worldwide and specially in US as a healthy oil and food supplement for human and animal consumption due to its favorable oil composition, and high protein, fiber, and antioxidant contents. Despite these benefits and its growing public demand, very limited gene sequence information is currently available in public databases. In this project, we generated 90 million high quality 150bp paired-end sequences from the chia leaf and root tissues. The sequences were de novo assembled into 103,367 contigs with average length of 1,445 bp. The resulted assembly represented 92.2% transcriptome completeness. Around 69% of the assembled contigs were annotated against the uniprot database and represented a diverse array of functional and biological categories. A total of 14,267 contigs showed significant expression difference between the leaf and root tissues, with 6,151 and 8,116 contigs up-regulated in the leaf and root, respectively. The sequence data generated in this project will provide valuable resources for future functional genomic research in chia. With the availability of transcriptome sequences, it would be possible to identify genes involved in the important metabolic pathways that give chia its unique nutritional and medicinal properties.

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Functional genomics capabilities for transcript profiling and metabolic modeling in KBase (0300-013)
Hall 2

The Department of Energy Systems Biology Knowledgebase (KBase; http://kbase.us) is an open, web-accessible computational environment for systems biology research focused on microbes, fungal, plants and the microbial

communities. KBase provides a range of integrated biological data types and associated analysis tools (Apps) that include gene expression analysis, metabolic modeling, comparative genomics and functional genomics. The user-friendly KBase Narrative Interface offers researchers and bioinformaticians a range of analysis tools and data resources that accelerate the pace of functional genomics research by allowing large-scale sample processing, expression-level quantification and integration of gene expression profiles with downstream functional analysis including clustering of expression profiles based on different algorithms, ontology enrichment, metabolic networks and gene regulation. KBase currently has 78 plant genomes from the JGI Phytozome database, and 134 fungal genomes from the JGI MycoCosm database. KBase has several data resources that originated from the PlantSEED project which combines plant comparative genomics, functional annotation of enzymes, and reconstruction of plant primary metabolism for individual species. Plant-specific compounds and reactions, collected from public sources such as KEGG, MetaCyc, and AraCyc, have been integrated into PlantSEED and made available in KBase, where they can be used for plant metabolic modeling.KBase is also actively engaged with the external community to help us improve the available tools and workflows for functional and comparative genomics. These capabilities are directly relevant to important DOE research goals such as optimizing biomass production in biofuel feedstocks. KBase is funded by the Genomic Science program within the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research.

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Genome-wide identification and classification of WD40 superfamily genes in peach (0300-004)
Hall 2

The WD40 transcription factor family is a superfamily found in all eukaryotes that plays important roles in regulating growth and development. Based on our knowledge, to date, WD40 superfamily genes have been identified and characterized in several plant species. Little information is available on the WD40 superfamily genes in peach. In this study, we identified 220 members of the WD40 superfamily in the peach genome, and these members were further classified into five subfamilies based on phylogenetic comparison with those in Arabidopsis. The members within each subfamily had conserved motifs and gene structures. The WD40 genes were unevenly distributed on chromosomes 1 to 8 of the peach genome. Additionally, 58 pairs of paralog WD40 members were found on eight chromosomes in peach, and 242 pairs of orthologous WD40 genes in peach and

Arabidopsis were matched. The 54 selected putative WD40 genes in peach had the diverse expression patterns in the red-fleshed and white-fleshed peach fruits at five developmental stages. Prupe.6G2110800.1 was located only on the cytomembrane, while Prupe.1G428200.1 and Prupe.I003200.1 were located on both the cytomembrane and nucleus. Prupe.1G5580700.1 was densely located around the nuclear rim but relatively faintly located in the nucleoplasm; Prupe.5G11060300.1 was located in the nucleus and cytomembrane with strong signals but was located with weak signals in the cytoplasm; and Prupe.8G2120400.1 and Prupe.1G0530600.1 were mainly located in the nuclear envelope and cytomembrane but were relatively faint in the nucleoplasm. This study provided a foundation for further functional verification of WD40 genes in peach.

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Gramene subsites: pangenome browsers for crops (0300-012)
Hall 2

Continued advances in sequencing/assembly technologies are generating an abundance of high quality reference assemblies within crop species, ushering a transition from single-genome to pan-genome research approaches. With this transition, communities will need ready access to pre-computed comparisons of genome assemblies to identify and characterize common and variable regions. To accommodate this need, the Gramene comparative genomics project is developing Gramene subsites, each dedicated to the study of individual crop groups. We will describe current status on four pangenome subsites that support rice (http://oge.gramene.org), maize (http://maize-pangenome-ensembl.gramene.org), sorghum (https://www.sorghumbase.org) and grapevine (http://vitis.gramene.org). A key feature of pan-genome subsites is the application of uniform annotation protocols to minimize methodological artifacts, and the application of Ensembl and Gramene infrastructures for comparative analysis and visualization. Extending Compara gene tree output, we define conserved syntelog sets and assign conservation scores based on the proportion of genomes with membership in each set. We then score individual genomes for presence/absence and copy-number variation, additionally supported by whole genome alignments. Using related approaches in the Oryza genus, we showed that, compared to ancient families, recently emerged genes have higher rates of evolution, higher lability, more limited expression, prevalence in pericentromeric regions, reduced coding-length, and enrichment for stress-response functions. We gratefully acknowledge support from grants NSF#1744001, NSF#1127112, and USDA-ARS#58-8062-7-008.

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Gramene: Unifying Comparative Genomics and Pathway Resources for Plant Communities (0300-011)
Hall 2

At Gramene (http://www.gramene.org), we integrate publicly available datasets and synthesize added value resources to address the knowledge gap that exists between plant genomes and the function encoded within them, thus enabling comparative functional analysis.Gramene provides integrated search capabilities and interactive platforms to visualize gene features and gene neighborhoods for about 2.3 million genes from 61 plant genomes, 224 million SNPs mapped to 12 reference genomes, over 93,000 phylogenetic trees with 1.9 million genes, expression profiles, pathways, and references to literature and external resources. Gramene's Plant Reactome hosts 298 reference pathways curated in rice, and projected to 82 species by orthology. Visualizations of EBI Expression Atlas data, from over 0800 experiments, are integrated into the search results panel, facilitating visualization on both, plant genome and pathway browsers. More than 4,000 public RNA-Seq studies are aligned to plant genomes on our genome browser. Gramene is committed to open access and reproducible science based on the FAIR data principles. We are a phylogenomic resource, built upon best-of-class open source software, Ensembl, Reactome, and Expression Atlas infrastructure platforms. Integrating across these platforms, Gramene has developed a powerful and flexible document-based architecture that enables advanced searching via a web-service accessible by a variety of programming languages; each platform supporting web-based and programmatic access through application programming interfaces (APIs). Extensive use of ontologies, database cross-references, common data formats, metadata, community engagement and open-source software promotes interoperability within the ecosystem of informatics data and services. Gramene is supported by an NSF grant IOS-1127112, and partially from USDA-ARS (1907-21000-030-00D).

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In silico characterization of plant microRNAs in large-scale natural variation studies. (0300-009)
Hall 2

Elucidating the molecular mechanisms underlying highly desirable agronomical traits is fundamental for crop improvement and dealing with the challenges of plant breeding bottlenecks. To date, most large-scale genetic variation studies comparing crop species and their existing wild relatives and landraces had focused predominantly on DNA variations within protein-coding genes. Besides protein-coding genes, active noncoding species such as microRNAs play very critical roles in gene regulation, and yet the possible contribution of plant microRNAs present in these wild relatives and landraces, but are absent in the reference crop species are generally not explored. We propose a computational method for discovering neglected microRNA gene loci in large-scale natural variation studies. Next-generation sequencing (NGS) reads derived from the wild relatives or landraces are initially aligned to their crop reference genomes using the BWA short reads aligner. The subset of unmapped reads from the alignment step are cataloged using the SAMtools package, and then de novo assembled using the SPAdes de Bruijn graph assembler. Using the einverted program, inverted repeats loci in the assembled genomic sequences are subsequently annotated. Next, mature plant microRNA sequences obtained from the miRbase repository are aligned to the inverted repeats sequences using the BLAST program. BLAST hit loci are then evaluated for microRNA hairpin signature, by using the RNAfold program. Using the proposed method, our preliminary analysis detected several candidate microRNA gene loci from a variety of crop plant species.

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RNA-sequencing reveals influence of a systemic suckercide, maleic hydrazide on tobacco transcriptome (0300-006)
Hall 2

Maleic hydrazide (MH) is a systemic suckercide (a chemical that inhibits shoot bud growth) which is widely used in farming of crops, such as tobacco (Nicotiana tabacum) and tomato, to control the growth of axillary shoots following topping (removal of apical buds). However, the influence of MH on tobacco gene expression and its molecular mechanism of action are not well studied. Here, we described the influence of MH on transcriptomic landscape of apical (ApB) and axillary buds (AxB) of "chemically topped" (un-topped plants treated with MH) Burley tobacco. RNA-sequencing (RNA-Seq) analysis revealed that, compared to the untreated control, 573 (132 upregulated, 441 downregulated) and 2,632 (2,174 upregulated, 458 down-regulated) genes were differentially expressed in ApB and AxB of chemically topped tobacco, respectively. Gene ontology (GO) analysis showed that upregulated genes in ApB were enriched for phosphorelay signal transduction, leaf proximal/distal pattern formation, and regulation of timing of transition from vegetative to reproductive phase, whereas downregulated genes were more related to meristem maintenance, cytokinin metabolism, cell wall biosynthesis, photosynthesis, and DNA metabolism. In MH-treated AxB, GO terms related to defense response and oxylipin metabolism were over-represented for upregulated genes, whereas cell cycle, DNA metabolism, and cytokinin metabolism were over-represented for downregulated genes. Furthermore, genes related to biosynthesis and signaling of phytohormones, including auxin, cytokinin, jasmonic acid, and gibberellins, were affected by in ApB and AxB of MH-treated tobacco. Collectively, the RNA-seq analysis provides insights into global changes in gene expression profiles and possible molecular mechanism of action of MH on apical and axillary bud growth in tobacco.

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Transcriptome analysis of Korean fir (Abies koreana) in response to elevated carbon dioxide and high (0300-001)
Hall 2

Transcriptome analysis of Korean fir (Abies koreana) in response to elevated carbon dioxide and high temperatureIncreasing temperature and carbon dioxide (CO2) concentration are key climate change factors that could affect plant growth and

development, but the molecular mechanisms regulating responses to these factors are still poorly understood. To broadly survey genes with altered expression during combined heat and CO2 stress, RNA samples were prepared from needles of Korean fir subjected to high CO2 and heat. RNA-sequencing analyses revealed that the expression of a large number of transcripts was affected under high temperature. Intriguingly, the transcriptomic results showed fewer gene expression changes under a combination of both high CO2 and temperature compared with heat treatment alone. Gene ontology analysis revealed the differentially expressed transcripts were mainly associated with metabolic process, binding and cell terms. The expression profiles of transcripts involved in metabolic pathways and cellular responses were identified using MapMan analysis, which revealed that CO2 and heat stress induced transcript expression related to light reactions, biotic and abiotic stress responses, and development. Additionally, transcription factor genes known to be important for abiotic stress responses, such as ERF, bHLH and NAC, were identified. The reliability of the observed expression patterns was confirmed by quantitative RT-PCR. The genetic knowledge acquired here should be very useful for future studies of the molecular adaptation of this tree species to simultaneous environmental stresses.

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Uncovering Chinese cabbage flowering time genes responsible for differences in bolting time in respo (0300-005)
Hall 2

Flowering time is an important economical trait of leafy vegetable crops. Despite Chinese cabbage (Brassica rapa ssp. pekinensis) is a typical species which requires vernalization to flower, little is known about the flowering-time genes and flowering mechanism under vernalization in Chinese cabbage. Here, we conducted genome-wide transcriptome analysis to determine concerning flowering time genes that govern flowering time using two Chinese cabbage inbred lines, '04004' (early bolting) and '50' (late bolting). On the basis of previously identified 223 flowering time-related (Ft) genes in this species, 104 and 74 of these Ft genes were differentially expressed (DEG) between the early bolting '04004' and late bolting '50', respectively with or without vernalization treatment. In particular, most of Ft DEGs were associated with circadian/light signaling/photoperiod (C/L/P) pathway. Subsequently, expression of the Ft DEGs was also validated by real-time reverse transcription PCR (qRT-PCR). The expression levels of flowering integrator genes BrFT1/2 and BrSOC1/2/3 were highly expressed in early bolting '04004' line, while transcripts of flowering repressor BrFLC1/2/3/4 were greatly expressed in late bolting '50' line. In addition, the expression levels of major enhancers and repressors between two lines closely corresponded with the different bolting time of

the two lines. These results suggest that the vernalization process is conserved between Chinese cabbage and Arabidopsis.

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Primary Poster Presenter: Areum Lee

Variant-Scope: A Mutation Detection Software for Wet-Lab Biologists (0300-010)
Hall 2

Identification of bona fide DNA mutations in a sequenced sample is crucial for gene function discovery studies. The ascendency of next-generation sequencing (NGS) technologies have tremendously impacted the progress of scientific discoveries in molecular biology. However for the typical bench scientist, the computational task of determining DNA variants in large-scale NGS sequencing datasets may be daunting. In an era of ubiquitous advances in computing and cyberinfrastructure availability, enabling wet-lab scientists to independently perform computational analysis on datasets generated from their experiments without the need for detailed understanding of the core hardware and software is a very desirable goal. We describe a turnkey DNA variant-detection software for the automated analysis of NGS sequencing datasets called Variant-Scope. Variant-Scope is a Python-based software package which provides an intuitive user-interface (UI) and includes a set-up wizard, status viewer, and a range of summary charts and figures for the experimental study. Variant-Scope incorporates cutting-edge NGS analysis software such as BWA (for reads alignment), SAMtools (for variant detection), BEDTools, and SnpEff (for variant-effect annotation), and is available as a package to users running Windows, MacOS, or Linux. Variant-Scope is a very flexible package and can handle the analysis of whole-genome sequences of single individuals or large populations. The package is freely available and has been successfully utilized for the detection of both Fast-Neutron and EMS-induced DNA variants in a variety of mutagenized plant populations.

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A new method for the identification of herbal material using DNA barcoding and structural analyses (0300-150 (Screen 10))
Hall 2

Authentic identification of plants is essential for exploiting their medicinal properties as well as to stop the adulteration and malpractices with the trade of the same. The chief objective of this study was to identify a herbal powder obtained from a herbalist in the local vicinity of Rajkot, India, using deoxyribonucleic acid (DNA) barcoding and molecular tools. The DNA was extracted from a herbal powder and selected Cassia species, followed by the polymerase chain reaction (PCR) and

sequencing of the rbcL barcode locus. Thereafter the sequences were subjected to NCBI-BLAST analysis, followed by the protein 3D structure determination of the rbcL protein from the herbal powder and Cassia species namely Cassia fistula, Cassia tora and Cassia javanica (sequences obtained in the present study), Cassia roxburghii, and Cassia abbreviata (sequences retrieved from Genbank). Further, the multiple and pairwise structural alignment were carried out in order to identify the herbal powder. The nucleotide sequences obtained from the selected species of Cassia were submitted to Genbank (Accession No. JX141397, JX141405, JX141420). The NCBI BLAST analysis of the rbcL protein from the herbal powder showed an equal sequence similarity (with reference to different parameters like E value, maximum identity, total score, query coverage) to C. javanica and C. roxburghii. In order to solve the ambiguities of the BLAST result, a protein structural approach was implemented. The protein homology models obtained in the present study were submitted to the protein model database (PM0079748-PM0079753). The pairwise structural alignment of the herbal powder (as template) and C. javanica and C. roxburghii (as targets individually) revealed a close similarity of the herbal powder with C. javanica. Hence, the integrated use of DNA barcoding and protein structural analyses could be adopted, as a novel rapid and economic procedure, especially in cases when protein coding loci are considered.

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One-step GWAS analysis identifies loci for rice photosynthetic traits (0300-149 (Screen 8))
Hall 2

Increasing the yield potential of rice is a vital requirement to feed the world's growing population. One way to achieve this is by improving rice photosynthetic efficiency. Here, we used the indica diversity panel, which was established within the 'Phenomics of Rice Adaptation and Yield Potential' (PRAY) project. This panel was cultivated under irrigated field conditions in the Philippines, to investigate leaf anatomy and several photosynthetic parameters. Our study focuses on a new genome-wide association model using the 700k SNP high-density array with the aim to discover new genes associated with traits contributing to higher photosynthetic activity field conditions. This new association model is known as the one-step model which can simultaneously examine all raw data, such as all genotypes, all phenotype records (individual plants) and account for spatial variation and replication in a single step as opposed to conventional approaches that generally use the mean/median values of the phenotypic traits causing probable error inflation for the association analysis. Our results from the one step approach show an increase in statistical power as each individual plant is considered instead of using the means of each accession. This model allows the identification of previously undetected loci affecting photosynthetic parameters such as water use efficiency (WUE), Leaf intercellular CO2 concentration (Ci) and Leaf

chlorophyll content (SPAD). In recent years, this type one-step approach was efficiently implemented for many animal models but to our knowledge this is the first time this model has been applied to plant species and has yielded greater power and precise estimate values. To conclude, this approach has facilitated further exploration of the genetic diversity present in the PRAY indica panel, and helps towards the development of higher yielding rice varieties.

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Genes & Genomes: Epigenetics

Trithorax Group Proteins Act Together with a Polycomb Group Protein to Maintain Chromatin Integrity (0300-151 (Screen 13))
Hall 2

PcG and trxG proteins have been shown to act antagonistically to epigenetically regulate gene expression in eukaryotes. The trxG proteins counteract PcG-mediated floral repression in Arabidopsis, but their roles in other developmental processes are poorly understood. We investigated the interactions between the trxG genes, ATX1 and ULT1, and the PcG gene EMF1 during early development. Unexpectedly, we found that mutations in the trxG genes failed to rescue the early-flowering phenotype of emf1 mutants. Instead, emf1 atx1 ult1 seedlings showed a novel swollen root phenotype and massive deregulation of gene expression. Greater ectopic expression of seed master regulatory genes in emf1 atx1 ult1 triple than in emf1 single mutants indicates that PcG and trxG factors together repress seed gene expression after germination. Furthermore, we found that the widespread gene derepression is associated with reduced levels of H3K27me3, an epigenetic repressive mark of gene expression, and with globally altered chromatin organization. EMF1, ATX1, and ULT1 are able to bind the chromatin of seed genes and ULT1 can physically interact with ATX1 and EMF1, suggesting that the trxG and EMF1 proteins directly associate at target gene loci for EMF1-mediated gene silencing. Thus, while ATX1, ULT1, and EMF1 interact antagonistically to regulate flowering, they work together to maintain chromatin integrity and prevent precocious seed gene expression after germination.

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Primary E-Poster Presenter: Li Pu

A WRKY transcriptional factor is post-translational modified by acetylation and regulates flowering (0300-018)
Hall 2

WRKY transcription factors are one of the largest families of transcriptional regulators in plants and are involved in responses to biotic/abiotic stresses and development by targeting to the W-box. We found that a WRKY protein (WRKYX) is a transcription activator and is involved in flowering regulation. The gene activation ability of WRKYX can be inhibited by a histone deacetylase. Furthermore, we found that WRKYX can be acetylated both in vitro and in vivo. The acetyl-mimic and non-acetyl-mimic WRKYX show different cellular localization in Arabidopsis. The wrkyx loss-of-function mutant is early flowering under both long day (LD) and short day (SD) conditions. In addition, the transcript level of FLC was down-regulated in wrkyx compared to Col-0 wild type. Together, these results suggest that WRKYX can be acetylated and plays a critical role in controlling flowering time in Arabidopsis.

Primary Poster Presenter: Yuan-Hsin Shih

CHROMATIN CHANGES ASSOCIATED TO TRANSGENERATIONAL PRIMING IN Phaseolus vulgaris L. (0300-014)
Hall 2

Plants have evolved some defense mechanisms to cope with pathogen challenge, one is priming, which is a robust strategy that improves the defensive capacity of plants. During priming the plant is sensitized for enhanced defense, and provides a long-lasting, broad-spectrum resistance to stress.Nowadays there are numerous reports concerning the molecular bases of priming, as well as the generational priming mechanisms (e.g. accumulation of inactive mitogen-activated protein kinases, histone methylation or acetylation at the promoter regions of defense-related genes, DNA methylation/demethylation). Furthermore, some studies have shown that the priming status of a plant can be inherited to its offspring (transgenerational priming). However, information related to the molecular bases of transgenerational priming is still scarce and it has been mainly focused on DNA modifications.Chromatin modifications associated with the priming phenomenon, such as changes in histones modifications and nucleosomal rearrangements, are still in early studies. Accordingly, our main goal was to analyze in the common bean the transgenerational defense priming mechanism associated with chromatin modifications, during three generations. In this work, we evaluated nucleosomal rearrangements and enrichment of histone modifications (H3K4me3 and H3K36me3) at the transcriptional start sites of primed genes, throughout different generations. We show that, in addition to reduction in pathogen growth, leaf

damage and transcriptional activation of defense related genes, transgenerational priming is associated with chromatin modifications (e.g. histone modifications and nucleosome positioning).

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Dissecting the Roles of Plant-Specific RNA Polymerases IV and V in Soybean (0300-022)
Hall 2

Small RNA-mediated transposon silencing by DNA or histone methylation is a universal phenomenon in eukaryotes. In plants, RNA-directed DNA methylation (RdDM) is mediated through 24nt-siRNAs and two plant-specific RNA polymerases, Pol IV and V. Whereas disruption of Pol IV or V in Arabidopsis shows normal development, developmental defects are observed in maize and tomato including abnormal leaves, flowers, and sterility. To study the function of Pol IV and V in soybean, a major seed crop, we generated RNA-interference (RNAi) lines targeting the largest and second largest subunit of Pol IV and V (gmnrpd1, gmnrpe1, gmnrp(d/e)2). Similar to Arabidopsis, these RNAi lines showed reduced 24nt siRNA accumulation. Interestingly, we identified ~3,000 regions with enhanced 21-22nt siRNA accumulation in the gmnrp(d/e)2 and gmnrpe1 mutant lines. These RNAi lines were also more resistant to Phytophthora sojae infection suggesting a role for soybean Pol V in biotic stress response and plant defense. Our results indicates that RdDM in general, and Pol V, in particular, is important for soybean immune response.

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DNA methylation profiling to identify biomarkers of age and non-infectious bud failure in identical (0300-019)
Hall 2

Aging is a universal phenomenon; however, perennial plants seem to defy aging as they experience cycles of dormancy, vegetative, and reproductive growth while exhibiting prolonged longevity. The aging process is poorly understood and research neglected for perennial plants such as fruit and nut trees, despite the negative impacts aging can have on growth, yield, and reproductive fitness. Phenomena of aging include alterations to the epigenome via changes in DNA methylation patterns that can impact expression and function of genes and gene products, leading to

development of aging related disorders. Therefore, patterns of differential DNA methylation can potentially function as biomarkers of aging and predict aging related disorders in plants. To test this, DNA methylation profiling was performed in almond, an economically important nut crop for the US that exhibits an aging related disorder known as non-infectious bud failure (BF). Whole-genome bisulfite sequencing was conducted to search for differentially methylated regions (DMRs) using monozygotic twin almond pairs discordant for BF exhibition. Sequencing reads were mapped to the reference genome, and methylated bases were called to distinguish genome-wide methylation patterns. Identified DMRs and other quantifiable features derived from the bisulfite sequencing were analyzed to determine their association with phenotypic data from and within the twin pairs, namely BF exhibition. Expression patterns of genes nearby or overlapping identified DMRs in the twin pairs are currently being analyzed via qRT-PCR. Results thus far suggest DNA methylation profiles are associated with BF-exhibition in the germplasm tested and indicate an impact of DNA methylation on lateral meristem development. These and further results will provide information to almond breeding, propagation and production efforts and expand our understanding of BF exhibition, as well as provide a basis to interrogate aging in other Rosaceae species.

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Epigenetic and transcriptional reprogramming during petunia flower development directs VOC synthesis (0300-016)
Hall 2

Volatile organic compounds (VOCs) are important secondary metabolites contributing to plant defense and communication, as well as reproductive success through attracting pollinators. In Petunia hybrida cv. Mitchell, production and release of floral VOCs, enriched in volatile phenylpropanoid/benzenoid products, is tightly controlled both during the day-night cycle and flower developmental process to potentiate pollination, peaking at dusk two days post-anthesis. While precisely timed production and release of the mixture of floral volatiles requires coordination of multiple complex and interlinked metabolic pathways, the regulatory mechanisms directing VOC pathways remain poorly understood. To investigate the dynamics of the transcriptome and epigenome during the period of corolla development when VOC production and release is initiated, data were analyzed from previously published RNA-Seq and newly generated ChIP-Seq collected from unopened petunia buds and from flowers two days post-anthesis. Genes encoding enzymes of the shikimate and phenylpropanoid pathways or producing volatile compounds/intermediates derived from these pathways are strongly induced while genes involved in other pathways drawing away phenylpropanoid precursors, like

those producing anthocyanin compounds or responsible for lignification, show significant decreases in transcript levels. ChIP-Seq was utilized to profile two histone marks associated with active gene expression, H3K4me3 and H3K9ac, at the same time points during corolla development. It was found that H3K9ac deposition is significantly associated with transcriptional activation of the VOC pathway and acts at specific gene loci encoding important enzymes and transcriptional regulators. Taken together, this work reveals a dramatic reprogramming of the epigenome and transcriptome to direct flow of metabolites through the shikimate and phenylpropanoid pathways toward VOC production as an underlying process in petunia flower maturation.

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Epigenetic footprints of CRISPR/Cas9-mediated genome editing in plants
(0300-021)
Hall 2

CRISPR/Cas9 has been widely applied to various plant species accelerating the pace of plant genome editing and precision breeding in crops. Unintended effects beyond off-target mutations are still somewhat unexplored. We investigated the degree and patterns of epigenetic changes after gene editing. We examined DNA methylation changes in the gene edited promoters of naturally hypermethylated genes (AT1G72350 and AT1G09970) and hypomethylated genes (AT3G17320 and AT5G28770) in Arabidopsis. Transgenic plants were developed via Agrobacterium-mediated transformation. Homozygous edited lines were selected from segregated T2 plants by in vitro digestion assay using ribonucleoprotein complex. Bisulfite sequencing comparisons were made between paired groups of edited and non-edited plants and genes. DNA methylation of the edited plants in the locus-specific edited genes was compared to that of the control plants. We found that directed mutagenesis via CRISPR/Cas9 resulted in no unintended alterations morphologically and epigenetically. Phenotypes of wild-type, transgenic empty vector, and transgenic edited plants were similar. Epigenetic profiles revealed that methylation patterns of promoter regions flanking target sequences were identical among wild-type, transgenic empty vector, and transgenic edited plants. There was no effect on mutation type on epigenetic status. We also evaluated off-target mutagenesis effects in the edited plants. Potential off-target sites containing up to 4-bp mismatch of each target were studied. No off-target mutagenesis was detected in candidates of potential off-target sites. Our results showed that CRISPR/Cas9 did not leave epigenetic footprints on either the immediate gene edited DNA and flanking DNA or off-target mutagenesis.

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Isolation of pure dme mutant central and egg cells using CRISPR-Cas9 system (0300-020)
Hall 2

DEMETER(DME) is a type of DNA demethylase related to maternal-specific imprinting. Because dme mutants show seed abortion phenotype, it is hard to maintain dme homozygous mutant line and collect pure dme mutant cells. Recently, it is reported that sequential transformation method using CRISPR-Cas9 system enhance gene targeting in Arabidopsis thaliana. To isolate pure dme mutant central and egg cells, we designed constructs containing GFP using this method. By putting pCC or pEC-GFP-terminator in DME region, we can identify cells that have GFP expression but no DME expression. Through manual isolation method picking GFP expressing cells, we can get pure dme mutant central and egg cells. Although this method is limited to gamete cells, it is meaningful in that we can get pure dme mutant cells only maintaining dme heterozygous line. Analyzing collected pure mutant cells will offer the chance to understand functions of DME in central and egg cells of Arabidopsis thaliana.

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Primary Poster Presenter: Seunga Lee

Reprogramming of Rice epigenome during cold stress response (0300-152 (Screen 1))
Hall 2

Reprogramming of cellular function in response to external stress stimuli is one of the important mechanism by which plants adapt to environment. The functional reorganisation occurs primarily via an alteration in the expression of a multitude of stress-inducible genes. The highly complex structure of chromatin imparts resistance to several nuclear processes including transcription. Several covalent modifications at the N-terminal tails of histones and changes in DNA methylation together generate epigenetic code to promote accessibility of nuclear factors to their cognate binding site. Owing to its tropical nature, Oryza sativa L. ssp. indica is highly sensitive to low-temperature conditions. Rice cells perceive cold stress by changes in membrane rigidity, which leads to increased electrolytic leakage, ultimately triggering expression of cold-responsive genes. The present study focuses on the role of H3K27 modifications in the differential cold stress response of indica rice. Genome-wide study on changes of H3K27 modification demonstrated enrichment of H3K27 acetylation and RNA polymerase II occupancy under cold

stress at the promoter and upstream regions of cold-responsive genes. Interestingly, a strong existence of the "H3K27me3/ac Switch" was detected in the promoter/upstream region of such loci. Furthermore, co-ordinated interplay between Polycomb group (PcG) and Trithorax group (TrxG) of proteins was observed in regulating the transcription "off" and "on" state of stress responsive gene. These findings indicate an important role of epigenetic control in the transcriptional activation of different inducible genes of rice in response to abiotic stress.

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The Catalytic Core of DEMETER Guides Active DNA Demethylation in Arabidopsis (0300-017)
Hall 2

The Arabidopsis DEMETER (DME) DNA glycosylase demethylates the maternal genome in the central cell prior to fertilization, and is essential for seed viability. DME preferentially targets small transposons that flank coding genes, influencing their expression and initiating plant gene imprinting. DME also targets intergenic and heterochromatic regions, and how it is recruited to these differing chromatin landscapes is unknown. The C-terminal DME catalytic core consists of three conserved regions required for catalysis in vitro. We show that the catalytic core of DME guides active demethylation at endogenous targets, rescuing the developmental and genomic hypermethylation phenotypes of DME mutants. However, without the N-terminus, heterochromatin demethylation is significantly impeded, and abundant CG-methylated genic sequences are ectopically demethylated. We used comparative analysis to reveal that the conserved DME N-terminal domains are only present in the flowering plants, whereas the domain architecture of DME-like proteins in non-vascular plants mainly resembles the catalytic core, suggesting that it might represent the ancestral form of the 5mC DNA glycosylase found in all plant lineages. We propose a bipartite model for DME protein action and suggest that the DME N-terminus was acquired late during land plant evolution to improve specificity and facilitate demethylation at heterochromatin targets.

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Primary Poster Presenter: Yu-Hung Hung

The LDL1/2-HDA6 histone modification complex is involved in regulation of the long non-coding RNAs (0300-023)
Hall 2

In recent years, the eukaryotic long non-coding RNAs (lncRNAs) have been identified as important factor in expression regulation and involved in a wide variety of essential biological processes, including the chromatin regulation, alternative splicing, gene dosage compensation, genomic imprinting, and nuclear organization. In plants, lncRNAs have been found to be associated with flowering and light response. Recently, a large number of lncRNAs have been identified in plants, but only very few of them have been studied in detail. Furthermore, regulation mechanism of the lncRNAs also still remains largely unknown. Recent research revealed that the Arabidopsis H3K4 demethylases LYSINE-SPECIFIC DEMETHYLASE 1-LIKE 1/2 (LDL1/2) and HISTONE DEACETYLASE 6 (HDA6) can act synergistically to regulate gene expression by histone deacetylation and H3K4 demethylation. From RNA-seq and ChIP-seq assays, we identified that the global expression of lncRNAs was highly increased in hda6/ldl1/2 triple mutant plants, and the increased lncRNAs expression was associated with H3 acetylation (H3Ac) and H3K4 di-methylation (H3K4me2) changes. Moreover, we also identified that HDA6 and LDL1 shows more binding on the promoter regions of lncRNAs than protein coding genes. These results revealed that the expression of lncRNAs is associated with H3Ac/H3K4me2 change regulated by the LDL1/2-HDA6 histone modification complex.

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Primary Poster Presenter: Fu-Yu Hung

The trxG factor ULT1 regulates Arabidopsis development as well as biotic and abiotic stress response (0300-015)
Hall 2

In eukaryotes, Polycomb group (PcG) and trithorax group (trxG) factors oppositely regulate gene transcription on a wide scale through chromatin modifications, with PcG factors repressing and trxG factors activating the expression of their target genes. Although plant trxG factors regulate many developmental and physiological processes, their downstream targets are poorly characterized. We have used transcriptomics to identify genome-wide targets of the Arabidopsis thaliana trxG factor ULTRAPETALA1 (ULT1) during vegetative and reproductive development, and compared them with those of the PcG factor CURLY LEAF (CLF). Our analysis reveals that genes involved in development and transcription regulation are over-represented among ULT1 target genes. In addition, stress response genes and plant defense response genes such as those in glucosinolate metabolic pathways are enriched, revealing a previously unknown role for ULT1 in controlling biotic and abiotic responses. Finally, many ULT1 target genes can be oppositely regulated by CLF, suggesting that ULT1 and CLF may have antagonistic effects on plant growth and development in response to various endogenous and environmental cues.

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Transcriptome changes and hormone accumulation associated with TSA-induced somatic embryogenesis (0300-024)
Hall 2

Epigenetic modifications, including histone acetylation, play a significant role in the regulation of the genes involved in the embryogenic reprogramming of plant somatic cells. In support for this belief, we observed that treatment of the Arabidopsis explants with trichostatin A (TSA), an inhibitor of histone deacetylases, results in somatic embryogenesis (SE) induction (Wójcikowska et al. 2018). Here, RNA-seq analysis was used to identify the histone acetylation-regulated genes controlling the TSA-induced somatic embryogenesis. The explants (immature zygotic embryos) of Columbia-0 were cultured in vitro on the control (E0) and supplemented with 1 μM TSA (ET) medium. RNA was collected from 0, 5 and 10 day-old cultures and the RNA-seq libraries were sequenced with Illumina's HiSeq sequencer. In total, 27,581 genes were analyzed and expression of 12.9% (3,558) of them was shown to be significantly modulated on ET vs E0 medium. Within the TSA-modulated transcripts, 24 genes were found to be related with hormone biosynthesis including those involved in biosynthesis of indole-3-acetic acid, IAA (e.g. TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1, YUCCA10 and NITRILASE2), salicylic acid, SA (SALICYLIC ACID INDUCTION DEFICIENT2), abscisic acid, ABA (NINE-CIS-EPOXYCAROTENOID DIOXYGENASE6, BETA-GLUCOSIDASE HOMOLOG1) and jasmonic acid, JA. In line with this result, UPLC-ESI-MS/MS analysis indicated a significant accumulation of IAA (5 fold), SA (5-70 folds) and ABA (10-120 folds) during TSA-induced SE. In contrast, the JA level was significantly decreased in this culture that might result from high expression of the CYP94B3 gene of negative impact on accumulation of the biologically active JA. The obtained RNA-seq data provide a valuable platform for identification of the histone acetylation-regulated genes of decisive function in the SE induction.This work was supported by a research grant from the National Science Centre in Poland (OPUS13 2017/25/B/NZ1/01615).

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Primary Poster Presenter: Barbara Wójcikowska

Genes & Genomes: Gene Regulation and Transcriptional Networks

Targeted Endoplasmic Reticulum Localization of Storage Protein mRNAs Requires the RNA-binding Protei (0300-153 (Screen 1))
Hall 2

The transport and targeting of glutelin and prolamine mRNAs to distinct subdomains of the cortical endoplasmic reticulum (ER) is a model for mRNA localization in plants. This process requires a number of RNA-binding proteins that recognize and bind to mRNA cis-localization (zipcode) elements to form messenger ribonucleoprotein (mRNP) complexes, which then transport the RNAs to their destination sites at the cortical-ER. Here, we present evidence that the rice (Oryza sativa) RNA-binding protein RBP-L, like its interacting RBP-P partner, specifically binds to glutelin and prolamine zipcode RNA sequences and is required for proper mRNA localization in rice endosperm cells. A T-DNA insertion in the 3′ untranslated region resulted in reduced expression of the RBP-L gene to 10–25% of that in the wild-type. Reduced amounts of RBP-L caused partial mis-localization of glutelin and prolamine RNAs and conferred other general growth defects, including dwarfism, late flowering and smaller seeds. Transcriptome analysis showed that RBP-L knockdown greatly affected the expression of prolamine family genes and several classes of transcription factors. Collectively, these results indicate that RBP-L, like RBP-P, is a key RNA-binding protein involved in mRNA localization in rice endosperm cells. Moreover, distinct from RBP-P, RBP-L exhibits additional regulatory roles in development, either directly through its binding to corresponding RNAs or indirectly through its effect on transcription factors.

Primary E-Poster Presenter: Li Tian

Identifying regulators and the regulatory network of Kranz anatomy development through Laser-Capture (0300-154 (Screen 2))
Hall 2

C4 leaves are characterized by the Kranz anatomy, in which the vascular bundle is surrounded by one layer of organelle-rich bundle sheath (BS) cells, which is then surrounded by one layer of radially arranged mesophyll (M) cells. Past histological and cell lineage studies in maize revealed that Kranz development starts from three contiguous ground meristem cells, but little is known about the genes and the molecular mechanism involved in Kranz anatomy development. To identify key regulatory genes involved in Kranz development, we compared the tissue specific transcriptomes of different developmental stages of maize embryonic leaf including: 5 stages (ground meristem tissues with only P1, 3, 4, 5, or 6 BS GM cells) of Kranz ground meristem (GM) cells; 4 stages of palisade-like (P1, 3B, 4B, and 5 BS stage) M cells; 2 stages of undifferentiated M ground meristem (1M, 2M) cells by LCM. We obtained high-quality RNAs, and then RNA-seq data. Principal components analysis (PCA) showed that early Kranz and M cells exhibited distinct mRNA populations. These data sets indicate that Kranz and M cells have distinct gene regulatory

networks because they arise from distinct genetic origins and that the captured cell types show sufficient diversity at the mRNA level. Differential gene expression and weighted correlation network analysis (WGCNA) identified candidate coexpression modules and gene coexpression networks involved in Kranz development. GO analysis indicated that these modules were enriched for genes involved in anatomical structure, leaf, shoot development, etc. In situ hybridization validated several genes expressed in early Kranz anatomy. Finally, we predicted putative cis-regulatory elements in upstream gene sequences from each gene and validated the predictions by Y1H and protoplast transient assay. Moreover, we constructed a network related to Kranz development. These results provided much insight into the transcriptional regulation of Kranz anatomy development.

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Primary E-Poster Presenter: Wen-Yu Liu

On the challenge of gene regulatory network inference in soybean flowering control (0300-155 (Screen 12))
Hall 2

Plants synchronize various aspects of developmental and physiological transitions with seasonal environmental changes, including flowering transition that determines reproductive success and productivity of plants. To better understand flowering response to environmental fluctuations in soybean at the regulatory network level, we first elucidated global gene expression patterns under different photoperiod regimes. Transcriptomic signatures of the known maturity loci E1, E2, E3 and E5 in the NILs exhibited unique roles of the E loci in flowering control and identified candidate genes that were controlled by the E loci. To clarify the regulatory gene network controlling soybean photoperiodic flowering, we developed the network inference algorithmic package CausNet. CausNet is implemented in Python 3 and is freely available at https://github.com/Veggente/soybean-network. CausNet captured several regulatory interactions controlling soybean flowering transition that were previously reported, and provided with the predicted soybean circadian clock network and flowering gene networks. While the predicted circadian clock network showed robustness to photoperiods, the flowering gene networks differed drastically under long day and short day, consistently with the photoperiodic nature of soybean flowering control. We demonstrated the predicted regulatory roles of GmCOL1a and GmCOL1b in the flowering gene network using RNA interference. Next, we expanded the above approaches to different combination of photoperiod and temperature conditions. Our preliminary observations show many circadian clock genes exhibit higher amplitude under high temperature conditions, suggesting prominent implications of temperature for the circadian clock. Our results provide novel insights and testable hypotheses in the complex molecular mechanisms of flowering control in soybean and lay a framework for de novo prediction of biological networks controlling important agronomic traits in crops.

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Primary E-Poster Presenter: Yoshie Hanzawa

Analysis of development related differentially expressed genes in Gα and Gβ mutants of Brachypodium (0300-157 (Screen 10))
Hall 2

Heterotrimeric G-protein signaling networks govern wide range of growth and development pathways in plants. The G proteins comprise of Gα, Gβ and Gγ subunits, forming heterotrimeric complex with GDP attached to the Gα subunit, representing its inactive form. The activation of Gα subunit results from the exchange of bound GDP to GTP that leads to dissociation of GTP-Gα from the Gβγ subunits. Different G-protein subunits between dicots and monocots suggests the existence of lineage-specific regulatory pathways, the details of which remain mostly unknown. The majority of mechanistic studies are limited to the dicot model Arabidopsis thaliana. To gain insights in the G-protein regulatory pathways in monocots, we performed a detailed transcriptomic analysis of plants expressing reduced levels of Gα and Gβ genes in a monocot model Brachypodium distachyon. Gα- and Gβ-RNAi plants, grown under normal conditions exhibit significant developmental differences compared to the WT plants. RNA-seq analysis identified a total of 689 genes that were differentially expressed both in Gα- and Gβ-RNAi plants compared to the WT plants. Apart from these common genes, 973 genes were specific for Gα-RNAi and 253 genes specific for Gβ-RNAi, suggesting that Gβ governs wider range of developmental processes as compared to Gα, some of which might be independent of the Gα function. The detailed analysis also revealed that Gα-regulated genes are involved in the control of cell wall and cell membrane related components whereas Gβ not only governs cellular growth related genes but also hormone signaling pathways. These findings from RNA-seq analysis and detailed growth analysis suggest the critical roles of G-proteins in affecting growth and development phenotypes in Brachypodium and its similarities, and differences from the dicot model Arabidopsis.

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Primary E-Poster Presenter: Nikita Bhatnagar

Identifying Genome-Wide Regulators of Heat Responses Using High-throughput and Quantitative Pooled (0300-156 (Screen 6))
Hall 2

Heat stress jeopardizes plant growth, reduces crop yields, and hinders biofuel production. This problem will only exacerbate as global warming progresses. Despite this, the mechanisms employed by photosynthetic cells to regulate heat responses remain poorly understood. To engineer heat-tolerant crops and algae for

food and biofuel, a thorough understanding of how plant cells regulate heat stress is required. The eukaryotic, unicellular green alga Chlamydomonas reinhardtii is an excellent model organism to study many important cellular processes in photosynthetic organisms. Chlamydomonas has several prominent advantages to study heat regulation in photosynthetic cells, e.g. haploid genome, fast growth, simpler gene families, and homogenous heat treatment. A genome-saturating, mapped, indexed mutant library of Chlamydomonas has recently be generated, enabling both reverse and forward genetic screens. Besides the mapped insertion site, each mutant has a unique DNA barcode inserted in the genome, allowing for high-throughput and quantitative tracking of growth rates of individual mutants in pooled cultures. We employed the algal mutant library and the quantitative phenotyping tool to screen for Chlamydomonas mutants with altered sensitivities to various heat treatments. Through the genome-wide screens, we generated a list of genes with potential roles in algal heat responses. We are using the gene list to identify novel regulators that are important for heat responses in photosynthetic cells and further investigating some selective heat-sensitive mutants identified in the screens to elucidate function of the disrupted genes. This research will help us understand functional genomics and cellular mechanisms that govern heat responses in photosynthetic cells. It will provide information to engineer thermotolerant algal strains for biofuel production and the information gained in Chlamydomonas can be transformed into land plants to improve crop thermotolerance.

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Primary E-Poster Presenter: Ru Zhang

Decoding ABI3 gene regulation and its novel role in dehydration stress and recovery response in Arab (0300-158 (Screen 6))
Hall 2

Global climatic changes pose a continuous threat to plants in forms of unfavourable environmental cues. Plants are thus exposed to increased frequency of abiotic and biotic stress conditions, challenging their growth and productivity. As a part of our effort to understand the mechanism of dehydration stress signalling, we have unravelled a novel role of ABI3, a B3 domain containing transcription factor. ABI3 was originally identified as a seed-specific transcription factor that mediates ABA signalling. Our work has shown that, in Arabidopsis ABI3 gets expressed in response to dehydration stress and during early phases of stress recovery. Interestingly, ABI3 auto-activates its own transcription as a part of dehydration stress response, through specific consensus cis-elements present in its promoter region. Dehydration stress signalling is mediated by ABI3 through upregulation of an array of downstream genes, most prominently the LEA group of genes and some CRUCIFERIN genes, already known to be involved in the desiccation phase of seed maturation. As a part of gene regulation ABI3 recruits itself to the upstream

regulatory regions of its target genes. This is associated with nucleosomal rearrangement in the promoter region of the genes along with histone modifications that lead to transcription activation in response to dehydration stress and stress recovery. Deletion of ABI3 fails to upregulate expression of these genes in a dehydration stress-responsive manner, and the mutant plants show compromised recovery from dehydration stress. In summary, our work has decoded a novel role of ABI3 in dehydration stress signalling, beyond its known role in seed physiology. These findings add a new dimension to our understanding of stress signalling pathways and indicate the immense potentiality of ABI3 as a general mediator of dehydration stress and stress recovery response.

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A Dof transcription factor in durian (Durio zibethinus) regulates auxin biosynthesis (0300-058)
Hall 2

Durian (Durio zibethinus Murr.) is an economically important climacteric fruit crop native to Southeast Asia. Two commercial durian cultivars from Thailand, Monthong and Chanee exhibit different sensory characteristics and ripening behaviours. However, our actual knowledge regarding the mechanisms underlying this different ripening behaviour is limited. Due to the possible role of auxin in climacteric fruit ripening, we measured indole-3-acetic acid (IAA) levels during ripening of both cultivars and observed a significantly higher IAA level in Chanee than in Monthong, concurring with the greater expression levels of auxin biosynthetic genes (L-tryptophan aminotransferase 1 (TAA1) and the indole-3-pyruvate monooxygenase (YUCCA4)). Interestingly, we scanned the promoter regions of TAA1 and YUCCA4 and identified 40 and 34 cis-regulatory motifs (AAAG/CTTT), respectively, known as the binding site for Dof transcription factor family. Our genome-wide analysis identified 24 durian Dofs (DzDofs), 15 of which were expressed in the fruit pulp. Gene expression analysis revealed differential expression of DzDofs during ripening in Monthong and Chanee cultivars. Comparing the expression levels of fruit pulp-expressed DzDofs between these cultivars revealed ten potential cultivar-dependent Dofs, among which DzDof 2.2 showed a significantly greater fold increase at every ripening stage in Chanee than in Monthong. Transient expression of DzDof2.2 in Nicotiana benthamiana leaves significantly upregulated the expression levels of N. benthamiana TAA1 and YUCCA4 and, therefore, confirmed the transcriptional regulation of these genes by DzDof2.2. Higher expression levels of DzDof2.2 in Chanee could enhance its auxin levels during ripening. Higher auxin levels in Chanee could activate auxin-mediated transcription, contributing to the faster ripening of this cultivar compared to Monthong through earlier initiation of the ethylene response (auxin-ethylene crosstalk).

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Primary Poster Presenter: Supaart Sirikantaramas

Activating the Truncated 90bp CaMV 35S promoter by Protoplasting, 5-Azacytidine and Salycilic Acid. (0300-063)
Hall 2

Domain A of the CaMV35S promoter contains only 90 bases from the transcriptional start site, it promotes transcription in root tips, and it collaborates with other domains to create the well known strong and constitutive full-length CaMV 35S promoter. Within the 90 bp region, two direct TGAGC repeats from −82 to −62 provide potential TGA2 transcription factor binding locations that influence RNA production. When Domain A (the Δ90 region) was fused to the chloramphenicol acetyl transferase reporter gene, and the circular plasmid DNA electroporated into tobacco or carrot protoplasts, substantial CAT activity increased to over 50% chloramphenicol substrate conversion after 48h. This Δ90-CAT reporter activity was severely curtailed by either 20 µg/ml Actinomycin D, or cycloheximide (1.8 mM). For comparisons sake, we created stable and single-insert transgenic plants containing the Δ90-CAT reporter, that where selfed, and selected to at least the T4 generation. Leaf pieces of these Δ90-CAT leaves were floated on M&S medium for 24-48 hours, and CAT activity found to be only two - four percent of chloramphenicol substrate acetylation. However, co-incubation of these same leaves with 25 µM 5-Azacytidine, 1 mM salicylic acid, or both compounds enhanced CAT acetylation activity up to 25% within 48h. When the Δ90-CAT transgenic plants themselves were converted into protoplast, CAT activity rose up to 40% in protoplasts after 48h incubation. The Δ90 Domain A region of the CaMV 35S promoter appears to be activated in a protoplast environment. Because leaf tissues can emulate the Δ90-CAT initiation as seen in protoplasts, salicylic acid recruitment of the TGA2 factors with other transcriptional activators, and DNA demethylation may represent important factors impacting Domain A's functionality in both protoplasts and in wounded plants.

Co-author(s): Kyle Rowe,
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Primary Poster Presenter: John Thomas

An O-GlcNAc modified protein plays dual roles in RNA splicing and transcription to control developme (0300-068)
Hall 2

O-GlcNAcylation is an important post-translational modification found on thousands of proteins in animals that couples metabolic and energy status to the regulation of numerous cellular processes. A large number of O-GlcNAc modified proteins in Arabidopsis are known or putative transcriptional regulators, chromatin modifiers and splicing factors. The function of these O-GlcNAc modified proteins and their

roles in O-GlcNAcylation mediated regulations remain largely unstudied. This list includes a protein of unknown function in Arabidopsis, AtACINUS, which is widely conserved in eukaryotes and its animal counterpart is proposed to play an important role at the interface of transcription and splicing. Knocking-out AtACINUS and its closest homolog AtPININ caused severe growth defects including dwarfism, enhanced seed dormancy with hypersensitivity to abscisic acid, and late flowering. Transcriptomic analysis of acinus pinin identified hundreds of genes with altered expression levels or retention of specific introns, including increased expression of the flowering repressor FLC and intron retention in the ABH1 and HAB1 transcripts encoding negative regulators of ABA signaling, consistent with the late-flowering and ABA-hypersensitive phenotypes of the mutant. Interactome analysis indicated that AtACINUS is associated with numerous transcriptional regulators, histone modifiers and the splicing machinery as well as the RNAs of ABH1 and HAB1 and the promoter DNA of FLC, supporting direct involvement of AtACINUS in AS of ABH1 and HAB1 and transcription of FLC in a complex protein network. The AtACINUS-dependent splicing of HAB1 is altered in an O-GlcNAc transferase mutant, spy, suggesting that O-GlcNAc modification affects AtACINUS splicing activity. Our study demonstrates that AtACINUS is a hub in the protein networks that link nutrient signaling with chromatin remodeling and RNA splicing in the regulation of developmental transitions of seed germination and flowering.

Primary Poster Presenter: Yang Bi

Boolean logic gates for sophisticated control of plant gene expression

To produce economically valuable chemicals and metabolites in plants, and gain control of plant activity in response to the environment, new genetic tools and regulatory switches are required. This project aims to generate modular synthetic biology components for targeted control of plant gene expression. Sophisticated switches will be designed that enable complex logic operations to be performed in plants as well as the ability to selectively activate and repress desired plant pathways in a user-defined manner. In addition to providing new genetic tools for advancing basic research, the transcriptional circuits will enable targeted control of important secondary metabolite pathways for food and pharmaceutical industries. It is hoped that greater control of plant activity in response to endogenous signals or environmental and chemical stimuli will also produce smarter and more resilient crops.

Chair and Concurrent Symposium Speaker: Adil Khan, Ryan Lister, Kemal Kazan, Karam Singh

Primary Poster Presenter: Brendan Kidd

Characterization of Alternative Polyadenylation & Gene Expression Profile in the tcab1 Arabidopsis M (0300-066)

Hall 2

Using the power of bioinformatics and next generation sequencing, we are using a molecular genetics, genomic, and transcriptomic approach to understand how the machinery inside the nucleus affects the development of Arabidopsis thaliana. The TELOMERASE CAJAL BODY PROTEIN 1 (TCAB1, known as WRAP53β in humans) is a WD40 domain containing protein that acts as a platform to facilitate protein and nuclear structure interactions that occur in the cell. Moreover, a nuclear structure closely related to these processes is the Cajal Body (CB). The CB takes part in modifications of different types of ribonucleoproteins (RNPs) involved in the maturation of the splicing apparatus, the survival of motor neuron (SMN) complex and the telomerase RNP machinery. Interestingly, it is known that TCAB1 plays an important role in the localization and trafficking of these complexes to the CB. Therefore, having a detrimental impact when in absence. My project focuses on the machinery of TCAB1 regarding to gene expression and alternative polyadenylation events through PAT-seq (PolyA tag followed by high-throughput sequencing). Specifically, we are interested in looking at the changes in gene regulation that TCAB1 affects directly and/or indirectly through its function.

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Primary Poster Presenter: Antonio Serrano Rodriguez

Characterization of Transcription Factors Regulating Cotton Fiber Elongation (0300-036)
Hall 2

Cotton fiber is the single-celled structure elongated from seed epidermis, the development of cotton fiber and Arabidopsis trichome share similar but not identical regulatory mechanisms. In allotetraploid cotton genomes there are more than 20 genes encoding the GL2-type HD-ZIP IV factors, among which HOX3 is a key regulator of fiber cell elongation. Silencing of GhHOX3 expression in Gossypium hirsutum blocked cotton fiber elongation. To further dissect the regulatory pathway, we isolated genes regulated by GhHOX3, among which one encodes a member the Paclobutrazol Resistance (PRE) family known to promote cell growth. There are 26 PREs in the allotetraploid cottons, compared to five in cacao. The PRE family expansion in Gossypium may have played a role in cotton fiber evolution. Differential expression of homoeologous genes in polyploids is important to plant adaptation and phenotype innovation. PRE1 expression is specific to cotton fiber and upregulated when the fiber undergoes rapid elongation. Interestingly, in the cultivated tetraploids only A-homoeologue is expressed. We found that natural variation in the canonical TATA-box has caused the subgenome-biased gene expression, representing a mechanism underlying the selection of homoeologous genes, and the polymorphisms in the PRE1 promoter have contributed to spinnable fiber formation. In addition, we have used the CRISPR/Cas9 system to edit the HOX3-regulated genes, including those of ARF and TCP transcript factors, which

may create new germplasm for cotton breeding. Keywords: Gossypium, cotton fiber, HOX3, PRE1, transcription factors

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Primary Poster Presenter: XiaoYa Chen

Co-expression studies of the LAFL-network reveal novel roles during late embryogenesis (0300-048)
Hall 2

The seed, and seed storage compounds such as starch, oil, and proteins, has a fundamental role in human society. Seeds consist of an embryonic plant, endosperm and a seed coat and are the final products of a large and complex network of transcription factors governing the spatial and temporal expression of an even broader set of target genes. These networks are generally highly conserved among plant species and show a high level of functional redundancy. An extensive set of seed development regulators have so far been identified, but the redundant and transient nature of these often makes it difficult to study their function in detail. Among these are LEAFY COTYLEDON1 (LEC1), a member of the nuclear factor YB family, and the B3-family members LEAFY COTYLEDON2 (LEC2), ABSCISIC ACID INSENSITIVE3 (ABI3) and FUSCA3 (FUS3). It has previously been shown that these transcription factors are involved in early embryogenesis (LEC1) and seed filling and maturation (LEC2, ABI3, and FUS3).In this study, we utilise transient gene expression in Nicotiana benthamiana to investigate the combinatorial effect of LEC1 together with LEC2, ABI3, and FUS3 on the leaf transcriptome. We show that the addition of LEC1 results in changes of the transcriptomes induced by LEC2 and ABI3, but not FUS3, toward more specialised functions. We also show that ABI3 has LEC1-dependent functions regulating genes involved in the transition between globular and heart stage during embryogenesis as well as the start of oil accumulation through the activation of WRINKLED1. Furthermore, we demonstrate that LEC1 specifies the activation of late embryogenesis abundant genes through LEC2, indicating a shift in the role of LEC2 during embryogenesis. This study also yields further insight into the set of common regulatory targets of the B3-family of transcription factors.

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Primary Poster Presenter: Per Snell

Conditional regulatory logic for the Arabidopsis TCA cycle (0300-034)
Hall 2

As a hub of central carbon metabolism, regulation of the tricarboxylic acid (TCA) cycle is crucial to coordinate flux to neighboring metabolic pathways for optimizing

growth and development. TCA cycle regulation in plants has largely been studied at the level of the protein or metabolite including post-translational modification, allosteric feedback of enzymes, and metabolite channeling by organizing sequential enzymes into metabolons. However, transcriptional regulation of the TCA cycle in Arabidopsis, and even more broadly, in multicellular organisms, is largely unstudied. Using an enhanced yeast one-hybrid platform, we identified a large number of transcriptional regulators. These predict differential control of TCA targets in the various cellular compartments, potentially enabling flexibility to alter the pathway. Furthermore, no general regulators of the TCA cycle were identified via co-expression analyses, providing an immediate indicator of a novel paradigm for how multicellular organisms regulate this critical metabolic pathway. We selected 17 candidate TFs for conditional transcriptional regulation of the TCA cycle. In total, mutants of all 17 genes were shown to have perturbed TCA cycle function, with a subset having responses that were dependent on specific TCA cycle intermediates. One third of these TF mutants influence growth in a salt stress-dependent manner, and mutations in almost half led to perturbations in the abundance of C, N or C:N ratios. Thus transcription of TCA cycle genes are controlled in the plant to allow fine-tuning of metabolism to meet energetic demands of diverse cell types under various environmental constraints.

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Primary Poster Presenter: Michelle Tang

Contribution of time of day and the circadian clock to the heat stress responsive transcriptome in A (0300-025)
Hall 2

In Arabidopsis, a large subset of heat responsive genes exhibit diurnal or circadian oscillations. However, to what extent the dimension of time and/or the circadian clock contribute to heat stress responses remains largely unknown. To determine the direct contribution of time of day and/or the clock to differential heat stress responses, we probed wild-type and mutants of the circadian clock genes CCA1, LHY, PRR7, and PRR9 following exposure to heat (37oC) and moderate cold (10oC) in the early morning (ZT1) and afternoon (ZT6). Thousands of genes were differentially expressed in response to temperature, time of day, and/or the clock mutation. Approximately 30% more genes were differentially expressed in the afternoon compared to the morning, and heat stress significantly perturbed the transcriptome. Of the DEGs (~03000) specifically responsive to heat stress, ~ 70%

showed time of day (ZT1 or ZT6) occurrence of the transcriptional response. For the DEGs (~10400) that are shared between ZT1 and ZT6, we observed changes to the magnitude of the transcriptional response. In addition, ~2% of all DEGs showed differential responses to temperature stress in the clock mutants. The findings in this study highlight a significant role for time of day in the heat stress responsive transcriptome, and the clock through CCA1 and LHY, appears to have a more profound role than PRR7 and PRR9 in modulating heat stress responses during the day. Our results emphasize the importance of considering the dimension of time in studies on abiotic stress responses in Arabidopsis.

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Primary Poster Presenter: Emily Blair

Cytokinin regulation of plant potassium levels requires CRF6 (0300-035)
Hall 2

Cytokinin is a plant hormone essential to plant growth and response. Cytokinin Response Factor 6 (CRF6) is an Arabidopsis cytokinin responsive AP2/ERF transcription factor involved in regulation of several cytokinin controlled processes, such as senescence with additional links to abiotic stress. Recent studies of CRF6 function have focused on its regulation by oxidative stress pathways and that connection back to cytokinin. Surprisingly less work has been done looking at the genes CRF6 regulates via the cytokinin signaling pathway. Here we used transcriptome analyses (Affymetrix ST1.0 Arabidopsis microarrays) to identify these cytokinin-downstream targets genes of CRF6 by comparing the transcriptomes of wildtype, crf6 mutant and CRF6 overexpressor lines in the presence and absence of cytokinin. Differentially expressed genes (DEGs) affected by cytokinin in WT and not the crf6 background were identified as potential CRF6-dependent cytokinin regulated targets. The majority of CRF6-dependent genes show repression by cytokinin, suggesting that CRF6 functions as a negative regulator, as previously predicted. While the functions of CRF6-dependent target genes is varied, several members show an interconnected relationship to ion transport, specifically potassium (K+). We have further verifed that three major potassium transporters (HAK5, SKOR, and NRT1.5) are repressed by cytokinin in a CRF6-dependent manner. Additionally investigation of these K transport mutants show altered physiological responses to cytokinin treatments. Further connections between cytokinin regulation of potassium levels and CRF6 were made by measuring ion levels with Inductively-Coupled Plasma: Optical Emission Spectrophotometry (ICP-OES). Findings connecting cytokinin to potassium levels through CRF6 will be discussed.

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Primary Poster Presenter: Ariel Hughes

CYTOSOLIC CHAPERONIN MEDIATES SIGNALINGS OF VERNALIZATION AND COLD ACCLIMATION IN ARABIDOPSIS (0300-049)
Hall 2

Vernalization is one of the most important mechanisms to determine the timing of flowering in plants. In Arabidopsis, PRC2-mediated epigenetic repression of strong floral repressor gene, FLOWERING LOCUS C (FLC), is critical for vernalization. PHD finger domain protein VERNALIZATION INSENSITIVE3 (VIN3) was reported as one of the key component in POLYCOMB REPRESSIVE COMPLEX (PRC2)-mediated FLC suppression. When plant is exposed to vernalization, expression of VIN3 is gradually elevated according to cold period. However, how plant perceives long-term cold is not known yet.To reveal upstream components of VIN3, we generated a transgenic line with the minimal promoter of VIN3 fused to GUS reporter gene and performed ethyl methane sulfonate mutagenesis. We have isolated several mutants which show increased or decreased expression of VIN3 after vernalization treatment. p161 was isolated as vernalization insensitive mutant, which shows decreased expression of VIN3. The p161 mutant failed to accumulate a repressive histone marker, H3K27me3, on FLC locus after vernalization. The mutant failed to suppress FLC transcript level, thus flowering time was not accelerated as wild type. By positional cloning, we found a point mutation within the gene encoding a subunit of chaperonin in p161.In addition to impaired VIN3 induction, p161 has pleiotropic defects under long-term cold treatment. Long term cold treated p161 shows reduced chilling tolerance, accumulated anthocyanin, and decreased tubulin abundance. Our RNA-seq analysis using long-term cold treated p161 mutant reveals downregulation of several genes including C-REPEAT BINDING FACTOR (CBF1) and COLD-REGULATED PROTEIN 15a (COR15a), which have critical roles for cold acclimation. These results lead us to propose that chaperonin complex mediates vernalization and cold acclimation.

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Primary Poster Presenter: Goowon Jeong

Differential subgenome regulation manipulates staged fiber-cell differentiation in allotetraploid co (0300-042)
Hall 2

Cotton as the world’s most important naturally textile crop shares more than one third of the world textile fiber market, and cotton fiber is also an excellent experimental system for studying single-cell differentiation and elongation in plants. But little know about the genetic regulation during fiber development limited the

genetic improvement of this cash crop. Here, a genome-wide association study (GWAS) was performed in an allotetraploid cotton (Gossypium hirsutum), and 28 genetic loci associated with fiber quality-related traits were identified. 15,330 expression quantitative trait loci (eQTLs) were found to involved in the transcriptional regulation of 9,282 genes by sequencing fiber transcriptomes of 251 accessions. 13 causal genes were prioritized for differential fiber quality in a transcriptome-wide association study (TWAS) by integrating the cis-eQTLs and GWAS data. Differential genetic regulation patterns between two subgenomes were revealed by characterization of distal eQTLs. Notably, an eQTL hotspot (Hot216) on chromosome D11 establishing a genome-wide genetic network regulating the expression of 962 genes. Hot216 was found to be transcriptional regulation of genes responsible for cell wall synthesis, which contributes to fiber length via modulating developmental transition of rapid fiber elongation to secondary cell wall synthesis. This study uncovers subgenome genetic regulation manipulating staged fiber differentiation and sheds further light on the notion of spatiotemporal modulation of secondary cell wall synthesis for cultivating cotton producing superior fiber.

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Primary Poster Presenter: Zhonghua Li

Direct and Indirect Targets of the Arabidopsis Seed Transcription Factor ABSCISIC ACID INSENSITIVE3 (0300-044)
Hall 2

Arabidopsis thaliana ABSCISIC ACID INSENSITIVE3 (ABI3) is a transcription factor in the B3 domain family. ABI3, along with B3 domain transcription factors LEAFY COTYLEDON2 (LEC2) and FUSCA3 (FUS3), and LEC1, a subunit of the CCAAT box binding complex, form the so-called LAFL network to control various aspects of seed development and maturation. ABI3 also contributes to the abscisic acid (ABA) response. We report on chromatin immunoprecipitation-tiling array experiments to globally map binding sites for ABI3. We also assessed transcriptomes in response to ABI3 by comparing developing abi3-5 and wild type seeds and combine this information to ascertain direct and indirect responsive ABI3 target genes. ABI3 can directly express and repress its target genes' transcription and some intriguing differences exist in cis motifs between these groups of genes. Directly regulated targets reflect ABI3's roles in seed maturation, desiccation tolerance, entry into a quiescent state and longevity. Interestingly, ABI3 directly represses a gene encoding a microRNA (miR160B) that targets AUXIN RESPONSE FACTOR (ARF)10 and ARF16 that are involved in establishment of dormancy. The interplay between ABI3, the other LAFL genes, and the VP1/ABI3-LIKE (VAL) genes that are involved in the transition to seedling development are examined and reveal complex interactions controlling development.

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Primary Poster Presenter: Ran Tian

Distal Regulatory Element of FLOWERING LOCUS C Allows Plants to Distinguish Different Types of Cold (0300-050)
Hall 2

For plants, to recognize how long the cold lasts is crucial to timely flowering. Many plants require long-term cold for reproductive phase change to adjust its flowering time to the spring. In contrast, intermittent cold delays flowering to avoid prematurity. Both processes are known to occur through FLOWERING LOCUS C (FLC), a strong floral repressor. In the intermittent cold, FLC is upregulated to inhibit floral transition. During the long-lasting cold, however, the expression of FLC is suppressed while the antisense transcript of FLC, COOLAIR, is increased. Here we identify a distal regulatory element which allows such distinct responses. Several regulatory element-like sequences are distributed over FLC genomic locus. Only two of those elements, located in 3'-intergenic region of FLC, are directly activated by cold-induced transcription factors. Increase of such transcription factors can trigger FLC transcription in both sense and antisense direction. However, during the long-term cold, mutation in cold-induced transcription factors turns off the induction of COOLAIR and delays FLC suppression in early stage. These results indicate that cold-induced transcription activators, which can bind to distal regulatory element of FLC, regulate expression of FLC and COOLAIR differentially such like dominant direction of transcription is altered according to the length of cold.

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Primary Poster Presenter: Myeongjune Jeon

DNA methylation patterns match with S-RNase expression dynamics during pistil development in Nicotia (0300-056)
Hall 2

To avoid inbreeding, some species of hermaphroditic angiosperms developed a genetic system known as self-incompatibility (SI), which is defined as the inability of a fertile hermaphroditic plant to produce zygotes after self-fertilization. In Nicotiana, the female determinant is the S-RNase, a ribonuclease specifically expressed in style that reaches its maximum expression level in developmental stages near to anthesis. Similar expression patterns are observed in other factors essentials for SI such as HT-B, 120K and NaStEP. Although the biochemical regulation behind the SI has been extensively studied, the knowledge addressing its transcriptional regulation is limited. Data obtained from the masculine

determinant SP11 of Brassica rapa and for the S-RNase of N. alata, Petunia axillaris, and Prunus dulcis, show that the transcriptional regulation of these genes includes: tissue-specific regulation related with their coding regions and their promoters, allele-specific trans-acting repressor elements and allele-specific DNA methylation.Since transcriptional regulation of S-RNase seems to be highly regulated in N. alata, our work focuses on the SC10-RNase allele in which we found a fragment of a reverse-transcriptase gen from the Gypsy family, this element resides approximately 0300 bases upstream the transcriptional start site (TSS) and its localization matches with a change in DNA methylation patterns in three different tissues, including style in anthesis, style from young bud an leave. Results indicate that CHH methylation of this region is increased in style in anthesis, where the S-RNase transcript is more abundant, while in immature tissue with very low S-RNase expression, both symmetric methylation marks (CG and CHG) were predominant. DNA methylation in the CHH context in mature pistil might be related with the presence of the retrotransposon fragment which could be implicated in the S-allele S-RNase specific transcription regulation.

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Primary Poster Presenter: RENATA SALCEDO

Examining the evolution and genetic control of CAM photosynthesis in Sedum (0300-030)
Hall 2

Water use efficiency (WUE) of agricultural crops is becoming of increasing significance as the world grows hotter and drier. In order to meet our increasing global food demands, we need to produce more food on less land and with less water. In Crassulacean Acid Metabolism (CAM) photosynthesis, carbon fixation occurs nocturnally in order to minimize water loss, giving CAM plants more than double the WUE of C3 plants. The genetic basis of CAM is largely unknown, but may be useful for engineering improved WUE in crop plants. In this project, two species of Sedum, S. makinoi and S. mexicanum, are examined to unearth the genetic mechanisms controlling CAM. Using physiological data including stomatal aperture and titratable acidity, the type of CAM each species performs was determined. RNAseq was subsequently used to identify potential genes of interest that may control the regulation of CAM. The results of this research provide further information with which to elucidate the evolutionary origins of CAM, as well as its genetic regulators for future use in engineering applications.

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Primary Poster Presenter: Serena Lotreck

Expression Quantitative Trait Nucleotide Mapping Reveals Transcriptional

Regulatory Networks (0300-061)
Hall 2

Populus are being used for biomass production for a suite of industrial applications including biofuels conversion. Our understanding of biomass productivity and quality is limited by the fact that this complex trait requires the regulation and coordinated interactions of many genes. Identification of genetic networks regulating biomass productivity and quality remains largely unaccomplished and is urgently needed to inform genetic improvement of Populus feedstocks for biomass production and conversion. To uncover the genetic regulatory landscape in the woody perennial bioenergy crop Populus trichocarpa, we performed an expression quantitative trait nucleotide (eQTN) mapping enabled by the whole-genome resequencing and RNA-seq analysis of P. trichocarpa. natural variants. A panel of >8.2 million single nucleotide polymorphisms (SNPs) and nucleotide insertions and deletions (InDels) were obtained from whole-genome resequencing of 917 unrelated individuals of P. trichocarpa. Transcriptome data from 390 leaf and 444 xylem samples were analyzed and revealed that 16,030 and 15,496 genes, respectively, exhibited significant expression variation across the population. Through genetic mapping, cis- and trans-eQTNs were identified. Enriched transcription factor binding sites (TFBS) including cis-eQTN showed tissue-specific divergence. trans-eQTN analysis identified multiple hotspots that were significantly associated with expression of more than 100 putative target genes. Combined with genome-wide association studies (GWAS) of trait phenotype, the upstream regulators of these phenotype-associated genes and their regulatory network were identified. These analyses have provided a comprehensive understanding of the genetic regulatory mechanisms underlying complex traits.

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Primary Poster Presenter: Jin-Gui Chen

Flowering in Arabidopsis thaliana: Phosphorylation of FD by Calcium-Dependent Protein Kinases (0300-062)
Hall 2

Reproductive transition in plants (flowering) employs tightly controlled molecular mechanisms in response to environments to achieve flowering at the appropriate timing. Better understanding of the flowering mechanisms helps to cope with changing climate by decreasing crop failures while increasing crops' fitness. Flowering is controlled through the interaction of three main proteins – TERMINAL FLOWER 1 (TFL1), FLOWERING LOCUS T (FT), and the bZIP transcription factor FD. In Arabidopsis thaliana, TFL1 is expressed in the shoot apical meristem (SAM), while FT is produced in the leaves and transported to the SAM. The binding of FD

with FT or TFL1 forms a florigen complex (FT-FD) or an anti-florigen complex (TFL1-FD), respectively. The florigen complex induces the expression of floral meristem identity genes that lead to the development of flower organs, while the anti-florigen complex suppresses these genes. It has been suggested that phosphorylation of FD by CPK33, a member of the large Calcium-dependent Protein Kinase (CPK) family, is involved in the formation of a florigen complex (Kawamoto et al., 2015). We have identified another member of this family, CPK11, that strongly phosphorylated FD at the 282 threonine residue in the C-terminal FD in vitro. We hypothesize that CPK11 can phosphorylate T282 of FD in planta and that CPK11 and its close homologs share redundant roles in the regulation of a florigen complex. We cloned CPK4, the closest homolog of CPK11. CPK4, CPK11 and wild-type or mutant FD proteins were expressed and kinase assays were conducted. T-DNA insertion mutants of CPK4 and CPK11 were obtained and cpk4 cpk11 double mutants were created. In addition, transgenic plants overexpressing CPK4, CPK11 and wild-type or mutant FD proteins were produced. Further kinase assays and phenotypic analyses of these transgenic plants will elaborate on the effects of FD phosphorylation by CPKs in the flowering mechanisms.

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Primary Poster Presenter: Patrick Acosta

Gene regulatory networks underlying xylem identity in Solanum lycopersicum roots (0300-072)
Hall 2

Plant roots are excellent developmental models, as they are transparent, possess reproducible patterns of cell division and are responsive to environmental perturbations. Furthermore, roots are responsible for absorption of water and all non-photosynthetic nutrients. As key tissue in roots, xylem is also an important feature of all vascular plants. Xylem functions in long-distance transport of water and nutrients from roots to shoots in addition to giving plants structural support. Terminal differentiation of xylem is marked by the deposition of secondary cell wall polymers and programmed cell death. In Arabidopsis thaliana, the regulatory network controlling the synthesis of secondary cell wall in xylem cells was previously identified in our lab. However, the mechanisms that control specification of xylem identity in cultivated plants remains poorly understood.Our lab aims to understand the molecular mechanisms of xylem cell identity using Solanum lycopersicum (tomato) roots as a model. To elucidate gene putative regulatory networks that underlie tomato xylem identity, we integrated expression data from ribosome-associated transcripts (TRAP-Seq) and chromatin accessibility information (from ATAC-Seq). Using TRAP-seq, we have defined a set of tomato xylem-specific genes. Using ATAC-seq, we have identified genomic regions that have putative

regulatory activity. Within these accessible regions, we have also identified enriched transcription factor motifs that could point to potential upstream regulators of xylem cell fate.

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Primary Poster Presenter: Joel Rodriguez-Medina

GRAM domain protein GhGRE5 positively regulates cotton fiber initiation and elongation (0300-043)
Hall 2

Cotton fiber is the most important raw material for the textile industry. GRAM domain proteins have been well studied in Arabidopsis. Most reports focus on the role of GRAM domain proteins in biological process, such as seed development, inflorescence architecture and differentiation of Arabidopsis root epidermal cells. However, little is known on the roles of GRAM domain proteins in cotton fiber development so far. In this study, a cotton GRAM domain protein GhGRE5 that preferentially expressed in the root and fibers at 10 days post-anthesis (DPA) was cloned. Then transgenic cotton lines were generated to study how GhGRE5 regulates fiber development. Suppression of GhGRE5 inhibited fiber initiation, significantly suppressed fiber elongation, and reduced the fiber length. Other fiber quality indexes worsened, including uniformity index, strength, elongation and short fiber index, compared with the controls. However, over-expression of GhGRE5 promoted fiber elongation at stage of elongation, but the fiber quality of mature fiber did not changed obviously. Yeast two-hybrid and BiFC assay showed that GhGRE5 interacted with another GRAM domain protein GhGEM. Moreover, GhGEM could interact with the WD40-repeat protein GhTTG1, HD-Zip transcription factors GhHOX1 and GhHD1. It has been reported that GhTTG1 and GhHOX1 could effect trichome development of Arabidopsis. GhHD1 was involved in epidermal cell differentiation in cotton. These results suggest that GhGRE5 participates in fiber initiation and elongation as a positive regulator via the network consisting of GhGRE5, GhGEM, GhTTG1, GhHOX1 and GhHD1.

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Primary Poster Presenter: Zhengxiu Ye

GWAS analysis reveals genetic factor controlling bud flush in Populus trichocarpa (0300-059)
Hall 2

Many perennial plants become dormant to survive winter, the activity-dormancy cycle is crucial for both survival and growth of plants. To understand bud dormancy,

phenotyping of bud flush were applied in three common gardens of 1,146 Populus trichocarpa genotypes across multiple years. We then performed genome-wide association studies (GWAS) incorporating bud flush phenotypic data with 8,301,860 SNPs and InDels (minor allele frequency > 0.05) from 917 P. trichocarpa accessions and expression-based quantitative trait loci (eQTL) analyses to identify key regulators. All bud flush phenotypes are significant correlated with an InDel in the promoter region of a UDP-sugar transporter protein (PtUTr) after Bonferroni correction. This InDel is found to be an AT-Hook binding domain which often serves as a cis-acting element in plants. PtUTr leaf and xylem expression were significantly correlated with bud flush phenotypic data and it was regulated by cis-eQTLs containing the AT-Hook binding domain. This study provides insights into data-driven of gene function in woody species.

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Primary Poster Presenter: Kai Feng

Herbicide-induced Gene Expression System to Activate Embryogenesis
(0300-033)
Hall 2

Chemically-induced expression systems are useful both commercially and academically. The tetracycline repressor (TetR) is the basis for the most robust gene switch systems in eukaryotes. However, its use in plants is impractical since the ligands are antibiotics and light sensitive. TetR binds to the tet operator in a promoter, repressing expression of any gene regulated by this promoter. Binding of the ligand tetracycline to TetR creates a conformational change in the repressor, releasing it from the tet operator allowing transcription of the de-repressed gene to occur. Several rounds of gene shuffling of the tetracycline repressor (TetR) gene created mutants that bind sulfonylurea (SU) herbicides instead of tetracycline, creating an Ethametsulfuron Repressor (ESR). This ESR system was tested by de-repressing the fluorescent reporter DsRED in maize. ESR de-repression of transcription factors WUSCHEL and BABYBOOM in rice increased re-transformation and shoot formation frequencies. De-repression of WUSCHEL and BABYBOOM in maize led to differential expression of several genes involved in embryogenesis, cell cycle, and homologous recombination.

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Primary Poster Presenter: Maren Arling

Herbicide-induced Gene Expression System to Activate Embryogenesis. (0300-070)

Hall 2

Chemically-induced expression systems are useful both commercially and academically. The tetracycline repressor (TetR) is the basis for the most robust gene switch systems in eukaryotes. However, its use in plants is impractical since the ligands are antibiotics and light sensitive. TetR binds to the tet operator in a promoter, repressing expression of any gene regulated by this promoter. Binding of the ligand tetracycline to TetR creates a conformational change in the repressor, releasing it from the tet operator allowing transcription of the de-repressed gene to occur. Several rounds of gene shuffling of the tetracycline repressor (TetR) gene created mutants that bind sulfonylurea (SU) herbicides instead of tetracycline, creating an Ethametsulfuron Repressor (ESR). This ESR system was tested by de-repressing the fluorescent reporter DsRED in maize. ESR de-repression of transcription factors WUSCHEL and BABYBOOM in rice increased re-transformation and shoot formation frequencies. De-repression of WUSCHEL and BABYBOOM in maize led to differential expression of several genes involved in embryogenesis, cell cycle, and homologous recombination.

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High-Throughput Single-Cell RNA Sequencing of Arabidopsis Roots (0300-051)

Hall 2

Single-cell RNA sequencing (scRNA-seq) has been used extensively to define and compare gene expression in individual cells from animal tissues, but it has not been widely applied to plants. Here, we present our use of a commercially available droplet-based platform for high-throughput scRNA-seq to obtain more than 10,000 single-cell transcriptomes from Arabidopsis root cell protoplasts (Publication DOI: https://doi.org/10.1104/pp.18.01481)(PMID: 30718350). We find that all major tissues and developmental stages of roots are represented in this single-cell transcriptome population. Further, transcriptomes corresponding to distinct cell sub-populations and rare cell types, including putative quiescent center (QC) cells, were identified. A focused analysis of transcriptomes from the epidermal cells defined

individual cells progressing from meristematic through mature stages of root-hair and non-hair epidermal cell differentiation, and pseudotime analysis was used to infer the developmental trajectories for the root-hair and non-hair cell types. In addition, single-cell transcriptomes were obtained from two different root epidermal mutants, enabling a comparative analysis of gene expression at single-cell resolution and providing an unprecedented view of the impact of the mutated genes. Overall, this study demonstrates the feasibility and utility of high-throughput scRNA-seq in plants and provides a first-generation gene expression map of the Arabidopsis root at single-cell resolution.

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Primary Poster Presenter: John Schiefelbein

High-throughput single-cell transcriptome profiling of plant cell types
(0300-053)
Hall 2

Single-cell transcriptome profiling of heterogeneous tissues can provide high-resolution windows into developmental dynamics and environmental responses, but its application to plants has been limited. Here, we used the high-throughput Drop-seq approach to profile >12,000 cells from Arabidopsis roots. This identified numerous distinct cell types, covering all major root tissues and developmental stages, and illuminated specific marker genes for these populations. Additionally, we demonstrate the utility of this approach to study the impact of environmental conditions on developmental processes. Analysis of roots grown with or without sucrose supplementation uncovered changes in the relative frequencies of cell types in response to sucrose. Finally, we characterized the transcriptome changes that occur across endodermis development and identified nearly 0800 genes with dynamic expression as this tissue matures. Collectively, we demonstrate that single cell RNA-seq can be used to profile developmental processes in plants and show how they can be altered by external stimuli.

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Primary Poster Presenter: Benjamin Cole

Identification of Conserved Regulatory Modules in Dry and Fleshy Fruit Development (0300-041)
Hall 2

In the nightshade family (Solanaceae) there has been an evolutionary transition from an ancestral dry to a derived fleshy fruit, correlated with the divergence of the subfamily Solanoideae. To begin to understand the genetic basis of the transition, we are using a combination of transcriptome sequencing and targeted CRISPR knockouts of orthologues of the Arabidopsis transcription factor FRUITFULL. FRUITFULL is known from studies of Arabidopsis and tobacco to help pattern the dehiscence zone of fruits, but also plays a role in leaf shape and bolting. Previously, we performed RNA-seq on a developmental series of pericarps from wild-type cultivated tomato, Solanum lycopersicum, and its closest wild relative, S. pimpinellifolium. By identifying corresponding developmental stages, I have now extended this transcriptome sequencing work into dry-fruited desert tobacco (Nicotiana obtusifolia). I have also successfully generated CRISPR mutants in two of the four homologues of FRUITFULL in desert tobacco and will be reporting on preliminary phenotypes. Clustering of gene expression profiles from wild-type data shows suites of genes with similar patterns and suggests a common regulatory control. These regulatory modules will allow us to look for conservation in the genetic regulation of fruit development in other species with different histories of fruit evolution and in plant groups with similar fruit-type transitions.

Co-author(s): Kevan Elkins,
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Primary Poster Presenter: Alex Rajewski

Identification of drought and salt responsive mRNA and miRNA mediated networks in drought tolerant t (0300-029)
Hall 2

Plant tolerance to drought and salt stress is fine-tuned by a complex gene regulatory network. Understanding the molecular regulation of this polygenic trait is crucial for the eventual success to improve plant yield and quality. Prosopis juliflora (mesquite) is a weedy tree species of Fabaceace that is highly drought and can withstand high temperatures and leaf-to-air vapor pressure deficit. In the present study, we carried out integrated mRNA and microRNA transcriptome analysis of P. juliflora leaf and root tissues under drought and salt stress. mRNA transcriptome analysis identified a total of 3,062 annotated differentially expressed genes (DEGs). Overall, more transcriptomic changes were observed in root tissue compared to leaf tissue. The study identified genes commonly and differently regulated under drought and salt stress in leaf and root tissues. A high percentage of genes commonly downregulated by drought and salt stress specifically in root tissue were

coding for various ribosomal proteins. The study also indicated a possible role for 'centrins' in stress responses. microRNA transcriptome analysis identified a total of 416 differentially expressing miRNAs (DEMs) belonging to more than 157 families including 44 conserved miRNAs and 40 miRNA*s. miRNA target predictions were carried out using transcriptome data from mRNA sequencing. Among the unigenes, 2,182 transcripts were predicted to be targets for 396 known miRNAs. Gene ontology (GO) enrichment analysis and KEGG pathway analysis of the potential targets of the identified miRNAs were carried out to get a better idea of miRNA mediated gene networks under drought and salt stress. Five of the DEGs and three DEMs with their target genes were further validated by quantitative real time-PCR (qRT-PCR). Overall, this study provides a transcriptome-wide insight into the molecular basis of drought and salt stress tolerance and cross talk between these stresses in P. juliflora.

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Primary Poster Presenter: suja george

Identification of proteomes in Al-sensitive root tip cells of cherry tomato 'LA 2710' (0300-031)
Hall 2

Suping Zhou, Hui Li, Shaolan Yang, Theodore Thannhauser, Yong Yang The laser capture microdissection (LCM) - tandem mass tag (TMT) - proteomics analysis method was used to identify cell specific proteomes from root tips of cherry tomato 'LA 2710' and important proteins for Al stress tolerance. 80,000-100,000 cells were harvested from epidermal and outer cortical layers in transition zone of vertical micro-sections of root tips. TMT- quantitative proteomics analysis were conducted using 18 µg protein/sample which led to the identification of 3879 quantifiable proteins. These proteins were distributed in all subcellular organelles based on gene ontology (GO) analysis. Among the quantified proteins, 129 proteins showed significant changes in abundance ratio of Al treated/control groups. Functional analysis showed that the protein translation process was reduced, and antioxidant group was greatly enhanced in Al-treated root-tips. Through this study, we have developed an efficient LCM-TMT-Proteomics platform for the analysis of Al sensitive root cells. It also has a broad application for proteomics analysis of spatially separated cells from complex tissues. The proteomes from the Al-sensitive root cells are valuable resources for understanding and improving Al tolerance in plants. Funding of this project was provided by USDA-NIFA.

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Primary Poster Presenter: suping zhou

Investigating the dynamic localization of Arabidopsis AGO1 between the nucleus, cytoplasm and the ER (0300-067)
Hall 2

MicroRNA (miRNA)-mediated gene silencing is a central regulatory mechanism in eukaryotes. In plants, most miRNAs associate with ARGONAUTE1 (AGO1), forming an RNA-induced silencing complex (RISC) to downregulate target transcripts. Previous observations showed that mature miRNAs are likely loaded into AGO1 in the nucleus, and further mediate gene silencing in the cytosol and on the ER. However, it is still largely unknown how plant AGO1 shuttles between these compartments. Here we show that the N-terminal extension (NTE) region of AGO1 is essential for its functions and may play a role in the dynamic localization of AGO1. Unlike the highly conserved N-terminal, PAZ, MID and PIWI domains, NTE is variable among the 10 Arabidopsis AGOs. Truncated AGO1 lacking the NTE is unable to rescue the lethal phenotype of the ago1-36 mutant, while AGO1 missing the first half of the NTE can complement the phenotype of ago1-36, suggesting that the second half of the AGO1 NTE is vital for its function. Additionally, we found that the ago1-36 null mutant accumulates a group of miRNAs that are under-represented in the wild type. Future study will focus on the molecular mechanism by which the second half of the NTE regulates the subcellular localization of AGO1

Co-author(s): Xuemei Chen

Primary Poster Presenter: YE XU

Investigating two factors affecting growth in Arabidopsis: SOB3 pathway and brand of gelling agent (0300-064)
Hall 2

Using Arabidopsis as a model system, the goal of this research is to understand how light mediates seedling growth. One key group of genes involved in this process is the AT-HOOK MOTIF NUCLEAR LOCALIED (AHL) gene family. AHL29/SUPRESSOR OF PHYTOCHROME B-4 #3 (SOB3) directly regulates the auxin-associated genes YUCCA8 (YUC8) and members of the SMALL AUXIN UP RNA 19 (SAUR19) subfamily during seedling development. SOB3 is able to modulate hypocotyl elongation in seedlings by repressing transcription of the aforementioned genes. Since YUC8 and members of the SAUR19 subfamily are directly involved in phytohormone pathways, ChIP-Seq was used to test the hypothesis that SOB3 binds to the promoters of additional genes associated with plant-specific hormone pathways, such as brassinosteroids. Preliminary analysis shows that SOB3 directly binds to genes involved in auxin and brassinosteroid signaling, such as the SAURs, YUC8, and BRI1. RNA-Seq is currently being conducted to investigate the expression of such hormone-related genes in the SOB3 pathway.The above research was made possible by using agarose plates to grow Arabidopsis. As such, the gelling agent used in the making of these plates needs generate reproducible

and reliable results. The Neff lab uses the gelling agent Phytagel to make agarose plates. Recently, we identified phenotypic discrepancies in seedlings of known, published phenotypes on plates made with Phytagel. We discovered that Phytagel is now being made in China (Phytagel C). Since American-made Phytagel is no longer available for purchase, a replacement needed to be identified. Using Arabidopsis, the Neff lab compared the original Phytagel against Phytagel C, as well as two other widely used gelling agents, Gellan and Gelzan. We have found that Gellan has higher germination rates, higher root penetrance rates, and fluence rate responses that mirror published data. Therefore, the Neff lab has replaced Phytagel with Gellan for phenotypic experimentation.

Primary Poster Presenter: Caitlin Jacques

Investigations on the role of vernalization pathway genes during soybean flowering (0300-032)
Hall 2

Soybean (Glycine max) is one of the major oil-seed legume crops. It is a rich source of proteins and other nutrients for humans and animals. Success of flowering determines the yield in legume crops such as soybean. Despite the importance of flowering in defining yield in legumes, studies regarding the molecular pathways that control flowering in soybean are limited. Floral initiation is governed by the cross talk between many internal and external cues. Temperature is one of the important factors that control flowering. The promotion of flowering by cold treatment called ‘vernalization. This process is regulated by vernalization pathway genes and has been extensively studied in Arabidopsis thaliana. Soybean does not have quantitative/obligate vernalization requirement however, during the duplication event, it retained large number of vernalization genes. According to RNA sequencing study on soybean shoot apical meristem (SAM) undergoing floral transition [Wong et al.2013, PLOS ONE 8(6), e65319], many of these temperature responsive vernalization genes are differentially regulated. Using genomics approach, we have identified thirteen different vernalization pathway genes. These genes showed considerably high peptide sequence homology with their respective Arabidopsis homologues. Based on phylogenetic relationships, these genes were grouped into three different subfamilies namely VRN1 (eight paralogues), VRN2 and VRN5 (four paralogues). A fundamental gap in our knowledge remains in elucidating the function of vernalization pathway genes in soybean. Therefore, to study the role of soybean vernalization genes, we performed integrated genomics and molecular characterization. Expression analysis showed that vernalization genes are photoperiod responsive in soybean. Overexpression of soybean vernalization genes in Arabidopsis and Nicotiana toabcum showed both conserved and diverged functions.

Primary Poster Presenter: Sukanya Varape

KDEL Cysteine-EndoPeptidases CEP1 and CEP2 restrain root hair polar-

growth in Arabidopsis thaliana (0300-038)
Hall 2

In this work, the biological role Cysteine EndoPeptidases (CysEPs) in root hair and it is how is its transcriptional regulation is demonstrated in Arabidopsis thaliana. Abnormal phenotypes was detected in cep1 and cep2 mutants as well as in the CEP-overexpression induced lines. We tested if the expression of AtCEP1 and AtCEP2 proteins was specifically located in growing root hair cells. Both, AtCEP1 and AtCEP2 expression were confined to the epidermis root cells, and specifically to tricoblast cells and growing root hairs. Higher levels of AtCEP1 and AtCEP2 expression were observed in early stages of root hair cell development and significant lower levels in latter growth suggesting that both CEPs are required in actively cell expanding cells. The punctuated pattern of expression within the root hair cells suggested that these CEPs are targeted to the secretory pathway possibly to ER or/and Golgi compartments. On the other hand, there is no previous report of apoplast targeting of CEPs thus indicating that CEPs would act within the secretory pathway to process their substrates.In addition, AtCEP1 and AtCEP2 were also found to be functional in the novo emergence of adventitious root tips linked to EXT-degradation and under the control of NAC1 transcription factor. Our results suggest that AtNAC1 in controlling CEP expression during root hair growth. Overall, our results suggest that ER-resident proteins AtCEP1 and AtCEP2 positively regulated by NAC1 negatively impacted on polar-cell expansion in growing root hairs. It is plausible that AtCEP1 and AtCEP2 could act as components on the EXTs quality control program of O-glycosylation status and proper protein folding. Root hair cells provide an excellent model system to dissect the molecular components of the EXT O-glycosylation pathway required for polar-growth.

Primary Poster Presenter: Eliana Marzol

Knowing the cell wall signaling using an expansin-induced cell expansion system (0300-057)
Hall 2

A plant cell is surrounded by the cell wall which consists of cellulose microfibrils, matrix polysaccharides and minor proteins and play both mechanical support and restraint for the cell. For a plant cell to proceed division, expansion, and differentiation, intimate interactions between the cell wall and the protoplast would be required. Diverse cell wall-associated transmembrane kinases are indicative of the presence of the interface for these interactions. Mechanical changes in the cell wall, via this interface, would cause diverse downstream cytoplasmic or nuclear events for the cellular processes. Among many cell wall-modifying proteins, expansins are unique in that they reassemble the cell wall without apparent hydrolytic activity and cause cell expansion. We have adopted this expansin-mediated cell-wall modification to identify the events downstream of mechanical cell wall changes. In this study, we have expressed several types of expansin proteins by the glucocorticoid-inducible system in Arabidopsis seedlings and analyzed the time-dependent transcriptome changes. This analysis would give a

starting clue to understand the mechanism from cell-wall dynamics to cellular events.

Co-author(s): Hyung-Taeg Cho

Primary Poster Presenter: Hyodong Lee

Light-regulated genes participate in carotenoids synthesis in the dark-grown carrot storage root (0300-039)
Hall 2

Carotenoids are isoprenoid pigments that provide yellow, orange and red colors to flowers, fruits and vegetables. They contribute to light-harvesting and photoprotection during photosynthesis, serve as scavengers for oxidative damage and are essential for phytohormone synthesis. Carotenoids also play important roles in human health acting as vitamin A precursors and antioxidants.Carotenoid synthesis is induced by light during plant development (photomorphogenesis) and fruit ripening. Daucus carota, synthesizes and accumulates large amounts of carotenoids in its storage root grown in dark and contrary to other plants, light inhibits the synthesis of these pigments and storage root development. To understand the molecular processes that regulate the synthesis of carotenoids in the carrot root, we generate a de novo transcriptome between the root grown in light (R/L) and darkness (R/O). Unexpectedly, genes involved in Shade Avoidance Syndrome (SAS) and photomorphogenesis such as PAR1 and PIF3 were upregulated in the dark-grown root and down-regulated in light. In SAS, PAR1 interacts and inhibits PIF avoiding the excess of hypocotyl elongation. We determined that these genes were most expressed in orange varieties than in white ones, suggesting that these genes may be involved in carotenoid synthesis. Here we show the functional characterization of DcPAR1 and DcPIF3 through expression in A. thaliana and RNAi in carrot as well as in vivo nuclear PAR1:PIF3 interaction. DcPAR1 transgenic Arabidopsis seedlings present a dwarf phenotype while DcPIF3 show elongated hypocotyls. Interestingly, DcPAR1, contrary to DcPIF3 lines present higher level of carotenoids. Our results indicate that DcPAR1 and DcPIF3 participates in the synthesis of carotenoids and photomorphogenic development.Acknowledgments: Proyecto Fondecyt 1180747

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Primary Poster Presenter: Claudia Stange

MYB23 mediates a cell fate switch in the Arabidopsis root epidermis in response to ribosomal stress (0300-052)
Hall 2

The Arabidopsis root epidermis consists of hair and non-hair cells that differentiate in a position-dependent manner. The hair cells arise in H positions that are in contact with two cortical cells, while the non-hair cells arise in N positions that are in contact with one cortical cell. Underlying this unique cell patterning is a network of transcription factors, centered upon a MYB-bHLH-WD40 complex containing WEREWOLF (WER), GLABRA 3/ENHANCER OF GLABRA 3 (GL3/EGL3), and TRANSPARENT TESTA GLABRA 1 (TTG1). This study focuses on how this cell fate regulatory network is affected when ribosome biogenesis is impaired. We identified several mutants of ribosome biogenesis factors (RBFs) producing non-hair cells in H positions, implying a cell fate switch from hair cell to non-hair cell. Through multiplex genetic and molecular analysis, we discovered that this cell fate switch requires the MYB23 protein, a MYB family protein that is functionally redundant with WER. In wild-type roots, the MYB23 gene is specifically expressed in N position cells under direct regulation of the WER-GL3/EGL3-TTG1 complex. However, in RBF mutants, we observed additional MYB23 gene expression in both H and N positions that is independent of the central complex. Interestingly, this unspecific MYB23 gene expression requires the ANAC082 protein, a recently identified mediator for ribosomal stress responses. Thus, this study uncovers a novel role of the MYB23 protein in regulating root epidermal cell fate and provides the first mechanistic explanation for a molecular linkage between ribosome biogenesis and plant cell fate.

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Primary Poster Presenter: Wenjia Wang

Network walking charts transcriptional dynamics of nitrogen signaling by integrating validated and p (0300-073)
Hall 2

Charting a temporal path in gene networks requires linking early transcription factor (TF)-triggered events to downstream effects. Here, we scale-up a cell-based TF-perturbation assay to identify direct regulated targets of 33 nitrogen (N)-early response TFs that regulate 88% of N-responsive Arabidopsis genes. We uncover a duality where each TF can act as an inducer and repressor of specific sets of target genes, and known cis-binding motifs identified by in vitro assays are typically specific to regulation directionality. Validated TF-targets are used to refine precision of a time-inferred root network, connecting 145 N-responsive TFs and 311 targets. These data are used to chart network paths from direct TF1-regulated targets identified in cells to indirect targets responding only in planta via Network Walking. We uncover network paths from TGA1 and CRF4 to direct TF2 targets, which in turn regulate 76% and 87% of TF1 indirect targets in planta, respectively. These results have implications for N-use and the approach can reveal temporal networks for any biological system.

Primary Poster Presenter: Matthew Brooks

OsSPL6 represses signalling outputs of ER stress in control of panicle cell death (0300-047)
Hall 2

In plants, carotenoids play essential roles in light-harvesting processes and protect the photosynthetic machinery from photo-oxidative damage. In our previous studies, Orange gene (IbOr) from sweetpotato [Ipomoea batatas (L.) Lam] was isolated, which is involved in accumulation of carotenoids. IbOr protein with a holdase chaperone activity post-transcriptionally regulates phytoene synthase (PSY), an important enzyme in the carotenoid biosynthetic pathway. IbOr protects IbPSY stability, which leads to carotenoid accumulation and confers enhanced tolerance to heat stress at 47oC and oxidative stress in IbOr transgenic sweetpotato plants. In addition, IbOr interacts with oxygen-evolving enhancer protein 2-1 (PsbP), an extrinsic protein of the oxygen-evolving complex (OEC) of PSII, and the holdase chaperone function of IbOr can protect PsbP from heat-induced denaturation. In this study, substitution of a single amino acid (R96H) in a wild-type IbOr shows dramatically enhanced carotenoid accumulation by up to 30-fold in the transgenic sweetpotato calli. IbOr-R96H transgenic calli also showed enhanced tolerance to salt stress compared with IbOr-WT. To further explore the function of IbOr-R96H and its utilization to develop various industrial plants with enhanced carotenoid content and tolerance to abiotic stresses, transgenic sweetpotato plants overexpressing IbOr-R96H were successfully generated and are under characterization. We anticipate that IbOr-R96H transgenic sweetpotato plants will enhance production of carotenoids and various environmental stress tolerances for sustainable agriculture on marginal lands.

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Primary Poster Presenter: Qinglong Wang

PATL4 is an interacting partner of AtAPY1 and may be required for its growth-promoting functions. (0300-069)
Hall 2

Cells limit the concentration of extracellular ATP (eATP) in part through the activity of ectoapyrases (ecto -NTPDases), and two nearly identical Arabidopsis apyrases, AtAPY1 and AtAPY2, appear to share this function. High levels of eATP can block auxin transport and gravitropic growth in primary roots of Arabidopsis. Suppression of APY1 and APY2 increases [eATP] and suppresses auxin transport and growth in Arabidopsis. To further understand the function of AtAPY1 a yeast two-hybrid approach was used to identify AtAPY1-interacting partners. One of two membrane-bound proteins found to interact with AtAPY1 was PATL4 (Sec14p-like phosphatidylinositol transfer family protein), a plasma membrane-associated

protein, that is involved in auxin signaling and PIN1 polar localization. Its localizations in Arabidopsis primary root, lateral root primordia, and developing stomata are similar to the localization of AtAPY1, as judged by promoter-β-glucuronidase (GUS) staining. Both PATL4 and AtAPY1 are expressed in rapidly growing tissues and tissues that accumulate high auxin level. The physiological phenotypes of the patl4 mutant are similar to those of mutants suppressed in AtAPY1 expression. Additional studies are in progress to test whether the expression of PATL4 is required for AtAPY1 to promote auxin transport and growth.

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Primary Poster Presenter: Han-Wei Jiang

Phospholipase AIIIα regulates cell growth and confers virus resistance when overexpressed (0300-054)
Hall 2

Patatin-related phospholipase A (pPLAs) are major lipid acyl hydrolases that are classified into three groups. pPLAIII group members (α, β, γ, δ) are distinguished from other PLAs by a lack of canonical catalytic serine motif. Detailed studies of pPLAIIIβ and δ have been conducted but the enzymatic activity and cellular functions of pPLAIIIα have not yet been investigated in Arabidopsis. Here, using lipidomics we show that Arabidopsis AIIIα is able to hydrolyze a wide range of lipids including lysolipids and we report previously unknown functions of a phospholipase AIIIα based on characterization of knockout mutants and overexpressors (pPLAIIIαOEs). Fluorescence tagging of pPLAIIIα localized to the plasma membrane and that overexpression of pPLAIIIα reduced the level of a wide range of membrane lipids. pPLAIIIαOE resulted in reduced longitudinal growth displaying a short and stunted plant in all organs except overall increased seed size. pplaIIIα mutant displayed reduced length of trichome and increased hypocotyl. pPLAIIIαOE lines resulted in prolonged longevity with reduced ROS. Total lignin content was also significantly decreased in OE lines. A two-fold higher SA/JA ratio in the strongest pPLAIIIαOE line resulted in increased turnip crinkle virus resistance and enhanced expression of the defense gene PR1. These results thus show that Arabidopsis pPLAIIIα have distinct cellular functions in lowering lignin content and ROS, which in certain threshold level enhances virus resistance.

Primary Poster Presenter: Ok Ran Lee

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Primary Poster Presenter: Ok Ran Lee

Plant Reactome: Plant Pathway Network and Analysis Resource (0300-074)
Hall 2

Plant Reactome (https://plantreactome.gramene.org) is an open-source, manually curated pathway and network portal of Gramene database. It features cellular level pathway networks for 83 plant species ranging from unicellular autotrophs to higher plants. It hosts genetic, metabolic, signaling, transport, developmental and biotic/abiotic stress pathways for several model, crop, and evolutionarily important plant species. For reference species rice (Oryza sativa), manual curation involves generation of interactive network showing interaction among DNA, RNA, proteins, miRNA, complexes, and small molecules. Currently, database hosts ~300 curated rice pathways which are used to project similar events and pathways for other 82 species based on the gene orthology. The pathway clustering across the broad phylogenetic spectrum of photosynthetic organisms shows distinct gene-pathway association patterns reflecting evolutionary history and ploidy levels. Plant researchers can compare reference rice pathways with the projected pathways from any plant species from the available list to discover species-specific pathways, regulatory events, and loss/gain of various reactions, etc. Users can also get information about protein-protein interaction data, baseline and differential gene expression data within the pathway browser. Users can upload, analyze, and visualize omics datasets to identify gene-gene interaction data, differential expression of pathways and associated genes. Database entities also link to various external sources, such as UniProt, EMBL-EBI’s gene expression ATLAS, ChEBI, PubChem, PubMed, GO, BAR, IntAct, Planteome and various other plant genome databases. Users can download the data in various formats from the project web site and also access programmatically using APIs. This project is supported by the

NSF (IOS-1127112), the NIH (U41 HG003751), ENFIN (LSHG-CT-2005-518254), the Ontario Research Fund, and the EBI Industry Programme.

Primary Poster Presenter: Parul Gupta

Prediction of condition-specific regulatory maps in Arabidopsis using integrated genomic data (0300-071)
Hall 2

Recent advances in genomic technologies such as DNA Affinity Purification Sequencing (DAP-seq) and Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-seq) have generated large-scale, regulatory genomic data for the multiple plant species. To predict condition specific gene regulatory networks using these data, we developed the Condition Specific Regulatory network inference engine (ConSReg), which combines heterogeneous genomic data using sparse linear model followed by feature selection and stability selection. Using Arabidopsis as a model system, we constructed comprehensive and accurate maps of gene regulation under more than 50 experimental conditions. Our results show that ConSReg accurately predicted gene expressions with an average auROC of 0.84 across these testing datasets. Including ATAC-seq information significantly improves the performance of ConSReg across all tested datasets. We applied ConSReg to Arabidopsis single cell RNA-seq data of two root cell types (endoderims and cortext) and identified five regulators in two root cell types. Three out of the five regulators are supported by existing publications. Finally, we tested our approach in a rice gene expression dataset and were able to identify both known and novel regulatory motifs that control drought response in the rice genome. Our results demonstrated that integrating heterogeneous genomic data can provide novel insights into the regulation of condition-specific and single cell-specific gene expression.

Primary Poster Presenter: Song Li

PtrERF109 of Poncirus trifoliata functions in cold tolerance by directly regulating Prx1 involved in (0300-159 (Screen 10))
Hall 2

Ethylene-responsive factors (ERFs) have been revealed to play essential roles in a variety of physiological and biological processes in higher plants. However, functions and regulatory pathways of most ERFs in cold stress remain largely unclear. Here, we identified PtrERF109 of trifoliate orange (Poncirus trifoliata (L.) Raf.) and deciphered its role in cold tolerance. PtrERF109 was drastically up-regulated by cold, ethylene and dehydration, but repressed by salt. PtrERF109 was localized in the nucleus and displayed transcriptional activity, and the C terminus is required for the activation. Overexpression of PtrERF109 conferred enhanced cold tolerance in transgenic tobacco and lemon plants, whereas VIGS (virus-induced gene silencing)-mediated suppression of PtrERF109 in trifoliate orange led to increased cold susceptibility. PtrERF109 overexpression caused extensive transcriptional

reprogramming of several suites of stress-responsive genes. Prx1 encoding class III peroxidase (POD) was one of the antioxidant genes exhibiting the greatest induction. PtrERF109 was shown to directly bind to the promoter of PtrPrx1 (trifoliate orange Prx1 homologue) and positively activated its expression. In addition, the PtrERF109-overexpressing plants exhibited significantly higher POD activity and accumulated dramatically less H2O2 and were more tolerant to oxidative stress, whereas the VIGS plants exhibited opposite trends, in comparison with wild type. Taken together, these results indicate that PtrERF109 as a positive regulator contributes to imparting cold tolerance by, at least partly, directly regulating the POD-encoding gene to maintain a robust antioxidant capacity for effectively scavenging the ROS. Our findings gain insight into better understanding of transcriptional regulation of antioxidant genes in response to cold stress.

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Primary E-Poster Presenter: Ji-Hong Liu

Quantification of WRKY1 Alternative Splicing in Arabidopsis thaliana to Explore Phenotypic Plasticit (0300-065)
Hall 2

With recent developments in technology, research into transcriptional level regulation of plants has become more advanced. One method of transcriptional regulation occurs through the differential splicing of pre-mRNA transcripts, called alternative splicing. Alternative splicing allows for a single gene to encode various protein isoforms, which may have different functions. Transcription factor proteins bind to DNA with the help of other biological molecules, such as cofactors or other transcription factors, to up or down regulate the transcription of a DNA segment. Several transcription factors undergo alternative splicing to adjust the efficiency of transcription under stress conditions. One such transcription factor in Arabidopsis thaliana, called WRKY1, has two putative splice variants. WRKY1 is involved in plant drought and immune response. The aim of this project was to quantify the mRNA transcripts of WRKY1 under standard and stress conditions through the use of qRT-PCR.

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Primary Poster Presenter: Laura Janousek

Regulation of circadian clock genes by Heat Shock Transcription Factors in Arabidopsis (0300-060)
Hall 2

The circadian clock is an endogenous self-sustaining timekeeper that allows organisms to coordinate with fluctuating environmental conditions such as diurnal and seasonal changes in temperature and light to optimize fitness. Several factors involved in light perception and integration into the circadian clock in Arabidopsis are known. However, how temperature, specifically heat stress is perceived and

integrated into the clock is not fully understood. Preliminary data from yeast one hybrid studies show that select Heat Shock Transcription Factors (HSFs) bind to the promoters of the morning and daytime expressed clock genes (CCA1, LHY, PRR7 and PRR9). To gain a mechanistic understanding of the role of HSFs in temperature regulation of the circadian clock, we are functionally characterizing several members of the HSF family using molecular and genetic approaches. Based on our preliminary data, we validated the binding of select HSFs to the clock gene promoters using Chromatin immunoprecipitation and quantitative PCR. Reporter based assays and overexpression experiments at normal growth temperatures shows a decrease in transcript abundance of clock genes throughout the day, suggesting that these HSFs negatively regulate the expression of clock genes. In addition, we also observe alteration of clock properties such as phase and period as a result of HSF overexpression. Together, these results suggest a key role for HSFs in regulating clock function and responses to elevated environmental temperatures. The findings from this study will provide new mechanistic insights into how plants perceive and integrate temperature signals to the circadian clock, and potentially aid in the improvement of crop thermotolerance.

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Regulation of orchid floral scent (0300-037)
Hall 2

Phalaenopsis bellina has a charming floral scent and is widely used as a breeding parent for the scent phenotype. The main floral volatiles in P. bellina are monoterpenes. Previous studies have cloned and analyzed PbGDPS, the key enzyme involved in the monoterpenes biosynthesis in P. bellina. To study the regulation of floral scent biosynthesis in Phalaenopsis orchids, floral transcriptome libraries of scented P. bellina were constructed and compared to that of the scentless P. aphrodite. Significant differential expression of GDPS between P. bellina and P. aphrodite indicates that the elevated GDPS expression in the scented P. bellina is critical for its monoterpene accumulation. To identify the upstream factors regulating GDPS in Phalaenopsis orchids, we focused on the trans-factor first. By comparative transcriptome analysis, eight candidate transcription factors (TFs) out of 2360 TFs were showed to enhance differential expression between the two transcriptomes. Ectopic transient expression of these TFs in the scentless P. aphrodite revealed that PbbHLH4 most profoundly induces the monoterpene phenotype with a ~1000-fold increase of monoterpenoid production, while the other 4 showed minor effects. These results indicate its major role for monoterpene biosynthesis in Phalaenopsis orchids. Next, the cis-factor affecting the expression of GDPS was examined. By analyzing the GDPS promoter fragments isolated from 12 scented and scentless orchids, a dual repeat cis-element is present only in the Phalaenopsis orchids emitting monoterpenes. Serial deletions of the GDPS promoter fragment demonstrate that this dual repeat is crucial for its promoter activities. PbbZIP4 is isolated and it can only transactivate the GDPS promoter fragment

containing the complete or near-complete dual repeat. In addition, ectopic transient expression of PbbZIP4 induces production of monoterpenes in the scentless orchid. Furthermore, by exploring the external and internal signals regulating th

Primary Poster Presenter: HONG-HWA CHEN

Relief of sugar suppression through MYB and 14-3-3 protein interaction enhances plant growth, abioti (0300-026)
Hall 2

During plant growth and development, sugar provision and starvation contrastingly regulate gene expression both temporally and spatially, which plays a pivotal role in determining crop productivity. The mechanism regulating the reversible gene expression by the two opposing sugar availablility statuses in plants, and how the regulation impacts growth and productivity, has remained unknown. In cereals, α-amylase plays a central role in hydrolyzing starch to sugars to support seedling growth upon germination. Expression of the alpha-amylase gene (Amy) is up-regulated by sugar starvation and repressed by sugar provision. Our previous studies showed that sugar starvation induces expression and promotes nuclear import of the MYB-A transcriptional activator that interacts with the cis-acting element TA box and induces the Amy promoter in cereals. Here, we show that a sugar-inducible MYB-R transcription factor negatively regulates plant growth by competing with MYB-A for binding to the TA box and suppressing Amy promoter activity. We also discovered that sugar promotes nuclear import of MYB-R, whereas sugar starvation restricts MYB-R in the cytoplasm through specific interactions with 14-3-3 proteins. Moreover, phosphorylation at distinct serine residues in MYB-R regulates its nucleocytoplasmic shuttling and interactions with 14-3-3 proteins. Finally, we observed that expression of MYB-R is down-regulated by dehydration and heat, reflecting induction of Amy expression. Activation of Amy and suppression of MYB-R expression enhance plant growth, abiotic stress tolerance and grain yield in rice. Our findings not only reveal a hitherto undiscovered regulatory mechanism for switching on and off reversible gene expression by sugar homeostasis, which tightly regulates plant growth, stress tolerance and productivity, but also highlight MYB-R and Amy as targets for breeding stress-tolerant crops.

Primary Poster Presenter: Su-May Yu

The regulatory role of AGL18 in Arabidopsis embryogenesis. (0300-045)
Hall 2

The molecular mechanisms of Arabidopsis embryogenesis are still largely unknown. A MADS domain transcription factor (TF), AGAMOUS-LIKE15 (AGL15) accumulates mainly, although not solely, during embryogenesis. Ectopic expression of AGL15 stimulates development of somatic embryos from zygotic embryo explants. Previous reports stated that the product encoded by AGAMOUS-LIKE18 (AGL18) was structurally related and showed overlapping expression patterns with AGL15 in

Arabidopsis. Overexpression of both AGL15 and AGL18 in Arabidopsis produced analogous phenotypes, such as morphological variations and late flowering time. Here, we showed that like AGL15, constitutively expressed AGL18 promotes somatic embryogenesis from the shoot apical region of seedlings in liquid media containing 2,4-D. Moreover, AGL18 interacts with AGL15 to form a heterodimer by co-immunoprecipitation (Co-IP). In order to characterize the roles of the AGL18 transcription factor complexes at the molecular level, we studied genome-wide the direct targets of AGL18. We used chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing to obtain genome-wide DNA-binding sites of AGL18. The results demonstrate that AGL18 binds to thousands of sites in the genome in two biological replicates. Then we compared ChIP-seq data for AGL15 to the AGL18 data. Both data sets were generated from embryo culture tissues. A significant number of genes were bound by both AGL15 and AGL18. GO analysis revealed these genes were enriched for seed, embryo and reproductive development as well as hormone and stress responses. The results also suggest that AGL18 regulates its own expression through a positive auto-regulatory loop. The binding of AGL18 to cis-regulatory elements of other MADS-box genes, including AGL15 and AGL16, and expression analyses reveal that this protein is a key component in the regulatory transcriptional network to control embryogenesis in Arabidopsis.

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Primary Poster Presenter: Priyanka Paul

The Role of Transcription Factor LBD40 in Arabidopsis Embryogenesis
(0300-046)
Hall 2

Somatic Embryogenesis (SE) is an artificial process by which an embryo is derived from a single somatic cell or group of somatic cells which is regulated by key transcription factors (TF), including AGAMOUS-like 15 (AGL15). SE is a valuable means to regenerate transgenic plants to meet food demands or test gene function, but is poorly understood. Therefore, understanding regulatory mechanisms in SE is fundamentally important. One of the intriguing proteins with which AGL15 interacts is LBD40. LBD40 encodes a LATERAL ORGAN BOUNDARIES (LOB)-domain TF that is unique to plants and is specifically expressed during seed development, primarily in the embryo. The main objective of the research is to understand the mechanism of embryogenesis in Arabidopsis, specifically focusing on interaction between AGL15 and LBD40 in planta. Siliques and embryo culture tissue with epitope tagged transgenes are used for Chromatin-Immuno Precipitation (ChIP). The DNA binding regions of these proteins are assessed using ChIP-sequencing. In-vivo protein interaction of AGL15-LBD40 has been found using Co-immunoprecipitation. RNA seq results of 7-8 days old seeds from a lbd40/41 mutant line showed 335 genes as significantly expressed targets. The Gene Ontology (GO) enrichment analysis showed overrepresentation of biological processes that are associated with SE, suggesting importance of LBD40 in SE. The ChIP-Seq experiment along with RNA-seq of LBD40 will help us to understand better somatic embryogenesis.

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Primary Poster Presenter: sanjay joshi

Transcription Factor Regulatory Networks Controlling Soybean Seed Maturation (0300-040)
Hall 2

Soybean (Glycine Max) is the most produced and consumed oilseed in the world. The majority of storage compounds in the soybean seed accumulate during the maturation phase of seed development. Thus, understanding the initiation and establishment of seed maturation will allow for the development of strategies to improve soybean seed quality. Genome-wide transcriptome analysis allowed us to identify a set of co-expressed genes with a spatial and temporal expression pattern that correlates with the maturation program of the seed. Several transcription factors (TFs) that regulate seed maturation were identified in the cluster, including LEAFY COTYLEDON1 (LEC1), ABA INSENSITIVE3 (ABI3), BASIC LEUCINE ZIPPER67 (bZIP67) and ABA-RESPONSIVE ELEMENT BINDING PROTEIN3 (AREB3). We performed chromatin immunoprecipitation and differential gene expression analyses to identify potential target genes that are transcriptionally regulated by these TFs. Analysis of target genes showed a complex TF regulatory network in which different combination of TFs are involved in controlling distinct biological programs in soybean embryos, such as storage accumulation, photosynthesis and hormone signaling. Genome-wide analyses of TF binding sites (ChIP-Seq) and accessible chromatin regions (ATAC-Seq) suggest that distinct TF complexes are assembled in cis-regulatory modules to control the expression of target genes. DNA motif analyses suggests that the formation of TFs complexes in cis-regulatory modules are determined by a unique composition of DNA motifs. Transient assays in protoplasts isolated from soybean embryos have been used to validate the functionality of DNA motifs in cis-regulatory modules. We also observed that distinct sets of TF complexes are formed due their ability to physically interact to each other. Our results are providing a framework to understand the complex transcriptional regulatory networks that control distinct biological processes during soybean seed development.

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Transcriptional changes in response to elevated of superoxide dismutase

expression in tobacco (0300-028)
Hall 2

Stressful environmental conditions such as drought can cause oxidative stress damage by altering the equilibrium between production of reactive oxygen species (ROS) and cellular scavenging mechanisms. In plant chloroplasts, superoxide dismutase (SOD) constitutes the first line of defense against oxidative stress by catalyzing the conversion of superoxide, generated by the photoreduction of O2, to H2O2. In previous work, we and others showed that overexpression of chloroplast localized SOD in plants can enhance the antioxidant capacity of chloroplasts under stressful conditions. We also found that this transgenic intervention leads to ectopic activation of endogenous ascorbate peroxidase (APX) gene expression. To gain a better understanding of the compensatory changes in gene expression in plants that over-express SOD, we carried out transcriptomic analysis. Comparative analysis of RNA-seq data between transgenic tobacco plants that over-express chloroplastic Cu/Zn SOD with wild-type (WT) plants revealed a large number of differentially expressed genes (DEGs). Gene Ontology and KEGG pathway analysis showed that these DEG s were associated with abroad range of metabolic activities and involved in many biological process, cellular components, and molecular functions. In addition to oxidative stress responses, we found that transgenic plants have elevated expression of disease resistance genes transcripts compared to WT plants and therefore, we speculate that SOD could play an important role in biotic stress tolerance and/or signaling. In addition, SOD overexpression had a significant effect on the expression of several transcription factors and plant hormone signaling pathways. Reverse transcriptase qPCR confirmed the reliability of RNA sequencing data. Our results provide a foundation for further study of the physiological and molecular mechanisms affected by the ectopic expression of Cu/Zn-SOD in tobacco plants.

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Transcriptional control of Rubisco activase gene expression by heat stress in Arabidopsis (0300-027)
Hall 2

One of the major factors that inhibits photosynthesis during heat stress is deactivation of Rubisco, due to the thermal instability of its chaperone protein, Rubisco activase (RCA). Recovery of RCA activity is thought to be at least partly responsible for acclimation of plants to prolonged heat stress, and may occur through stabilization of RCA at both mRNA and protein levels. Previous studies in cotton and Arabidopsis show that RCA mRNA levels are stabilized post-transcriptionally by modification at their 3'-untranslated regions. Given that steady state transcript levels reflect a balance between synthesis and degradation, we questioned whether heat stress also induced RCA transcription. Using transgenic lines expressing modified Luciferase controlled by the RCA promoter, we found that

RCA transcriptional activity was significantly increased when plants were exposed to moderate heat stress (35 ºC). Interestingly, the magnitude of induction was dependent on the time of day rather than the duration of the heat treatment, suggesting that control of RCA transcription during heat stress may be coordinated by the circadian clock. Our in silico analysis of the RCA promoter identified several putative elements that may contribute heat regulation of RCA, including a well-conserved G-box element. We are now conducting transient expression assays in protoplasts, and site-directed mutagenesis, to examine the involvement of specific RCA promoter elements in the heat-stress response.

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Primary Poster Presenter: Xiaobo Sun

Transcriptomics Identifies Modules of Differentially Expressed Genes and Novel Cyclotides in Viola p (0300-055)
Hall 2

Viola is a large genus with worldwide distribution and many traits not currently exemplified in model plants including unique breeding systems and the production of cyclotides. Here we report de novo genome assembly and transcriptomic analyses of Viola pubescens using short-read DNA sequencing data and RNA-Seq from eight diverse tissues. First, V. pubescens genome size was estimated through flow cytometry, resulting in an approximate haploid genome of 455 Mbp. Next, the draft V. pubescens genome was sequenced and assembled resulting in 264,035,065 read pairs and 161,038 contigs with an N50 length of 3,455 base pairs. RNA-Seq data were then assembled into tissue-specific transcripts. Together, the DNA and transcript data generated 38,081 ab initio gene models which were functionally annotated based on homology to Arabidopsis thaliana genes and Pfam domains. Gene expression was visualized for each tissue via principal component analysis and hierarchical clustering, and gene co-expression analysis identified 20 modules of tissue-specific transcriptional networks. Some of these modules highlight genetic differences between chasmogamous and cleistogamous flowers and may provide insight into V. pubescens' mixed breeding system. Orthologous clustering with the proteomes of A. thaliana and Populus trichocarpa revealed 8,531 sequences unique to V. pubescens, including 81 novel cyclotide precursor sequences. Analysis of the RNA-Seq data for these cyclotide transcripts revealed diverse expression patterns both between transcripts and tissues. The diversity of these cyclotides was also highlighted in a maximum likelihood protein cladogram containing V. pubescens cyclotides and published cyclotide sequences from other Violaceae and Rubiaceae species. This work provides the most comprehensive sequence resource for Viola, offers valuable transcriptomic insight into V. pubescens, and will facilitate future functional genomics research in Viola and other diverse plant groups.

Primary Poster Presenter: Anne Sternberger

Genes & Genomes: Genetics

Genetic analysis of pod shattering in cowpea [Vigna unguiculata (L.) Walp] (0300-160 (Screen 4))
Hall 2

Cowpea [Vigna unguiculata (L.) Walp] is one of the most important food and nutritional security crops, providing the main source of dietary protein and folic acid for millions of people in sub-Saharan Africa and other parts of the developing word. Cowpea was domesticated in Africa, from the wild form V. unguiculata ssp dekindtiana. Domestication of cowpea has, in general, resulted in a determinate growth habit, increased pod and seed size, early flowering, and reduction of pod shattering. The shattering habit is an important adaptive mechanism for seed dispersal in wild species but causes severe reduction in yield in domesticated species. Non-shattering is still a breeding target in cowpea as the shattering habit is only reduced in certain domesticated accessions. To overcome this, there is a need to develop improved shattering-resistant varieties. Understanding the genetic basis of pod shattering is fundamental to the breeding of non-shattering cowpeas. Here, we investigated the genetic and cellular bases of pod shattering. A genome-wide association study was conducted using a panel of 368 cowpea diverse accessions from 51 countries, and regions associated with pod shattering were identified on chromosomes Vu03 and Vu11. Candidate genes underlying these regions were identified based on annotations of the reference genome IT97K-499-35. Cross sections of pod ventral sutures revealed different patterns between the shattering and non-shattering accessions. The same analysis will be conducted using different recombinant inbred lines derived from a cross between a wild and a cultivated cowpea. This study will determine the correlation between pod architecture and shattering resistance and will provide a basis for further fine mapping of genes involved in pod shattering.

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Developing a fertile ask1 mutant uncovers a comprehensive set of SCF-mediated intracellular functio (0300-161 (Screen 14))
Hall 2

Ubiquitin-mediated proteolysis is involved in many biological processes. In plants, SKP1-CUL1-F-box (SCF) complexes play important roles in selecting substrates for proteolysis. Among the four components in this complex, SKP1 defines as the bridge between F-box and CUL1 proteins. In this study, we demonstrate a vital role

for Arabidopsis SKP1-like1 (ASK1) in Arabidopsis embryogenesis and postembryonic development by developing a fertile ask1 mutant. Through 10 generations of backcrossing of a previous sterile ask1 mutant in Ler-0 with Col-0 and further four generations of selfing, we discovered that the new ask1 mutant was fertile albeit in low fertility. Applying this mutant, we identified that ask1 produced twisted rosette leaves, varied petals, few pollen grains with some being fertile, and large embryos and seeds. To further understand the depth and breadth of SCF-mediated intracellular functions, we analyzed the perturbation of both bud and open flower transcriptomes in ask1. Our RNA-Seq data suggested that ASK1-containing SCF complexes play a wide range of roles in plants, such as circadian rhythm, hormone signaling, stress responses, innate immunity and cellular development. To further verify these functions, we discovered that ask1 mutants were hyposensitive to auxin and salicylic acid and hypersensitive to abscisic acid compared to WT. In addition, red and far red light treatments both reduced the hypocotyl length of ask1 seedlings, confirming SCF-mediated photomorphogenesis. The up regulation of circadian rhythm pathways in ask1 resulted in late flowering. Consistent with the role of ASK1-containing SCF complexes in protein ubiquitylation, immunoblotting analysis detected a much lower amount of bud and floral proteins that were ubiquitylated in ask1 than that in WT. Taken together, this newly developed ask1 mutant encompasses a comprehensive set of phenotypes that reflect the broad regulatory functions of SCF complexes in plant growth and development.

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Genetic diversity and molecular marker screening for abiotic stress tolerance in weedy rice (0300-164 (Screen 3))
Hall 2

As global temperatures continue to rise and fluctuate, it is imperative that crop breeding programs continue to improve. In rice (Oryza sativa) it has been demonstrated that temperatures greater than 34C can cause spikelet infertility resulting in a yield reduction of up to 60%. In cold stress situations, temperatures below 17C can result in poor germination, seedling injury and reduced yield. In areas where flash flooding is unpredictable, submergence stress in rice fields can lead to a 10 – 100% yield loss. In areas where rice is the staple food product, impacts from cold, heat, and submergence stress could be devastating and felt for generations to come. Currently, rice breeding programs lack genetic diversity and suffer from a loss in traits through domestication. To combat these shortcomings, it has been suggested that weedy rice, a noxious subspecies of rice with increased competition within rice fields, may be used to discover new genes related to abiotic stress tolerance. In this study, a population of 54 kinds of weedy rice was selected and phenotypically screened for responses to cold, heat, and submergence stress

tolerance. Selected accessions that performed better than rice cultivars were used in a simple sequence repeat (SSR) marker study containing 30 SSR markers to discover markers that may lead to genes associated with the selected stresses. Screenings showed the complete selection of markers were 100% polymorphic with an average genetic diversity of 44% amongst the 54 weedy rice accessions, two cultivars, and three rice lines with allelopathic potential. This information could prove useful in the identification of genes that aid in tolerance to abiotic stresses.

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Primary E-Poster Presenter: Shandrea Stallworth

Transcriptome-based prediction of complex traits in maize (0300-165 (Screen 13))
Hall 2

The ability to predict traits from genetic information, known as Genomic Prediction (GP), can improve our understanding of the genetic basis of complex traits and has transformed breeding practices. In addition to genotype information, transcriptome data for entire breeding populations may be used for GP because transcript levels represent the integration of multiple cis and trans-regulatory variants. However it remains unclear the extent to which transcriptomic data can be used to predict plant phenotypes, particularly when the developmental stages differ when transcriptome and phenotype data are collected. Here we compare the predictive power of maize genetic markers and transcript levels collected at the seedling (V1) stage for predicting mature plant flowering time, height, and grain yield in a diversity panel containing 388 individual lines. Performance of models generated from transcript levels predicted plant phenotypes was well above random expectation and similar to that of genotype-based models. Although integrating both genotype and transcript data into one model did not improve predictive performance, we found that transcripts important for trait predictions were not located near important genetic markers and were not associated with important expression quantitative trait loci (eQTL). Thus, transcript-based models are identifying predictive expression variation that is not simply due to the genetic variation associated with these transcripts. Finally, transcript-based models identified 5 out of 14 benchmark flowering time genes as important for model predictions, while only 1 was identified by genotype-based models. This highlights that not only is transcriptome data useful for GP, but that it may be a missing link for understanding the genetic basis of traits controlled by epigenetic or complex regulatory variation.

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Genome sequence of the model rice variety KitaakeX and its use as a reference for fast neutron mutan (0300-166 (Screen 7))
Hall 2

The Kitaake variety (ssp. japonica), has emerged as a model for rice research. It is an extremely early flowering rice cultivar, easy to propagate, with good yield potential and eating quality. Here, we report the de novo genome sequencing and analysis of Kitaake.X, a Kitaake variety carrying the XA21 immune receptor. Our Kitaake.X sequence assembly contains 377.6 Mb, consisting of 33 scaffolds (476 contigs) with a contig N50 of 1.4 Mb. The assembly is complemented with detailed gene annotations of 35,594 protein coding genes. We identified 331,335 variations between Kitaake.X and the reference genome Nipponbare (ssp. japonica), and 2,785,991 variations between Kitaake.X and Zhenshan97 (ssp. indica). We also compared Kitaake resequencing reads to the Kitaake.X assembly and identified 219 small variations. We have generated a Kitaake mutant database called KitBase (http://kitbase.ucdavis.edu/), which includes genomic data, phenotypic data, and seed information for the 1504 fast neutron-induced Kiaake.X mutant lines. The availability of the high quality Kitaake.X genome and annotation reported here will greatly facilitate analysis of the FN mutants which was previously compared to Nipponbare as the reference genome. We aim to analyze ~3200 FN mutant lines by the end of year 2019 which will facilitate rice research.

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6:30 PM - 7:00 PM

Genome-wide association study leads to a future of firmer apples (0300-175 (Screen 1))

Apple (Malus domestica) is one of the world’s most valuable fruit crops and a promising candidate for marker-assisted selection (MAS) due to its lengthy juvenile

phase. To discover genotype-phenotype associations, we generated approximately 250,000 SNPs using genotyping-by-sequencing for the Apple Biodiversity Collection (ABC) located in Kentville, Nova Scotia, Canada. In 2017, phenotype data were collected from over 1,300 trees in the ABC, representing over 850 unique accessions. Accessions were also genotyped for several markers previously discovered using linkage mapping and currently used for MAS in apple. Genome-wide association study (GWAS) results confirmed the transcription factor NAC18.1, previously identified in a GWAS of the USDA apple germplasm collection, is a strong functional candidate for fruit firmness and harvest date. In comparison, no significant associations were identified for the firmness markers currently used in apple breeding. NAC18.1 is homologous to the NON-RIPENING (NOR) gene of tomato. While nor mutant fruits fail to ripen, transgenic complementation of nor with the apple NAC18.1 gene rescued its ripening defect, confirming NAC18.1's role as a conserved regulator of fruit ripening. These results demonstrate that GWAS in a diverse apple collection results in extremely high resolution mapping of putatively causal variants, which holds great potential for continued improvement of apples through MAS.

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Primary E-Poster Presenter: Zoë Migicovsky

A new gene for regulation of epidermal cell production in Arabidopsis cotyledons (0300-079)
Hall 2

Larger plant organs typically consist of greater numbers of cells than smaller plant organs. The Landsberg erecta (Ler-0) accession of Arabidopsis thaliana has more pavement cells, guard cells, and meristemoids than the Columbia-0 (Col-0) accession in the cotyledon, and cotyledons of Ler-0 are larger than those of Col-0 under certain growth conditions. Identification of the genes responsible for the epidermal cell number differences between Ler-0 and Col-0 will likely advance the understanding of the molecular mechanism regulating cell proliferation in plant organs. Towards identification of these genes, we conducted genetic analysis of F1 and F2 plants from the crosses between Col-0 and Ler-0. We found that the F1 plants showed an epidermal phenotype intermediate between those of Col-0 and Ler-0, indicating that the gene(s) in Ler-0 responsible for the epidermal phenotype

is(are) semi-dominant. The F2 plants segregated for the phenotypes of the Ler-0, Col-0, and the intermediate phenotypes. The ratio of non-Ler-0-like plants to Ler-0-like plants was ~7.2 : 1 (numbers of plants examined: 311 : 43). χ2 tests based on this ratio rejected the hypotheses that one gene or two unlinked genes underlie the Ler-0-like phenotype, which suggests that two linked genes underlie the Ler-0-like phenotype. A gene of interest is mapped to a ~0900kb region on chromosome 2 between At2G27130 and At2G29120. In this region, there is no gene that is known to be involved in the regulation of epidermal cell production. Therefore, a new gene in the regulation of epidermal cell production may be identified in the future. Interestingly, ER or At2G26330, the gene encoding a Leucin-rich repeat receptor kinase involved in the regulation of stomatal lineage formation, is close to this region. It seems plausible that the two linked genes suggested by the genetic analysis are ER and another gene residing between At2G27130 and At2G29120.

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Primary Poster Presenter: Jenny Swinton

Affecting Amino Acid Levels to Increase Methionine in the Lectin PHA-E of Phaseolus vulgaris (0300-083)
Hall 2

Developing countries around the world do not have access to regular sources of protein (e.g. poultry, fish, dairy products, etc.). Due to their availability, beans have become a staple food in many of these communities' diets to meet their daily protein intake. This crop is a great agronomic choice because they are able to be grown on small or large scale requiring minimal management. Though it is a dependable source of protein, the most abundant proteins are low in the essential amino acid methionine. Nutritional deficiencies can be attributed to the two major stores of protein in the bean, phaseolin and lectin; Lectin is the second most abundant seed protein. Its biochemical characteristics make it an effective anti-herbivory agent, and if the beans are not prepared properly, they lack nutritional value for human consumption. Several isoleucine sites were selected to be converted to methionine in the common black bean to improve the methionine content. Plasmids were prepared and transformed into wild type plants using Agrobacterium. The gene cassette was created and successfully introduced into T0 plants. The putative transformants are currently being tested to confirm they carry the expected modified gene. T1 plants will be tested in the near future. We will begin to investigate how the conversion of isoleucine to methionine affects the structure and function of the seed protein lectin. This research could elucidate how we can improve the nutritional value of essential foods in third world countries.This research was made possible by funding through the NIH grant #GM063787.

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Primary Poster Presenter: Ravi Madhwani

Association and QTL mapping reveal the genetic basis of solar tracking variation in wild and cultiva (0300-082)
Hall 2

Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation in eukaryotic organisms. SNP genotyping arrays are designed to optimize throughput, cost and efficiency of SNP detection. Ultimately, each genotyping application calls for an optimal balance between informativeness, cost, marker density, speed, flexibility, and data quality, which also differs across SNP genotyping platforms. The Cornell-IR LD Rice Array (C7AIR) contains 7,098 markers and represents a 2nd generation SNP array that improves upon the previously released C6AIR. The C7AIR is designed to detect genome-wide polymorphism within and between the five subpopulations of Oryza sativa (indica, aus, aromatic, tropical japonica, and temperate japonica) as well as between O. sativa and O. glaberrima, O. rufipogon and O. nivara. This SNP chip has been used at Texas A&M, Cornell and IRRI for genotyping >10,000 rice samples. In addition to successfully differentiating the five subpopulations of Oryza sativa, it also identifies introgressions from wild or exotic relatives, and is useful for QTL and association mapping in diverse materials. Using a diversity panel of 208 accessions, a genome-wide association analysis for days to flowering successfully employed the 7K array to identify key loci contributing to this trait in a field experiment in Beaumont, Texas. During the 2017 season, 6 significant loci were identified that contribute to days to flowering. Of these 6 loci, one co-localized with Hd3a and RFT1, two previously described flowering time genes. Due to the strong impact of environmental factors on flowering, this model was run again with solar radiation as a covariate. This increased the significance of QTL; however, these loci did not co-locate with previously described genes or with any significant loci identified in the basic model. Further validation of these flowering time genes may enable development of early-flowering varieties for U.S. rice production.

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Characterizing a rice diversity panel with a 7K SNP chip and flowering time evaluation (0300-077)
Hall 2

Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation in eukaryotic organisms. SNP genotyping arrays are designed to optimize throughput, cost and efficiency of SNP detection. Ultimately, each genotyping application calls for an optimal balance between informativeness, cost, marker density, speed, flexibility, and data quality, which also differs across SNP genotyping platforms. The Cornell-IR LD Rice Array (C7AIR) contains 7,098 markers and represents a 2nd generation SNP array that improves upon the previously released C6AIR. The C7AIR is designed to detect genome-wide polymorphism within and between the five subpopulations of Oryza sativa (indica, aus, aromatic, tropical japonica, and temperate japonica) as well as between O. sativa and O. glaberrima, O. rufipogon and O. nivara. This SNP chip has been used at Texas A&M, Cornell and IRRI for genotyping >10,000 rice samples. In addition to successfully differentiating the five subpopulations of Oryza sativa, it also identifies introgressions from wild or exotic relatives, and is useful for QTL and association mapping in diverse materials. Using a diversity panel of 208 accessions, a genome-wide association analysis for days to flowering successfully employed the 7K array to identify key loci contributing to this trait in a field experiment in Beaumont, Texas. During the 2017 season, 6 significant loci were identified that contribute to days to flowering. Of these 6 loci, one co-localized with Hd3a and RFT1, two previously described flowering time genes. Due to the strong impact of environmental factors on flowering, this model was run again with solar radiation as a covariate. This increased the significance of QTL; however, these loci did not co-locate with previously described genes or with any significant loci identified in the basic model. Further validation of these flowering time genes may enable development of early-flowering varieties for U.S. rice production.

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Characterizing Pre-Harvest Sprouting Using a Diverse Soft Winter Wheat Population (0300-086)
Hall 2

Pre-harvest sprouting (PHS) impacts soft and hard winter wheat production in all major United States wheat production regions when cold, wet conditions occur just before harvest. PHS reduces the grade, marketability and price per bushel of wheat by reducing test weight and functional properties of grain. Phenotyping for PHS is cost, labor, and time prohibitive and not many breeding lines are checked. We have gathered a set of ~200 soft winter wheat varieties adapted to grow in the

Northeastern U.S. spanning breeding lines from the early 1800's to present day. These varieties show a wide range of PHS resistance and will be used to unravel the genetic basis of pre-harvest sprouting to develop markers which are more cost-effective for breeders. Current genetic analysis indicates regions that may be important for PHS and provide new alleles for resistance.

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Primary Poster Presenter: Bryan Penning

Chloroplast Genome Isolation and Sequencing from Over 200 Accessions of Opuntia spp. (Cactaceae) (0300-093)
Hall 2

Prickly pear cactus (Opuntia ficus-indica) is a highly water-use efficient, highly productive, and climate-resilient biomass and bioenergy feedstock for semi-arid regions of the world. The Opuntia genus is the largest within the Cactaceae and is comprised of about 200 species. The global climate crisis is predicted to increase soil moisture deficits resulting in increased severity and duration of drought leading to reduced yield increases for many major crops. However, modeling studies demonstrate that Opuntia spp., which perform crassulacean acid metabolism (CAM) to improve their water-use efficiency (WUE) many folds compared with C3 and C4 photosynthesis-perfoming crops, shows great potential for food, forage, and biofuel production on semi-arid, abandoned, or degraded agricultural lands. The long-term goal of our research is to leverage existing cultivars, landraces, and wild selections of Opuntia ssp. through collaboration with the USDA-ARS National Arid Land Plant Genetic Resources Unit (NALPGRU) in Parlier, CA and optimize Opuntia germplasm selections to incentivize private industry participation. Specifically, we have developed a genotyping method based on complete chloroplast genome sequence information in order to resolve the genetic structure of the public Opuntia ssp. germplasm collection. The results from this effort will allow us to remove genetically identical accessions and resolve species designations in order to simplify curation efforts. Methods for the isolation and sequencing of the chloroplast genomes of over 200 accessions will be reported. These results will expose gaps in the collection and provide for molecular tools for future breeding and genome-editing efforts.

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Chromosome remodeling via genome reduction and tissue culture regeneration of potato (0300-085)
Hall 2

Genome instability is a disrupting phenomenon that can result from natural or artificial causes. The molecular mechanisms that trigger genome instability are not

well understood. At the same time, while the consequences of genome instability on plant breeding and evolution can be drastic, they remain poorly characterized. We characterize the extent and nature of genome instability after regeneration from tissue culture or after intraspecific haploid induction crosses of potato. Both processes resulted in chromosome remodeling at different frequencies, the outcomes of which are either truncated or shattered chromosomes. We show that regeneration from protoplasts without introduction of transgenes or genome editing tools was highly disruptive to genome integrity, whereas regeneration of transgenic potatoes using standardized procedures had a milder effect. In the context of haploid induction, we observed rare remodeling of single haploid inducer chromosomes that were retained in otherwise haploid plants, which is broadly consistent with other plant haploidization crosses. Overall, our results provide both a resource for investigating causes and outcomes of genome instability in plants and perspective on genomic instability incurred via routine experimental techniques in plant biology.

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Development of an InDel marker to distinguish the restorer gene Rf1/Rf2 simultaneously (0300-087)
Hall 2

CMS (cytoplasmic male sterility)/Rf systems provide both a practical tool for facilitating hybrid seed production and an ideal model for investigating nuclear-cytoplasmic interaction. In cotton, CMS-D2 with G. harknessii (D2-2 genome) cytoplasm and CMS-D8 with G. trilobum (D8 genome) cytoplasm are the two main CMS systems, with Rf1 and Rf2 as the restorer genes, respectively. Previous studies have proved that these two genes was located on the same chromosome (Chr_D05). In this research, an InDel marker that can be used to distinguish the restorer gene Rf1 and Rf2 simultaneously was developed. Multiple sequence alignment implied that 155 bp and 36 bp insertion existed in the Rf1 restorer line and Rf2 restorer line respectively at the same locus. This co-dominant markers was co-segregated with Rf1 and Rf2, as verified by a segregation analysis in the F2 (Rf1) and BC1F1 (Rf2) population. We subsequently used this marker to determine the allele status at the Rf1 and Rf2 locus in a backcross scheme for transferring Rf1 and Rf2. In this study, we developed a new marker to increase the marker density

in the restorer gene target region, which will be useful for the fine mapping of Rf1 and Rf2. The development of convenient and inexpensive co-segregating InDel markers will facilitate the marker-assisted selection of restorer lines carrying Rf1 and Rf2.

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Exposing the hidden half: Deeper Rooting 1 (DRO1) orthologs alter root architecture in Zea mays (0300-094)
Hall 2

Understanding the genetic control of root architecture holds untapped potential that could be harnessed for futurebreeding efforts. To date, only two genes controlling root architecture have been cloned: Pstol1 and Dro1 in O. sativa.Dro1 was identified in O. sativa landraces with steeper root angles and has been shown to increase yield under droughtconditions. Three orthologs of OsDro1 have been predicted in Zea mays, but their effect on root phenotype werepreviously unknown. Here we show that mutations in ZmDro orthologs lead to significant phenotypic differences,confirming they are involved in root growth in maize as well. In addition, since root architecture is highly variableacross the genus Zea, we hypothesized that ZmDro genes could play a role in modulating quantitative root phenotypes.We sequenced ZmDro orthologs across a diverse Zea panel including the NAM founder lines, a broad geographicsampling of landraces, and multiple teosinte species. We excavated roots of our panel at five weeks after germinationand used 2D imaging and feature extraction to quantify root architecture for comparisons with sequence- andexpression-level variation. Currently, we are exploring the potential connection between known phenotypic variation ofcrown root architecture and molecular level variation in the ZmDro orthologs using data from HapMap 3. Determiningthe link between genetic variation in ZmDro orthologs and root architecture across Zea will provide further insight intothe below-ground impacts of domestication and crop improvement and may ultimately help plant breeders select for anideal root architecture.

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Fine-scale dissection of a genetic locus governing cuticular hydrocarbon chain length on maize silks (0300-088)
Hall 2

The silk cuticular lipid metabolome includes at least 50 metabolites that are primarily linear hydrocarbons, fatty acids, and aldehydes ranging in chain lengths from 16 to 35 carbon atoms. To identify the genomic loci controlling the biosynthesis and accumulation of these metabolites, we performed metabolite-quantitative trait locus (mQTL) mapping across three years using the intermated B73xMo17 recombinant inbred line (IBMRIL) population. mQTL analysis of constituent traits, metabolite-class traits, and relative composition traits identified >0500 mQTLs that modulate the abundance and composition of the silk cuticular lipid metabolome, with a majority of mQTLs detected in more than one year. To connect this genetic network to the predicted biochemical network for surface lipid biosynthesis, identification of causal genetic polymorphisms or the ability to discriminate among competing candidate gene hypotheses is required. Here we report our progress in dissecting a 7 Mbp interval on chromosome 4 that accounts for 30% of the genetically explainable variance for each of 10 biochemical traits associated with hydrocarbon chain length, wherein the B73 allele increases the accumulation of hydrocarbons with chain lengths of 21 to 25 carbons. Two primary candidate genes residing within the locus encode a ketoacyl-CoA synthase involved in elongation of acyl-CoA molecules (hydrocarbon biosynthesis precursors) and a putative fatty acid reductase that converts the acyl-CoA molecules to aldehydes (hydrocarbon biosynthesis intermediates). Both isogenic dual testcross and heterozygous inbred family analyses were used to interrogate informative recombination events, narrowing the region of interest to 2 Mbp and 65 genes. Findings from transcriptomic analysis of silks in the parental lines was also used to evaluate the relative likelihood of multiple functional polymorphisms accounting for the observed effects on cuticular hydrocarbon carbon chain length.

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Functional analysis of a TDP domain and an SMAD/FHA domain in Tyrosyl-DNA phosphodiesterase 1 and th (0300-167 (Screen 2))
Hall 2

Tyrosyl-DNA phosphodiesterase 1 (TDP1) is a member of the phospholipase D (PLD) superfamily involved in phospholipid metabolism. TDP1 is a unique enzyme that hydrolyses the phosphodiester bond between the tyrosine residue of topoisomerase IB and 3’-phosphate of DNA in the repair of topoisomerase-

mediated DNA damage in eukaryotes. The gene encoding Tdp1 was first identified from a camptothecin (CPT) - sensitive Saccharomyces cerevisiae strain. Subsequently, it has been reported that a mutation in the human Tdp1 gene is involved in an autosomal recessive neurodegenerative syndrome, spinocerebellar ataxia with axonal neuropathy (SCAN1). In plants, the sequence alignment of TDP1 reveals a conserved C-terminal TDP domain and an N-terminal SMAD/FHA (forkhead-associated) domain, which is in contrast to those of yeast and human TDP1. AtTDP protein, as a homolog of human Tdp1, was identified in Arabidopsis thaliana. A loss-of-function AtTDP mutation leads to a dwarf phenotype due to reduced cell numbers caused by the accumulation of DNA damage and progressive cell death during Arabidopsis development. AtTDP protein and a truncated AtTDP mutant lacking the N-terminal FHA domain exhibit similar kinetic parameters. A basic amino acid sequence within the FHA domain of AtTDP protein is needed for nuclear localization. This report discusses the identification, characterization, and evolution of plant TDP1s is comparison to yeast and human TDP1.

Primary E-Poster Presenter: Hoyeun Kim

Genetic and Genomic Resources for Maize Transformation (0300-092)
Hall 2

Genome editing tools provide great potential for the elucidation of gene functions and crop improvements. In maize, however, current genetic and genomic resources as well as transformation capacity are insufficient for the full utilization of editing tools. We generated Nanopore long sequencing reads and BioNano physical mapping data to produce a reference-level genome assembly of a highly transformable maize inbred line A188. Genetic mapping using an F2 population of A188 and B73, an elite inbred line recalcitrant to callus culture or transformation, identified at least five genomic loci associated with callus culturability. In addition, hundreds of double haploids (DHs) and recombinant inbred lines (RILs) from the cross of A188 x B73 were generated, which will be used for validation of five association loci and selection for highly amenable maize lines with different combinations of the two genomes. Furthermore, using RNA sequencing data from diverse tissues of A188, including callus samples from multiple stages, we will construct gene regulatory networks to identify highly hierarchical regulators and hub genes that govern maize embryogenesis.

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Primary Poster Presenter: Sanzhen Liu

Heterosis in the allodiploid xBrassicoraphanus, a hybrid between Brassica rapa and Raphanus sativus (0300-081)
Hall 2

Heterosis is one of important biological phenomenon and its mechanism is remained to be elucidated. Intergeneric hybrid between Brassica rapa and Raphanus sativus has been introduced as an extreme example of heterosis. We generated synthetic hybrids from a cross between B. rapa cv. Chiifu and R. sativus cv. WK10039 both of them were used for reference genome sequencing for Chinese cabbage and big root radish, respectively. Heterosis phenotypes of these F1 hybrids were clearly observed as fresh weight, plant height, and maintenance of floral bud. On the other hand, flowering time of those hybrids were distributed as a normal segregating population spanning from the early flowering parent to the late flowering parent instead of homogeneous phenotype in usual F1 population. With advantages of clear heterosis phenotypes and genetic distance between parents, we performed transcriptome and metabolome analysis to understand the underlying mechanism of heterosis. Since the hybridized genomes are not allelic each other, classic models, including dominance, overdominance, and epistasis, only partly explain the heterosis in this organisms. Altered sugar metabolism was observed in terms transcriptome and metabolome. Differential expression of the primary and secondary metabolites related genes and altered metabolite profiles suggest breaking the balanced state of primary and secondary metabolism could be represented as heterosis.

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Primary Poster Presenter: Gibum Yi

Improving Fruit Quality of Tomato (0300-084)
Hall 2

Introgression lines from a cross between the cultivated tomato M82 cultivar and a wild relative, S. pennellii, have genes mostly from M82 but contain small pieces of DNA from S. pennellii. One introgression line, BIL 260, showed increased yield and fruit sugars with 1.5X more yield than M82, and a fruit BRIX of 4.9° compared to

3.8-4.0° in M82. Thus, the introgressed region of S. pennellii influences yield and BRIX in BIL 260. One of the genes originating from S. pennellii in BIL260 is a bHLH (basic Helix-Loop-Helix) transcription factor. The Arabidopsis homolog expresses in both flowers and leaves similar to the Tomato version, while the S. pennellii version is expressed at very low levels in flowers only. The coding region of this bHLH is the same between M82 and S. pennellii, and differences in the promoter region suggest that expression regulation of this bHLH could contribute to improved fruit BRIX. We identified CRISPR lines mutated at the bHLH gene in M82 and observed their phenotypes and genotypes. bHLH mutant lines had rounder leaves and lower leaf vasculature density similar to BIL260, but they had dramatic decrease in yield due to floral defects. Since bHLH is expressed mostly in flowers in S. pennellii, but in both flowers and leaves in M82, the mutation likely disrupted floral development in tomato which led to low fruit production. In future experiments, I plan to delete the promoter elements of bHLH that may be responsible for expression in leaves, while keeping expression in flowers and conduct transient expression assays in tobacco leaves. These experiments will help us identify what promoter elements are necessary for expression in leaves and allow modulation of this expression.

Primary Poster Presenter: Jennie Ahn

Intramolecular regulation of cell death signaling by mechanosensitive ion channel MSL10 (0300-095)
Hall 2

Programmed cell death (PCD) plays an important role in the development and stress responses of plants. We recently observed that PCD in Arabidopsis thaliana can be triggered by activation of the mechanosensitive ion channel MSL10, and we have been investigating the mechanisms by which this occurs. For example, we found that phosphorylation or a phospho-mimic version of MSL10's soluble N-terminus (MSL107D) prevents PCD, while a phospho-dead version of MSL10 (MSL107A) constitutively activates PCD. More recently, it was reported that the EMS-induced gain-of-function allele msl10-3G (rea1), which has a leucine substitution at Ser640 in the soluble C-terminus of MSL10, had dead lesions on its leaves (Zou et al, 2016). In unpublished data we have shown that the msl10-3G allele phenocopies phospho-dead MSL107A in size, hyperaccumulation of ROS, and gene expression profiles. In a phospho-mimetic (MSL107D) context, phenotypes associated with the S640L mutation including dwarfing, ectopic cell death, and H202 accumulation are suppressed, which genetically links the soluble domains of MSL10 together. These data suggest a model of MSL10 activation in which the C-terminus signals through the N-terminus, perhaps by promoting its dephosphorylation. Additionally, the distinct phenotype of msl10-3G plants were used in a suppressor screen to identify modulators of the MSL10-triggered PCD signaling pathway. I will describe new intragenic mutations that suppress the ability of msl10-3G to trigger cell death, and will discuss plans to characterize their impact on the activation of MSL10 signaling.

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Primary Poster Presenter: Jennette Shoots

Investigating oleosin dynamics during Arabidopsis thaliana development (0300-080)
Hall 2

Oilseed plants metabolize oil stored in seeds to provide fixed carbon for growth before the onset of photosynthesis. Seeds store fixed carbon in cytoplasmic oil bodies as triacylglycerol (TAG) surrounded by a protein-studded phospholipid monolayer. In Arabidopsis, the major seed oil-body proteins, oleosins (OLE1-5), are hairpin shaped and function to prevent oil body coalescence. During germination, oleosins are modified and degraded by the proteasome, and fatty acids released from oil body TAG are beta-oxidized by the peroxisome; both oil bodies and oleosin are fully degraded within a week of germination. However, the mechanistic details of oleosin modification and degradation are not fully understood. To identify genes involved in degradation of oleosin and/or oil bodies, we have mutagenized seeds expressing mNeonGreen-tagged OLE1 under its endogenous promoter and are screening for mutants with delayed oleosin degradation by identifying seedlings with prolonged fluorescence. We expect to recover new alleles of known mutants that slow oleosin degradation, including beta-oxidation and peroxin mutants. In addition, we expect to recover mutants defective in any proteins (Ub-protein ligases, retrotranslocation factors, etc.) specifically required for oleosin degradation. Further elucidation of the relationships between peroxisomes and oil bodies in Arabidopsis could lead to agricultural advancements such as increased seed oil content in crop plants, as well as insights into mammalian peroxisome-lipid droplet interactions.

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Metabolome-based genome-wide association study provides novel insights in cotton metabolism (0300-078)
Hall 2

Plants produce a variety of secondary metabolites to improve their fitness for survival under adverse environmental stresses. Here we report a non-targeted metabolome profiling of Gossypium hirsutum and a further metabolomic genome-wide association study based on ~2.6 million SNPs and more than 2,000 metabolite traits obtained from 292 diverse accessions. We identified 3,183 significant locus-trait associations ($p \leq 1*10\text{-}7$) across three environments. We functionally identified three candidate genes influencing metabolic traits. To test the relationship between content of metabolic and herbivory resistance, we have phenotyped the resistance level of diverse accessions of cotton to Aphis gossypii Glover and Adelphocoris suturalis Jackson. According to the correlation analysis result, we generated a

network that provides an insight into possible biological function of metabolites in resistance to biotic stress. Our study illustrates the power of combining non-targeted metabolomics with genome-wide association study in revealing the genetic basis of metabolic traits and detailed correlation analysis for uncovering metabolites with potential breeding value.

Primary Poster Presenter: HUAN SI

Morphological and Molecular Characterization of Mutants with Distorted Trichomes in Tomato (0300-091)
Hall 2

Trichomes are hair-like structures on the aerial surface of many plant species. Trichomes are well characterized for their function as physical and chemical defense against biotic and abiotic stresses. Despite the important roles of trichomes in plant defense, most of the genes for multicellular trichome formation including tomato remain unknown. To identify genes related to trichome development in tomato, we screened Micro-Tom mutant population generated by Ethylmethane sulfonate (EMS) mutagenesis and obtained four mutants with distinct trichome phenotypes compared with wild-type (WT) Micro-Tom plants. All mutants that we obtained have a similar trichome morphology with distorted and curled trichomes like previously known tomato mutants hairless (hl) and inquieta (ini). So we designated the new mutants as hl2, hl3, hl4, and hl5. Previously, we demonstrated that Hl and Ini genes are involved in the polymerization of actin cytoskeleton. Given similar trichome morphology among the new mutants, hl, and ini, we hypothesized that genes corresponding to the new four mutants are also related to actin polymerization. We performed reverse transcription (RT)-PCR from the new four mutant leaves using 16 specific primer set for actin-related protein (ARP)2/3 and WAVE complex genes which are involved in actin polymerization. All genes were amplified with expected size in the four mutants except for ARPC1 gene which showed different fragment size between WT and hl5 plants. cDNA sequencing of ARPC1 gene revealed that 82 bp was deleted in hl5 mutant. This result suggests that ARPC1 is hl5.

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Primary Poster Presenter: Jae-In Chun

Phenotypic Characterization of Fruit Quality Traits in a Mapping Population of Sweet Cherry. (0300-075)
Hall 2

Fruits quality parameters are essential for any new sweet cherry (Prunus avium L.) cultivars because they are directly related to consumer preference in important markets around the world, and economic profitability for the growers. It is for this reason, that determining the genetic components of these traits is a high priority for sweet cherry breeding programs. The present work aims to characterize a

segregating population phenotypically for fruit quality characters, in order to further identify QTLs and candidate genes related to these parameters in the segregating populations 'Bing X Lapins'. Towards this end, the following phenological traits were analysed: fruit equatorial width (cm), fruit weight (g), fruit firmness (durometer units), skin color, Flowering date and Maturity date (Calendar day "CD"). Phenotypic analyses of the mapping population from two seasons revealed significant segregation for quality traits such as: Diameter (Mean 2 cm and SD 0.46), Weight (Mean 6 g and SD 1.54), color (18% <2, 41% between 2-3 and 40% > 3), firmness (Mean 70 durometer units and SD 7.8), flowering date (Mean 265 CD and SD 4.5) , and maturity date (Mean 328 CD and SD 4.1). Further analyses of the segregating parameters in this population, should provide insight into the genetic regulation of important fruit quality parameters as well as molecular markers and associated candidate genes that may be incorporated into sweet cherry breeding programs.Keywords: Fruit quality, Ripening, QTLs, sweet cherry.Acknowledgments. This work was funded by the project Fondecyt No 1171016, Fondecyt No 1161377, the Conicyt Scholarship Grant ID: 21190238, CORFO PMG-cerezo 09PMG-7243 y 16PTEC-66646-P01.

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RNA-seq analysis in Southern Highbush Blueberry fruit reveals genes associated with 'crisp' texture (0300-089)
Hall 2

Texture is one of the most important breeding targets in Blueberry, as this trait has the potential to affect the commercially viable yield of a harvest, the postharvest quality of the fruit, and consumer satisfaction. A novel texture phenotype, identified as 'crisp' due to the crisp texture it imparts, has been identified in the UF Southern Highbush Blueberry (SHB) breeding program, and has been previously correlated with high fruit firmness. In this study, fruit from 384 germplasm generated by a 'crisp' x 'crisp' were evaluated both by sensory ('crisp') score and mechanical measures. From this sample five 'crisp' and five non-'crisp' accessions were chosen and further evaluated over a period of 3 years. Mechanical measures for both compression and bioyield force showed significant differences between the 'crisp' and non-'crisp' varieties. From these, two accessions, 236-37 and 236-84 were selected and used for RNA-seq to examine differential gene expression between the 'crisp' and non-'crisp' varieties in both pulp and peel. Results showed gene expression levels (as expressed in normalized read counts) for 206 genes in the peel and 156 genes in the pulp were significantly different ($P<0.05$) between the 'crisp' and non-'crisp' fruit. Annotations for 12 of these genes suggested their

association with the cell wall and cellular membranes, indicating that they are likely candidates for explaining firmness and "crispness".

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Sequence Polymorphisms Within DcOr are associated with Beta-Carotene Accumulation in Carrot (0300-076)
Hall 2

Carrot (Daucus carota) is one of the richest sources of the vitamin A precursor beta-carotene in the human diet. Two genes, Y and Y2 have been previously identified to be responsible for the majority of carotenoid accumulation in carrot roots. Y conditions all carotenoid accumulation in carrot roots, and one allele present in orange and yellow carrots harbors a 212 bp insertion in the gene. Y2 is known to condition the accumulation of alpha- and beta-carotene in carrot roots. The identity of Y2 is unknown, but Y2 has been fine-mapped to a 650-kb region on Chromosome 7. Recently, the Orange gene in carrot (DcOr) was identified in a genome-wide association study (GWAS) to also be significantly associated with carotenoid accumulation in roots. Molecular studies of Or in other plants, such as Arabidopsis, melon, and cauliflower have revealed mutations that result in increased sequestration of beta-carotene in tissues that are normally non-photosynthetic. Analysis of sequence polymorphisms within the CDS of DcOr found a SNP, which was nearly fixed in cultivated carrot, resulting in a substitution of a highly conserved Serine with a Leucine in OR. It is our hypothesis that during carrot domestication, a mutated DcOr allele was selected, alongside Y and Y2, for its unique ability to increase carotenoid accumulation in root tissue. Other mutations in DcOr are currently being investigated along with signatures of selection using a panel of wild and domesticated resequenced plant introductions (PIs) collected from different regions of the world. Additionally, patterns of Or expression are being analyzed in a mapping population of carrots identified to be fixed for Y and Y2 but still segregating for orange and yellow root color.

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The genetics of planting density-dependent branching in chrysanthemum (0300-090)
Hall 2

The architecture of chrysanthemum plants raised for cut flowers is strongly influenced by their ability to form branches. The genetic basis of branching was revealed through a quantitative trait locus (QTL) analysis of an F1 population generated by crossing the varieties 'Nannong Xuefeng' and 'Monalisa' grown under contrasting planting densities. Under the low planting density regime (E1), 12 additive QTL involving seven branching-associated traits were detected, while under the high planting density regime (E2), the number of QTL detected was only eight. One of the individual QTL accounted for over 10 % of the phenotypic variance. Of the 20 QTL, only one was expressed under both high and low planting density. A joint QTL analysis across the two environments identified two QTL which were separately detected in E1. A set of four QTL exhibiting additive × additive epistasis was identified, few of which had interaction with environments.

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Primary Poster Presenter: Wei Sun

Genetic diversity and population structure of Vaccinium floribundum in the Ecuadorian Highlands (0300-168 (Screen 12))

Mortiño (Vaccinium floribundum Kunth.), known as Andean blueberry, is a shrub belonging to the Ericaceae family. In Ecuador, this species is found in the paramos (a tundra-like ecosystem) between 1600 and 4200 masl. This plant is characterized by its nutritional and medicinal properties. The edible berries are used to prepare traditional food and the high content of polyphenols and anthocyanins have been shown to have antioxidant and anti-inflammatory effects. On a previous study, we reported the genetic diversity of mortiño found in three provinces from the northern Ecuadorian Highlands using 14 homologous microsatellites markers (SSR). However, due to the fragmentation of the ecosystem where these plants naturally grow, we expanded the area of study to have a better understanding of the genetic makeup of this species in the country. In the current study, we used 16 homologous microsatellite markers to analyze 100 samples from 27 localities distributed from the north to the south of the Ecuadorian Highlands. The expected heterozygosity (He=0.73) revealed a moderately high genetic diversity. Molecular variation analysis indicated that 70% of the genetic variability is found within populations and 30% among populations. Population structure results suggested four possible genetic clusters: the first composed of individuals from the north of the region, the second by individuals from the center, the third by individuals from the south, and the fourth by individuals from Quilotoa, in Cotopaxi and Azuay provinces. We propose possible hypotheses that could explain the genetic clusters found. This is the first study that comprehensively describes the genetic diversity and population

structure of the mortiño in Ecuador. The results obtained could serve as the basis for the development of conservation programs for an adequate management of this important biological resource, and for the conservation of the ecosystems in which the species is found.

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Primary E-Poster Presenter: Pamela Katherine Vega Polo

Genes & Genomes: Genome Editing

Combinatorial, pooled CRISPR knockout screens for plants (0300-169 (Screen 4))
Hall 2

Genetic screens with traditional mutagens such as EMS, fast neutrons, or T-DNA insertions have been used successfully for decades. However, these methods are limited by the time needed for self- and cross-pollinations, additional mutations caused by the mutagen and the laborious process of generating higher-order mutants. With the advance of CRISPR genome editing, the production of targeted gene knockouts is specific, scalable, and can be easily multiplexed. This has enabled us and others to perform in planta pooled CRISPR screens in tomato and rice. We are now actively developing and optimizing this technology, taking advantage of the multiplexing capability of CRISPR, to perform combinatorial knockout screens using Arabidopsis as a model system. This approach allows us to rapidly generate targeted mutant collections in practically any genotype or marker line of interest and potentially overcome genetic redundancy by targeting multiple members of a gene family or pathway. Here, we report the generation and use of a knockout mutant collection targeting ~20% of the proteases in Arabidopsis. For relatively small gene families (~20-40 genes) targeted by six gRNAs per vector, we can recover mutants in each individual gene and pairwise-gene combinations as well as a majority of the three-gene combinations with only a few hundred T1 plants. In the T1 and T2 generations, we consistently observed phenotypes such as leaf bleaching, dwarfism, seedling lethality and other developmental defects and rapidly associated these phenotypes with the incorporated gRNAs and mutated genes. Further refinements of this technology will utilize automated phenotyping platforms, generate even higher order combinations, and streamline the bioinformatics analysis.

Lightning Speaker: Thomas Jacobs

Genome editing strategies for rice improvement (0300-170 (Screen 12))
Hall 2

The precision and power of CRISPR/Cas-based genome editing has tremendous potential to accelerate crop improvement, especially if a few key bottlenecks can be addressed. These constraints include the need for better prediction of target genes and modifications, ways to overcome limitations in plant transformation and regeneration, and the need for non-transgenic approaches to assist in regulatory and consumer acceptance. Recently, Texas A&M AgriLife Research has supported the development of a Crop Genome Editing Lab at Texas A&M working towards optimizing a high-throughput gene editing pipeline and providing an efficient and cost-effective gene editing service for research and breeding groups. The lab is using rice as a model to test and optimize new approaches aimed towards overcoming current bottlenecks. For example, a wealth of genomics data from the rice community enables the development of novel approaches to predict which genes and target modifications may be most beneficial for crop improvement, taking advantage of known major genes, high-resolution genome-wide association mapping data, multiple high-quality reference genomes, and resequencing data from the 3,000 Rice Genomes Project. New analysis techniques can lead to numerous testable hypotheses with implications for rice improvement, which can then be rapidly validated using CRISPR editing techniques. Likewise, recent approaches demonstrating novel CRISPR delivery and regeneration methods can be tested to bypass the slow tissue culture process. Many of these approaches employ CRISPR/Cas9 protein + gRNA ribonucleoprotein complexes to provide a non-transgenic approach to genome editing, which will also help once edited products reach the commercialization stage. Thus, the vision of the lab is to use a newly optimized high-throughput gene editing pipeline for large-scale gene editing projects and to develop novel products for crop improvement.

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Primary E-Poster Presenter: Michael Thomson

1)Multiplex gNRA-CRISPR/Cas9 significantly improves genome editing efficiency in tetraploid alfalfa (0300-105)
Hall 2

Alfalfa (Medicago sativa) is a tetraploid legume with an obligate outcrossing reproductive system. Genome editing is more difficult in tetraploid species than diploids because of gene duplication resulting in a lack of complete gene knockout mutants. Recent advances in genome editing CRISPR/Cas9 has become a promising tool for efficient gene knockout in complex genomes. Despite the great achievement of CRISPR/Cas9 genome editing technology, the mutagenesis efficiency in alfalfa is still challenging. Our initial single gRNA-CRISPR/Cas9 genome

editing system in alfalfa led to very low mutagenesis efficiency with an obscure phenotype, suggesting the need for robust optimization. Thus, we assembled multiplex gRNA-CRISPR/Cas9 vector cassettes by a polycistronic tRNA-gRNA approach targeting stay-green gene in alfalfa. We tested three different CRISPR/Cas9 vector versions, namely 35S::Cas9, 35S::Cas9 with optimized gRNA scaffold and AtUbi::Cas9 as versions I, II and III, respectively. The genotyping analysis indicated that MsSGR mutagenesis efficiencies were 31%, 23% and 49% for vector versions I, II and III, respectively, four- to 10-fold higher than single gRNA efficiency. Among the three optimized multiplex gRNA-CRISPR/Cas9 vectors, version III (AtUbi::Cas9) showed the highest genotypic and phenotypic efficiency, with 49% and 50%, respectively. Interestingly, we have also obtained several tetra-allelic homozygous mutants with a stable strong phenotype, which indicates success in establishing a highly efficient multiplex gRNA-CRISPR/Cas9 genome editing system in alfalfa. Furthermore, the AtUbi::Cas9 CRISPR/Cas9 vector consistently exhibited superior knockout efficiency, with 44% to 75% in multiple alfalfa genes. The ability of current effective mutagenesis attained by a multiplex gRNA-CRISPR/Cas9 system in alfalfa could be an opportunity to target specific genome editing in such complex polyploidy plant genomes.

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Primary Poster Presenter: Tezera Watira

Antisense transcription and small RNA regulation of Arabidopsis Auxin Response Factor ARF12/22 gene (0300-104)
Hall 2

Auxin, being a master switch among all the phytohormones, regulates many plant growth and developmental processes. Auxin mediates its effect through various signaling pathways, specific to plant tissues/organs, which involves the participation of various plant-specific gene families like Auxin Response Factors (ARF), Aux-IAAs, etc. In Arabidopsis, ARF gene families comprise of 23 members in four clades differentiated in evolutionary terms by their being targeted by different microRNAs or small interfering RNAs (siRNAs). The ARF12/22 clade consists of seven highly homologous and tightly linked genes on chromosome1 expressed only in the female gametophyte and during embryogenesis. So far, the functions of ARF12/22 genes are not known and the prospects for genetic dissection limited by a lack of recombination between individual knock-out alleles. We are using genome editing (CRISPR-Cas9) approach to target ARF12/22 cluster and elucidate the roles of these genes in plant growth and development. Analogous to ARF10/16/17 clade of

genes, there exists a weak candidate miR160 binding site in the exon 8 of all the genes in the ARF12/22 cluster. Lending credence to the hypothesis that RNA interference is involved in ARF12/22 post-transcriptional regulation is the existence of a natural-cis-antisense transcript for ARF14 and sense and antisense siRNAs unique within, and conserved between, ARF12/22 paralogs clustered around the cryptic miR160 site. We have recent evidence from tobacco consistent with the hypothesis. We propose that ARF12/22 gene cluster could function as a 'target mimic' for miR160; preventing post-transcriptional gene silencing of other bona fide miR160 targets like ARF10, ARF16, and ARF17, thus regulating the developmental processes in plants. The significance of this work is a better mechanistic understanding of gene silencing associated with smRNAs and potential functions of ARF12/22 gene cluster in embryogenesis.

Co-author(s): Sunitha Sukumaran

Primary Poster Presenter: Pranav Dawar

CRISPR/Cas9-mediated knock-out of genes related to abiotic stress tolerance in alfalfa

Alfalfa (Medicago sativa L.) is Canada’s most widely grown perennial forage legume, with an estimated cropping area of approximately 4M Ha. The demand for ruminant products such as meat and milk is expected to grow substantially in coming years due to our ever-expanding population, and therefore high levels of forage crop production will be a necessity. However, adverse environmental conditions, which are expected to escalate in both their severity and frequency due to climate change effects, often have a severe negative impact on alfalfa production. Since the global demand for livestock is predicted to escalate in line with our population, and the land base for forage production is decreasing, there is an urgent need to exploit accelerated molecular breeding technologies in this species with the aim of developing alfalfa cultivars with improved resiliency to various types of abiotic stress in a timely manner. It has been shown previously that the down-regulation of several miRNA156 targets, including SPL8 and WD40-2, leads to an enhancement of drought and/or salinity tolerance in alfalfa. However, despite their potential economic benefit, public concern and regulatory constraints surrounding the use of transgenic crops can be problematic for implementing such germplasm. Genome editing based on CRISPR/Cas9 provides a tool that yields germplasm bearing a mutation that is virtually identical to that achieved using conventional breeding approaches such as chemical mutagenesis, which could mitigate these issues. While CRISPR/Cas9 has been shown to be effective in alfalfa previously, the efficiency of editing was very low. Therefore, the aim of this project is to target the M. sativa SPL8 and WD40-2 genes using CRISPR/Cas9 technology in an attempt to increase editing efficiencies, and to concomitantly develop novel germplasm for the downstream breeding of abiotic stress resilient alfalfa cultivars.

Chair and Concurrent Symposium Speaker: Surya Acharya,
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Primary Poster Presenter: Stacy Singer

CRISPR-Based Mutagenesis and Gene Knock-In in Protoplasts of Physalis pruinosa (0300-098)
Hall 2

CRISPR/Cas9 has enabled quick and precise generation of mutational gene knockouts. Delivery of CRISPR/Cas9 editing components into plant cells is routinely conducted by Agrobacterium tumefaciens-mediated transformation. This delivery method has substantial drawbacks in that it is relatively low-throughput and requires genomic integration of the Cas9 construct. For experiments that often result in low efficiency, such as promoter modification and gene knock-in, high throughput methods are necessary. Protoplast transformation is a suitable alternative for achieving high-throughput mutagenesis, and the large number of transformed protoplasts can circumvent the low efficiency of gene knock-in. We are developing a protoplast gene editing method for Physalis pruinosa, a semi-domesticated member of the Solanaceae family.First, we optimized protoplast isolation from Physalis pruinosa seedlings, and developed a quick, reliable method to screen gRNA efficiency using PEG-mediated transformation of a plasmid containing Cas9 and gRNA. After transformation, protoplasts were cultured in a liquid medium and microcallus formation was observed within 14 days. Microcalli were transferred to solidified medium, and formed larger callus within 30 days. In addition to CRISPR mutagenesis, we are improving gene knock-in methods. Though numerous studies have succeeded in gene knock-in using homology arms (HAs) of various lengths, nearly all attempts have yielded prohibitively low efficiency. Furthermore, the most efficient HA length for gene knock-in is unclear. By using progressively larger homology arms, we are attempting to determine the optimal HA length in order to achieve knock-in of short sequences. Initial results will be presented based on a 2-codon substitution in the coding sequence of green fluorescent protein, and a single-codon substitution in a native gene homologous to the brix mutation in Solanum lycopersicum.

Co-author(s): Linnell Randall,
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Primary Poster Presenter: Nathan Reem

Development of male sterile tomatoes by CRISPR/Cas9 genome editing system (0300-102)
Hall 2

The demand for tomato is steadily increasing worldwide, but production of tomato hybrid(F1) seeds through emasculation and cross-pollination is less economical as production costs are higher than sales costs. In this study, to improve efficiency of hybrid seed production and protect breeding materials, we try to produce various tomato male sterile materials using CRISPR/Cas9 systems. Through reports based on the transcriptome of spontaneous male-sterile mutant (ms1035) and male sterility in Arabidopsis and rice, twelve putative genes associated with ms were selected. Each guide RNA targeting putative ms genes was designed and fused into Two-genes-target CRISPR vector(pAGM4723, Addgene) which could edit two target genes simultaneously. And then, the tomato M82 was transformed by Agrobacterium tumefaciens EHA105 including the final CRISPR/Cas9 binary vector. Three months later, the regenerated T0 seedlings were transferred to soil and were grown in a greenhouse with 16/8 light/dark cycle at 24-28℃. Independent transgenic lines were confirmed by PCR analysis of NPTII gene and sanger-sequencing. Among 177 regenerated T0 plants, total 21 lines were edited, showing chaotic peaks after the target site. No differences in shape and size of flower organ between edited lines and WT were observed, but the pollen production of the edited lines has decreased. Pollen germination test of several T0 lines showed that germination rate of pollen was reduced in edited lines. Five T1 progeny plant lines are being analyzed to determine the mutations in the target site and observe male sterile phenotype.

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Primary Poster Presenter: JIHEE PARK

DNA-free genome editing via CRISPR-Cas9 Ribonucleoprotein complexes in potato plant (0300-100)
Hall 2

Potato (Solanum tuberosum L.) is the third most important food crop in the world and one of its key research goals is to breed resistant varieties to plant pathogens, which is a major threat to commercial potato production worldwide. Plant pathogens transmit effectors to plant cells to inhibit immune function. While many effectors inactivate immune regulators to inhibit immunity, some effectors play a role as negative regulators of plant immunity, positively affecting plant pathogen infection. Genes that act as negative regulators in the plant immune system are called susceptibility (S) genes. The clustered regularly interspaced short palindromic repeat -associated protein 9 genome editing system (CRISPR- Cas9) derived from the type II prokaryotic adaptive immune system is widely used for accurate genomic editing due to its ease of use and wide programmability. It is known as a simpler and more efficient editing tool than other programmable

nucleases such as ZFN and TALEN. The CRISPR-Cas system can be delivered to live cells via plasmid DNA, RNA and ribonucleoprotein (RNP). In particular, transcription through RNP can induce a decrease in off-target effect due to the transient expression of an enzymatic action with a short activation period. In addition, it is one of the ways to solve the GMO problem in plants because it can eliminate the possibility of insertion of recombinant DNA into the host plant genome. However, genome editing using protoplast-derived RNP method in plants has been reported to be induced with low efficiency. We set the appropriate conditions from protoplast extraction to regeneration in potato plants and induced regeneration at high efficiency. In addition, for the development of disease resistance potato, the susceptibility gene was induced by genomic editing using the RNP method and the indel-induced potato plants were obtained with a high-efficiency of over 30%.

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Primary Poster Presenter: Ki-Beom Moon

First generation genome editing in potato using hairy root transformation (0300-096)
Hall 2

Genetic transformation has become a bottle-neck for genome editing and cis-gene breeding in crop species. Hairy root transformation using Agrobacterium rhizogenes provides a rapid method to generate transgenic hairy root clones which express genome editing reagents, such as CRISPR/Cas and carry targeted mutations or other modifications. Regeneration protocols have been developed in a diploid, species of potato (2n = 2x = 24) which allows whole plants to be regenerated from individual transgenic hairy root clones that carry germline targeted mutations identified in the original hairy root clone with minimal effects of chimerism in a single generation. This novel approach to genetic transformation can accelerate generation and screening of useful modifications and creation of multiple whole plant events harboring similar germline modifications.

Primary Poster Presenter: Nathaniel Butler

Functional analysis of OVATE FAMILY PROTEINS (OFPs) in fruit shape formation in strawberry using CRI (0300-103)
Hall 2

Plant development is the process in which cell division in meristem provides new cells for expansion and differentiation of tissue to generate new organs. Genome-wide analyses of the downstream targets for SHOOTMERISTEMLESS (STM), which is required for the maintenance of the shoot apical meristem (SAM) in Arabidopsis,

have identified the OFP gene family. Members of the OFP gene family were found that involved in meristem differentiation in Arabidopsis and in fruit shape development in tomato. In strawberry, homologs of STM and OFP are abundant in receptacle during the later stages of fruit development. To further characterize the function of OFP, we are using the CRISPR/Cas9 genome editing system to knockout multiple endogenous OFP genes by both transient and stable transformation in strawberry. Phenotypic characterization of the gain-of-function and loss-of-function mutant lines as well as the genetic interaction between OFPs and STM would provide valuable information for understanding the function of proteins that are involved in the organ development. Our study using CRISPR/Cas9 technology also provides an incredibly useful tool for functional analysis of genes in plants and offer opportunities for improving the desirable traits in strawberry.

Co-author(s): Tie Liu

Primary Poster Presenter: Mingxi Zhou

Gene editing through de novo induction of meristems on seedlings (0300-107)

Hall 2

Plant gene editing begins by delivering gene editing (GE) reagents to somatic plant cells in culture, using either the gene-transferring bacterium, Agrobacterium tumefaciens, or physical means such as particle bombardment. Edited cells are then induced to differentiate into whole plants by exposure to various combinations of plant hormones, namely auxin and cytokinin. Regeneration of plants through tissue culture is not ideal for large-scale, high-throughput production of gene edited plants. The process is often inefficient, requires considerable time, works with limited genotypes, and causes unintended changes to the genome and epigenome. Methods that circumvent these limitations would greatly enhance the ability to create edited plant lines. By editing the stem cells within plant meristems, all tissues derived from the meristem would be expected to contain GE events of interest, leading to vertical transmission. However, direct modification of existing meristematic tissue has proven challenging as it has been historically recalcitrant to genetic modification. Combinations of developmental regulators like WUSCHEL (WUS) and SHOOT MERISTEMLESS (STM), amongst others, have been implicated in the patterning and formation of shoot meristems. Co-opting these types of patterning regulators, a new meristem can be generated from transformed somatic tissues. Using our method of fast treated Agrobacterium co-culture (Fast-TrACC), various combinations of WUS, STM and ISOPENTENYL TRANSFERASE (IPT) were found to facilitate de novo meristem generation in the model species Nicotiana benthamiana. Combining these developmental regulators with GE reagents provides the potential to establish an edited shoot directly from somatic tissue in order to avoid tissue culture. In this regard, de novo meristem induction promises to alleviate the tissue culture bottleneck, allowing for larger collections of genetically engineered germplasm to be assembled to solve agricultural problems.

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Primary Poster Presenter: Ryan Nasti

Gene-editing in Arabidopsis thaliana using a novel Cas12a ortholog (0300-106)
Hall 2

Gene-editing in plants such as Arabidopsis thaliana using CRISPR-based endonucleases such as Cas9 is currently limited by the requirement of large constructs for multiplex targeting as well as inflexible PAM motifs. Here, we assess the activity of a novel Cas12a (Cpf1) variant in A. thaliana, Mb3Cas12a, and demonstrate editing at both canonical (TTTV) and non-canonical PAM sequences (VTTV and CTV). Additionally, using a single crRNA array, we demonstrate simultaneous gene editing of multiple genes within a single generation. The ease of multiplex-gene editing, coupled with relaxed PAM recognition, establish Mb3Cas12a-mediated genome editing as an attractive alternative to Cas9-based editing systems in plants.

Co-author(s): Robert Schmitz

Primary Poster Presenter: William Jordan

H2O2-based method for rapid detection of transgene-free CRISPR/Cas9 genome-edited plants in rice (0300-099)
Hall 2

Genome-editing (GE) techniques such as CRISPR/Cas9 have been widely used in crop functional genomics and improvement. To efficiently deliver the guide RNA and Cas9, most studies still rely on Agrobacterium-mediated transformation, which involves a selection marker gene. However, several limiting factors may impede the efficiency of screening transgene-free genome-edited plants, including the time needed to produce each life cycle, the response of selection reagents, and the labour costs of PCR-based genotyping. To overcome these disadvantages, we developed a simple and high-throughput method based on visual detection of antibiotics-derived H2O2 to verify transgene-free genome-edited plants. In transgenic rice containing hygromycin phosphotransferase (HPT), H2O2 content did not change in the presence of hygromycin B (HyB). In contrast, in transgenic-free rice plants with 10-h HyB treatment, levels of H2O2 and malondialdehyde, indicators of oxidative stress, were elevated. Detection of H2O2 by 3,3’-diaminobenzidine (DAB) staining suggested that H2O2 could be a marker efficiently distinguishing transgenic and non-transgenic plants. Analysis of 24 segregating progenies of an HPT-containing rice plant by RT-PCR and DAB staining verified that DAB staining is a feasible method for detecting transformants and non-transformants. Detection of genome-edited plants showed that transgene-free genome-edited plants were faithfully validated both by PCR and the H2O2-based

method. Moreover, HyB induced overproduction of H2O2 in leaves of Arabidopsis, maize, tobacco, and tomato, which suggests the potential application of the DAB method for detecting transgenic events containing HPT in a wide range of plant species. Thus, visual detection of DAB provides a simple, cheap and reliable way to efficiently identify transgene-free GE and HPT-containing transgenic rice.

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Primary Poster Presenter: Chwan-Yang Hong

Improvement of Gene Delivery and Mutation Frequency in CRISPR-Cas9 Wheat Genomics via Biolistics

The discovery of the CRISPR-Cas9 gene editing system has revolutionized the field of plant genomics. Compared to previous technologies, CRISPR-Cas9 allows for direct and site-specific gene edits for extensive improvements in crops like wheat. Wheat is a moderately recalcitrant species for tissue culture and genetic modification. It is utilized as a protein and carbohydrate source more than any other crop in developing countries. As global wheat consumption increases, it is important to focus on its advancement through genome editing. Despite the advantages of ease in designing gRNA and the relative low cost of the CRISPR-Cas9 system, there are still hurdles to overcome in low mutation frequencies, specifically in hexaploid bread wheat. Currently, wheat genomics projects require a high output of T0 transformants in order to generate the desired mutations. In conjunction with gene delivery and transformation efficiency, the mutation rate bottleneck has the potential to slow down advancements in genome editing of wheat. In this study, we report our findings of efforts to increase gene mutation rates in the wheat cultivar Fielder in order to make CRISPR-Cas9 a more direct and stable gene editing system via biolistics.

Chair and Concurrent Symposium Speaker: Jaclyn Tanaka,
Snigdha Poddar,
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Primary Poster Presenter: Myeong-Je Cho

Modifying Bean Lectin to Improve Digestibility (0300-108)
Hall 2

Beans are a staple food in lesser-developed countries. They are a great source of complex carbohydrates, nutrients, vitamins, minerals, and proteins. Lectins are an abundant bean storage protein. They are carbohydrate-binding proteins involved in defending plants against insects and pathogens. When beans are uncooked or

undercooked and consumed, lectins bind to carbohydrates in the gut surface. The glycoconjugates accumulate forming a coating on the digestive tract preventing proper absorption of nutrients. The goal of our research is to reduce the anti-nutritional properties of lectins by modifying glycolysation sites throughout the protein. We used the bean Phaseolus vulgaris var Negro Jamapa in our experiments. We focused our studies on the E subunit of phytoheamagglutinin (PHA-E). Using BLASTX we found three potential N-glycosylation sites in the PHA-E sequence. Plasmids Asn-22-Ser, Asn-103-Ile, and Asn-134-Ser were made utilizing site-directed mutagenesis to modify a glycolysation site (Asn-X-Thr/Ser). Asn-22-Ser, Asn-103-Ile, Asn-134-Ser were digested, the fragments separated by gel electrophoresis, and purified using a gel purification kit. The purified modified lectin sequence was ligated into a binary vector. The reconstructed plasmid was electroporated into Agrobacterium tumefaciens and colony PCR was used to verify positive colonies. A verified Agrobacterium colony for each reconstructed plasmid was used to innoculate a two-week-old Negro Jamapa. T0 generation seeds were analyzed for size. We have planted the T0 seeds and our next steps are to test gDNA for mCherry using PCR. We believe our approach to modifying one glycolysation site at a time will increase the digestibility of the bean without inhibiting the defensive qualities of the lectin. JH was supported by NIGMS T34 08395 to MEZ.

Co-author(s): MariaElena Zavala

Primary Poster Presenter: Jesus Hernandez

Optimizing Soybean DGAT1 for increasing oil via CRISPR-editing (0300-097)
Hall 2

Androgenesis via anther culture is the efficient system for producing haploid and doubled haploid (DH) plants in maize. Especially maize anther culture is the most promising system to speed up the maize breeding program. In general, the anther culture is affected by the primary factors: genotype, environmental element, stages of anther development, and anther culture medium conditions for the callus induction and plantlets in maize. In this study, various genotypes (Korean maize, tropical and temperate inbred lines) were used for embryogenic callus induction and plant regeneration. And also we tested using the early stage of anther development as explants. After 3~4 weeks on modified callus induction medium, embryogenic calli were showed. The highest frequencies of embryogenic callus and plant regeneration were obtained when the immature anthers at the early stages were used. The optimum anther length for embryogenic callus formation and plantlets efficiency was at 0.5~1 mm. This study suggested that optimum maize anther length and genotypes in the anther culture were useful for producing enough haploid or doubled haploid plantlets in maize breeding.

Primary Poster Presenter: Laura Wayne

Polyphenol oxidase (PPO) in lettuce: a genome modification target using CRISPR/Cas9 system (0300-101)
Hall 2

In lettuce (Lactuca sativa L.) one of the principal problems is its susceptibility to enzymatic browning, with polyphenol oxidase (PPO) activity being one of the main responsible. Polyphenol oxidase is an enzyme encoded in the nucleus that is subsequently transported to the plastids. PPO are usually located in the thylakoid membrane of chloroplasts and in non-green plastid vesicles. Otherwise, the phenolic substrate is located in the vacuole. Subsequent to the damage generated, browning occurs because these subcellular compartments are lost, generating the oxidation of phenolic compounds to quinones. Here we are using CRISPR/Cas9 technology to get a new variety anti-browning phenotype through the edition of PPO gene. The cloning of PPO from lettuce cv. Salinas 88 was performed successfully and the deduced peptide showed molecular characteristics corresponding to PPOs from other plant species. Three single guide RNA (SgRNA) were designed in order to generate an editing and consequently a loss of function of PPO gene. We perform a stable transformation of cotyledons from lettuce cv. Salinas 88 Agrobacterium-mediated using the CRISPR/Cas9 constructs. In addition to contribute in the characterization of PPO gene in lettuce, we perform a subcellular localization experiment. To evaluate this enzyme localization, tobacco leaves were transiently-transformed with an expression vector and analyzed using confocal microscopy.Acknowledgements: Funding FONDECYT postdoctoral project Nº3170674

Co-author(s): Patricio Arce-Johnson

Primary Poster Presenter: Daniella Utz

Genes & Genomes: Molecular Evolution/Comparative Genomics

Characterization of BTB E3 Ubiquitin-Ligase Gene Families in Viridiplantae (0300-171 (Screen 9))
Hall 2

Ubiquitylation, the attachment of ubiquitin to proteins to mark them for degradation by proteasomes, is crucial for proper organism function. One family of complexes that play a role in this process are the BTB/Cullin 3/RBX E3 ubiquitin-protein ligases, which catalyze attachment of ubiquitin to target proteins. The BTB (Bric-a-Brac, Tramtrack, Broad Complex) domain-containing proteins are the target-adapters, binding to the proteins to be ubiquitylated via motifs appended to the BTB domain. Genes encoding BTB proteins have been identified in wide range of eukaryotic organisms (including fungi, protists, animals, and plants) but the BTB gene families in different organisms show great variability in size and composition. In land plant genomes thus far studied BTB gene families are large (~75-150

members) and complicated (with multiple BTB subtypes encoded based on the presence of a diverse set of putative target-binding motifs). We are interested in when the BTB family composition seen in the higher plants may have arisen. To help answer this question we have identified the complete BTB families in 21 representative species in the Viridiplantae clade (including land plants and algae). Our analyses thus far have shown that while the size of the BTB families in land plant genomes vary significantly (~40 to more than 200 members), they all share a similar set of BTB types (as defined by domain organization). The chlorophyte algal genomes we have analyzed encode a distinctly different set of BTB types, but interestingly the one charophyte genome analyzed (Klebsormidium nitens NIES-2145) encodes several BTB types otherwise only seen in land plants. Collectively these data show that there have been dramatic changes in both the size and composition of this E3 ubiquitin-ligase gene family during Viridiplantae evolution and suggest that the BTB types seen in land plants had already begun to emerge in the charophyte ancestors.

Co-author(s): Zachary Jacobson

Primary E-Poster Presenter: Derek Gingerich

A Genome-Wide Association Study: Searching for Candidate Genes in Wild Soybean for Crop Improvement (0300-124)
Hall 2

Cultivated soybean, Glycine max (G. max), is an important crop consumed as a plant-based protein for humans and livestock worldwide. G. max was derived from wild soybean Glycine soja (G. soja) through domestication events which occurred in Central China 6,000-9,000 years ago. It is thought that several bottleneck events, owing to artificial selection, have greatly decreased the genetic variation in our current domesticated crop. G. soja genomes can shed light on the evolutionary history between the two species and the changes that have occurred in gene function and phenotype, and provide genetic resources for trait improvement of G. max. Aiming at identification of genes and polymorphisms responsible for phenotypic differences within the G. soja population, we conducted a Genome Wide Association Study (GWAS). Using a panel of 192 G. soja accessions widely distributed in China, South Korea, Japan and Russia, we conducted phenotypic measurements of important agronomic traits including plant height, flowering time, seed filling time, and the time it takes for seed pods to mature at 20 °C and 30 °C. We obtained about 180K single nucleotide polymorphisms (SNPs) segregating in the panel using a Genotype By Sequencing approach by aligning a total of 380M reads to the G. max reference genome. GWAS analysis was carried out using a mixed linear model in TASSEL to calculate association between SNPs and phenotype data. Candidate genes of significant association were identified, including Glyma.20g144400 that showed a strong association with flowering at 20 °C, and Glyma.11G105300-Glyma.11G105500 that were prime candidates for flowering at 30 °C. Characterization of expression and function of these genes are underway.

Genes and polymorphisms identified in this study will aid in understanding early domestication processes, traits that may have been artificial selected for during domestication events, and may be beneficial for improvement of soybean cultivars.

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Eric Sedivy

Primary Poster Presenter: Angela vela

A rice beta-amylase gene appears to encode a nuclear transcription factor and a chloroplast enzyme (0300-112)
Hall 2

In most plants, starch accumulates in plastids during the day to provide the carbon and energy necessary to sustain metabolism at night. Starch hydrolysis is catalyzed by members of the β-amylase (BAM) family, which in Arabidopsis thaliana includes nine structurally and functionally diverse members; this work will focus on BAM2 and BAM7. AtBAM2 is a plastid-localized enzyme that is only active in the presence of KCl, and it is a tetramer that exhibits a sigmoidal substrate saturation curve with a Hill coefficient of over 3. Phenotypic analysis of T-DNA mutant plants suggests that AtBAM2 does not play a significant role in leaf starch degradation. AtBAM7 is a catalytically inactive, nuclear-localized transcription factor with an N-terminal BZR1-like domain. Sequence alignments show that the BAM domains of AtBAM7 and AtBAM2 are closely related. Analysis of the genomes of 46 flowering plants revealed 12, including Amborella; some monocots; and basal eudicots, that have a BAM7-like gene but lack a BAM2-like gene. Upon closer inspection, the BAM domains of these BAM7 genes are more similar to AtBAM2 than they are to the BAM domain of AtBAM7. They also share all of the functional residues that we identified in AtBAM2, some of which are different in AtBAM7. Moreover, an in-frame methionine near the 3’ end of the first intron of these BAM7 genes is the putative N-terminus of BAM2-like proteins that begin with predicted chloroplast transit peptides. We hypothesize that these genes contain two transcriptional start sites that drive the expression of two functionally different proteins. Using rice as a model, we designed cDNAs of the long and short forms of rice BAM7 (OsBAM7) for expression in E. coli, and preliminary evidence suggests that the purified short form of OsBAM7 is catalytically active and shares properties with AtBAM2 such as KCl sensitivity.

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Primary Poster Presenter: Claire Ravenburg

Characterization of the auxin conjugate amidohydrolase orthologue family from several hornwort speci (0300-111)
Hall 2

We have previously characterized a liverwort auxin conjugate hydrolase (MpILR1) that appears to be the molecular ancestor for tracheophyte M20D peptidases. Our current studies focus on the most recently evolved bryophytes, i.e. the hornworts, that emerged about 25 million years after liverwort. We have identified five auxin conjugate amidohydrolases in four different species (Phaeoceros carolinianus, Megaceros tosanus, Megaceros vincentianus, and Paraphymatoceros hallii). The genes for two of these enzymes have been cloned and characterized from P. carolinianus (PcILR1, PcILR2), and we are presently investigating a third from the species P. hallii (PhILR). Sequence analysis suggests that all five enzymes have greater than 60% similarity to tracheophyte amidohydrolases, while phylogenetic analysis supports the hypothesis that the bryophyte and tracheophyte hydrolases are derived from a common ancestor. PcILR1 and PcILR2 (98% homology) appear to be the result of a relatively recent gene duplication in P. carolinianus. Enzyme studies of PcILR1 and PcILR2 suggest an increase in activity and substrate recognition above that demonstrated by the more ancient MpILR1, although no mean hydrolytic activity was observed to be as great as any tracheophyte hydrolase previously studied. We hypothesize that the increased activity in the hornwort hydrolases may be due to a change in the residue at Amino Acid 244. Two of the hornwort orthologues (MvILR and MtILR) have a Glycine replacing the Serine244 that is conserved in tracheophytes, while PcILR1/2 and PhILR have the same residue replaced by an Alanine. These Glycine and Alanine residues may represent an intermediate evolutionary state with a less hydrophobic amino acid in place of the highly hydrophobic Leucine244 in MpILR1 and the polar Serine244 in tracheophytes. Continued enzymatic studies of the other orthologues may suggest whether or not that hypothesis is supported.

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Cis-regulatory mutation responsible for flower color variation in Phlox drummondii (0300-120)
Hall 2

In numerous systems, mutations in cis-regulatory elements, including promoters and enhancers, have been shown to cause significant phenotypic changes by altering gene expression. Recently, cis-regulatory mutations have garnered support as important contributors to morphological evolution and population divergence, but there are few examples that link genotype to phenotype in natural systems. Phlox drummondii, a well-established system for reinforcement speciation, exhibits light-blue flowers in allopatry and dark-red flowers in sympatry with a closely-related species. Previous research has shown that flower color variation in P. drummondii is caused by cis-regulatory differences at two independently assorting loci that function in the anthocyanin biosynthesis pathway. In this study, we aimed to determine the location of functional variation in the promoter region of the F3’5’h

gene that is responsible for flower hue. We performed agrobacterium-mediated transient transformations with four lengths of promoter-reporter constructs to test gene expression within P. drummondii petal tissue and to determine where the causal promoter mutation lies. This study will aid in understanding the cis-regulated evolutionary mechanisms behind P. drummonii's floral variation and in comparing molecular evolutionary mechanisms within the angiosperm clade.

Primary Poster Presenter: Bridget Bickner

Comparative analysis of ABA-dependent gene regulatory networks across the Brassicaceae reveal innova (0300-127)
Hall 2

Plants are exposed to a wide range of abiotic stresses in the environment including drought, freezing, and soil salinity. Over time, some plants adapt strategies to these stressors. A common type of stress tolerant plant is a halophyte, which lives along ocean shorelines or near hypersaline lakes. Currently, the gene regulatory networks that underlie a halophyte's adaptation to stress is still unknown. Studies in stress-sensitive plants like domesticated crops and Arabidopsis have identified the plant hormone ABA (abscisic acid) as a major regulator of plant stress response. Upon the onset of stress, transcription factors downstream of ABA signaling, known as ABRE binding factors (ABFs), activate stress-response genes by binding to cis-regulatory elements known as ABA response elements (ABREs). While components of the ABA-mediated gene-regulatory network have been established in stress-sensitive plants, the regulatory map may look different in the halophytes. Using root growth assays, I found that halophytes are less sensitive to the growth inhibitory effects of ABA than glycophytes. To identify molecular level differences that mediate growth regulation under stress, I used RNA-Seq and DNA affinity purification (DAP-Seq) and found that halophytes differ in the spatial and temporal expression of genes upon ABA treatment. Some of the halophyte specific genes are associated with previously identified stress tolerance pathways in glycophytes such as cell wall modification and toxin catabolism. Additionally, the expression pattern differences are partially mediated by changes in the ABF binding landscape, identified using DAP-Seq. These findings suggest a transcriptional rewiring of ABA-mediated gene-regulatory networks in the halophytes that may greatly contribute to their enhanced tolerance to environmental stress.

Primary Poster Presenter: Ying Sun

Comparative genomics of rice with contrasting photosynthesis and grain production under salt stress (0300-109)
Hall 2

Unfavourable environmental conditions, including soil salinity, lead to decreased rice (Oryza sativa L.) productivity, especially at the reproductive stage. In this study, we examined 30 rice varieties, which revealed significant differences in the photosynthetic performance responses under salt stress conditions during the

reproductive stage, which ultimately affected yield components after recovery. In rice with a correlation between net photosynthetic rate (PN) and internal CO2 concentration (Ci) under salt stress, PN was found to be negatively correlated with filled grain number after recovery. Applying stringent criteria, we identified 130,317 SNPs and 15,396 InDels between two "high-yield rice" varieties and two "low-yield rice" varieties with contrasting photosynthesis and grain yield characteristics. A total of 2,089 genes containing high- and moderate-impact SNPs or InDels were evaluated by gene ontology (GO) enrichment analysis, resulting in over-represented terms in apoptotic process and kinase activity. Among these genes, 250 were highly expressed in reproductive tissues, and most were annotated as receptor-like protein kinases. These findings highlight the importance of variations in signalling components in the genome and these loci can serve as potential genes in rice breeding to produce a variety with salt avoidance that leads to increased yield in saline soil.

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Comprehensive analysis of mitogen-activated protein kinase cascades in chrysanthemum (0300-118)
Hall 2

Mitogen-activated protein kinase (MAPK) cascades, an important type of pathway in eukaryotic signaling networks, play a key role in plant defense responses, growth and development. We characterized 6 MKK genes and 11 MPK genes in chrysanthemum based on transcriptomic sequences. Phylogenetic analysis and conserved motif analysis of the MKK and MPK families in Arabidopsis thaliana, Helianthus annuus and Chrysanthemum morifolium were performed, and classified these genes into four groups. qRT-PCR analysis demonstrated that CmMKKs and CmMPKs exhibited various expression patterns in different organs of chrysanthemum and in response to abiotic stresses and phytohormone treatments. Furthermore, a yeast two-hybrid assay was applied to analyze the interaction between CmMKKs and CmMPKs and reveal the MAPK cascades in chrysanthemum. Our data led us to propose that CmMKK4-CmMPK13 and CmMKK2-CmMPK4 may be involved in regulating salt resistance and in the relationship between CmMKK9-CmMPK6 and temperature stress.

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Discovering the genes that are involved in postharvest senescence in broccoli (Brassica oleracea) (0300-110)
Hall 2

The plant hormone abscisic acid (ABA) plays an important role in many processes related to survival in stressful environments. ABA can inhibit formation and growth of leaves and is involved in stress induced senescence. We have recently identified a transcription factor ABIG1 that is necessary for stress-induced senescence in Arabidopsis (Liu et al, eLife, 2016). We have identified a number of genes that involved in this pathway by using systems biology and quantitative genetic approaches. We are unveiling the regulatory networks through which ABIG1 act to control growth in plants in response to stresses. ¬-Additionally, the cultivated Brassica species, which are the group of crops most closely related to Arabidopsis. Previous comparative research between Brassica oleracea and Arabidopsis thaliana genome identified numerous one-to-one segmental relationships and genome duplications. This is particularly interesting because little is known about the molecular mechanism of postharvest senescence in Brassica species including broccoli, cabbage, cauliflower. To carefully characterize the effects of environmental stresses on postharvest broccoli, we are working on identifying candidate transcripts, related proteins and metabolic compounds that accurately reflect the physiological age or freshness of broccoli and the other Brassica species during postharvest storage using transcriptomics, proteomics and metabolomics approaches. The long-term goal of the proposed research is to aid development of an innovative tool to accurately estimate the freshness of produce and to generate germplasm for breeding new varieties with stress tolerance and postharvest color retention in Brassica vegetables. Such a tool would allow a new level of postharvest logistics, supporting availability of high-quality, nutritious, fresh produce.

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Primary Poster Presenter: Tie Liu

Dissecting the genetic basis of seasonal reproduction in a basal land plant, the moss Physcomitrella (0300-114)
Hall 2

Many plants make use of environmental cues, such as daylength and temperature, to synchronize reproductive development with favorable climatic conditions, thereby increasing their reproductive success. Although the genetic mechanisms that regulate reproductive development in response to seasonal cues are largely conserved across flowering plants, it is not known whether this conservation extends beyond angiosperms to other land plant lineages. We are using the model moss Physcomitrella patens to probe the evolutionary origin of seasonal regulation

in land plants, with the long-term goal of determining whether a core mechanism evolved in the common ancestor of all land plants, or if convergent mechanisms arose separately in distinct land plant lineages. We are coupling transcriptomic and genomic analyses of a set of P. patens ecotypes that vary in reproductive timing in response to seasonal cues with traditional mutagenesis screens to identify and characterize the genetic networks that underpin seasonal regulation of sexual reproduction in this basal land plant. Using a combination of cross-population genome-wide measures of selection and differential co-expression network analysis comparing ecotypes across daylength and temperature conditions, we have narrowed in on candidate genes homologous to upstream components of angiosperm temperature and daylength induced flowering time pathways. We have generated CRISPR-Cas9 mutants in four gene families and are currently evaluating their phenotypes. In parallel, we designed a mutagenesis screen, which has yielded heritable mutants with striking differences in the timing of onset of reproductive development in response to seasonal cues.

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Primary Poster Presenter: Karen Hicks

Evolution of specific receptor-target interactions in karrikin and strigolactone signaling pathways (0300-126)
Hall 2

Plants have evolved various hormonal signaling systems to regulate their life processes. However, many aspects of the evolution of plant hormone signaling remain obscure. Here, we use the signaling pathways of strigolactones (SLs) and karrikins (KARs) as models to understand the evolution of hormone signaling pathways. SLs are endogenous hormones, whereas KARs are plant growth regulators found in smoke. SL and KAR signaling mechanisms are surprisingly similar: both pathways consist of homologous receptors, D14 and KAI2, and target repressors within the SMAX1-LIKE (SMXL) family, and share an F-box protein MAX2. The activated receptors work with MAX2 to promote degradation of SMXLs, regulating different downstream responses such as germination or shoot branching. Recent studies propose that the SL signaling mechanism evolved from duplication of an ancient KAR signaling pathway. However, genetic evidence shows that SL and KAR signaling components are not interchangeable and there is biochemical

evidence for specific interactions between the receptors and their targets. We hypothesized that coevolution of KAI2 with SMAX1 and D14 with SMXL6/7/8 enabled the SL signaling pathway to emerge and prevent crosstalk with KAR signaling. To determine the basis for receptor-target specificity, we generated SL receptor and repressor mutants and evaluated their effects on protein-protein interactions. We isolated the candidate regions from SMXL7 through yeast two-hybrid analysis using alanine scanning and random mutagenesis. The key regions of SMXL7 will be swapped with the corresponding regions of SMAX1 to create chimeric proteins responsive to KARs. The sequences and structures of the proteins will also be compared to analyze their evolutionary changes. This will be a clue to understanding how SL signaling mechanisms diverged from KAR signaling and coevolved to have their own functions exclusively, and ultimately how plant hormone signaling evolved divergently and coevolutionarily.

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Primary Poster Presenter: Sun Hyun Chang

Evolution of the LATD/NIP gene root and nodule meristem function (0300-113)

Hall 2

Legumes can form two types of root lateral organs; lateral roots and nodules. Nodules are an important source of fixed nitrogen for legumes as a result of the symbiotic relationship with nitrogen-fixing bacteria. Medicago truncatula LATERAL ROOT ORGAN DEFECTIVE/ NUMEROUS INFECTIONS and POLYPHENOLICS (LATD/NIP) (MtNPF1.7) is a nitrate transporter. The Medicago truncatula latd mutant is defective in primary and lateral root meristems, as well the formation of symbiotic nodules, and these defects are due to a meristem defect.Our phylogenetic analysis indicates that the LATD/NIP gene originated in the angiosperms at the base of the eudicots. The evolutionary history of LATD/NIP is known; however, the evolution of its gene function is not. Interestingly, the origin of the LATD/NIP gene predates the origin of root nodulation, although it functions in nodule development in M. truncatula. So, it is unknown if the root and the nodule meristem functions are a single function or separate functions. Furthermore, if they are separate functions, they could be acquired at once or sequentially. To address these questions, we are testing the ability of LATD/NIP orthologs from different species to rescue the Medicago truncatula latd mutant. We are cloning these genes into Medicago truncatula latd mutant roots and observing primary and lateral root elongation and development of nodules. Our primary results show that at least the root function was acquired before the divergence of nitrogen-fixing clade in fabids, prior to the evolution of root-nodule fixation. Functional complementation of mutant lines with orthologs should provide a clearer evolutionary understanding of the gene’s function. In this way we hope to understand at what point the LATD/NIP gene gained its ability to control root and nodule development in Medicago truncatula.

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Primary Poster Presenter: Berke Tinaz

Evolution of WRKY Transcription Factors Across Oryza Species (0300-119)
Hall 2

The WRKY family of transcription factors are widely spread throughout the plant kingdom and play diverse roles in stress responses, growth, and development. To study how the WRKY family has diversified, we examined its evolution within closely related lineages that have been actively selected for and with members adapted to broad biogeographic ranges under various selection pressures. The Oryza genus is an ideal model due to its long history of domestication, globally recognized economic importance, and central role as a model system for monocots. Putative WRKY proteins were identified using HMMER3.2v2 through screening of 11 Oryza genomes against a Hidden Markov model constructed from previously identified Oryza sativa WRKY proteins. 1,018 WRKYs were identified across the species which included O. barthii (African wild rice), O. glaberrima (African rice), O. brachyantha (grass rice), O. glumaepatula (Brazilian wild rice), O. meridionalis (Australian wild rice), O. nivara (Indian wild rice), O. punctata (red rice) and O. rufipogon (brownbeard rice). The WRKY orthologs identified through bidirectional BLASTp were found to be heavily concentrated in chromosomes 1 and 5. Through MEME analysis high sequence conservation was observed with most having single copies of WRKYGQK or WRKYGKK amino acid motif in the N-terminal terminus and a Cys-Cys-His-His zinc-finger motif in the C-terminus. Syntenic analysis of paralogs using CLfinder-OrthNet shows high density of WRKY paralogs between chromosome 11 and 12. Identification of positive selection using CODEML reveal ~76% of the orthologous WRKY proteins under different amounts of selective pressures, which depend on the types of WRKY domains and the evolution rate of each species. These results provide invaluable insight into the diversification of an important gene family under strong selective pressure as well as useful information for the biotechnological improvement of the developing world’s most valued food crop.

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Primary Poster Presenter: Anne Jinky Villacastin

Evolutionary Origins of Domesticated Sorghum (0300-125)
Hall 2

Sorghum is a versatile cereal crop that can be used for food, forage, and biofuel. Its ability to grow in hot, arid environments and nutrient poor soils make it particularly important in regions of the world with less-desirable farm-land and limited

resources. Domestication of sorghum took place between 8,000 and 5,000 years ago in central Africa and from there domesticated varieties spread throughout the African continent as well as into India, China, and the Middle East. As domesticated sorghum spread it also diverged, giving rise to different races: bicolor, caudatum, durra, guinea, and kafir. Because of its high diversity and extensive local adaptation, several hypotheses suggest the domestication of sorghum could have occurred more than once. Of particular interest is the possible independent domestication of a subgroup of the Guinea race called the margaritiferums. According to this hypothesis, the genetically distinct margaritiferums were derived from local populations of wild sorghum in western Africa before the arrival of the other domesticated guinea varieties around 1,000 BC. Alternatively, the single origin hypothesis suggests that the margaritiferums were a subset of previously domesticated guinea sorghums that later underwent extensive hybridization with local wild sorghum populations. We used publicly available genome wide SNP data to test these two hypotheses using a demographic modeling approach. Preliminary results were unable to differentiate between the two hypotheses based on overall likelihood. However, divergence time estimates from the single origin model were more in agreement with the current historical record. This model placed the emergence of the margaritiferums sometime between 4,000 and 3,000 years ago, which would support the idea that the margaritiferums diverged around the time guinea sorghums were present in western Africa. These findings help to provide insight into the origins of sorghum and the history of agriculture.

Primary Poster Presenter: Rachel Bartolomeo

Extensive intraspecific gene order and gene structural variations in upland cotton cultivars (0300-122)
Hall 2

Upland cotton is the world's most lucrative cash crop and is also an important model species for studying polyploidization. Here, seeking to substantially improve the resolution and overall quality of the available genome assembly for this allotetraploid species, we used data from both single-molecule long read and Hi-C sequencing technologies to generate assemblies for G. hirsutum L. acc.TM-1 and zhongmiansuo24 (ZM24) genomes. These new assemblies enabled unprecedentedly comprehensive comparisons, both between these two varieties, and between these tetraploid's and their diploid progenitor species. Moreover, a total of 127 inversions and 2,127 translocations were identified between TM-1 and ZM24. Strikingly, genomic comparison identified, and subsequent RIL population and germplasm diversity panel haplotype analyses confirmed, that large-scale inversions on chromosome A08 are widely distributed and have over time mediated the reduction of meiotic recombination that has ultimately driven distinctly genetically isolated haplotypes of G. hirsutum. These new upland cotton genome assemblies are likely to become the new benchmark for cotton functional genomics research, and the scientific insights from our comparative analyses contributed substantially to our

basic understanding of how genomic inversions influence meiotic recombination and thus lower genetic diversity in plant populations.

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Primary Poster Presenter: Zhaoen Yang

Gene neighborhoods and fusions as a tool for gene function discovery (0300-116)
Hall 2

Algae represent one of the most diverse, complex and understudied groups. Over 100 whole-genome sequences from algae are either published or soon to be published, but over 50% of proteins are of unknown function. For the other 50%,the reliability of many functional annotations is unknown. Our goal is integrating and leveraging available genomic and post-genomic (transcriptomic) data to decipher protein function and prioritizing targets for experimental characterization. Here, we utilize comparative genomics approaches to infer protein function from association, by identifying gene neighborhoods and gene fusions in algae. Ten chlorophyte algal genomes were used to find conserved gene neighborhoods, defined as: proximal orthologous genes within a 6 gene window, in a minimum of 4 species,. This resulted in 152 gene neighborhoods with potential functionally relevant neighborhoods including genes involved in carotenoid biosynthesis, photorespiration, thiamine metabolism, nitrogen recycling, oxidative stress responses, and arsenic detoxification. Gene fusions were identified by searching for separate domains that were fused in one open reading frame among the ten algal species, providing insight into some poorly characterized proteins. Several genes of unknown function within gene neighborhoods or fusions were chosen to follow up with experimental characterization, for validation of the functional link between the genes in these conserved neighborhoods.

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Primary Poster Presenter: Fatima Foflonker

Genomic fingerprinting of Camelina species based on γ-tubulin gene intron length polymorphism (0300-123)
Hall 2

False flax (C. sativa L. Crantz.) have been the focus of many investigations to date, but other species of this genus are underused despite their potential utility as germplasm donors for C. sativa genetic improvement. In addition, a limitation on broader use of C. sativa as a genetic model and for gene editing is its hexaploid nature. In this study, DNA profiling of six Camelina species (C. microcarpa, C. rumelica, C. hispida var. grandiflora, C. alyssum, C. laxa and C. sativa) was conducted using assessment of γ-tubulin intron length polymorphism. Because only two copies of γ-tubulin gene are represented in a diploid genome, ploidy can also be evaluated with this method. Among seven C. microcarpa accessions obtained

from USDA (http://www.ars-grin.gov/), two samples (PI650134, PI650135) possessed atypical γ-tubulin intron patterns. C. microcarpa had 4 amplicons of 507 bp, 528 bp, 557 bp, 620 bp. The PI650134 accession contained four amplicons, two being similar to C. rumelica - 510 bp and 578 bp, as well as 553 bp and 0700 bp fragments. The PI650135 accession had two fragments only (507 bp and 0600 bp), which are unique compared to other Camelina species. The analysis with SSR markers confirmed differences among all camelina species. Previously, Galasso et al. (2018) described accessions characterized by atypical β-tubulin intron length polymorphism. Brock et al. (2019) described the PI650135 accession as a new species C. neglecta. γ-Tubulin intron profiles of C. alyssum and C. sativa were very similar to C. microcarpa, which could be explained by their common origin. In C. rumelica, two fragments (510 and 578 bp) were found, while C. hispida possessed high number of fragments (about 10) of 498-956 bp length. Together, these results demonstrate high polymorphism of this species. Therefore we can confirm that C. neglecta and C. rumelica are diploid species and, as such, their use could simplify transformation or genome editing versus use of the hexaploid C. sativa.

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Primary Poster Presenter: Rostyslav Blume

Identifying SNPS Unique to Certain Groups in Sorghum bicolor (0300-117)
Hall 2

In more widespread species, distinct populations can acquire and maintain unique genetic mutations through genetic drift or selection on locally beneficial alleles. When these population-specific variants occur, they often have a low frequency in the species as a whole. This can lead to them being overlooked in many trait mapping studies; however, these alleles may drive local adaptation or show disease risk and are therefore important in connecting genotype to phenotype.In this study, we identified over 14 million SNPs using whole-genome re-sequencing data from a collection of 352 Sorghum bicolor individuals and created a custom program to isolate mutations that are specific to particular groups. Sorghum is a highly diverse crop with high levels of historical population structure, and modern-day sorghum can be further divided into distinct biotypes used for the production of grain, syrup or biofuel. Because modern sorghum types such as sweet or grain-type sorghums can be derived from any of the historical groups, different alleles could have been selected at different times, making it difficult to identify them without the correct context. With our program, this collection of over 0300 individuals can be parsed into many different custom groupings in order to search for mutations underlying present-day phenotypic differences within specific historical or geographical groups. We have also extended our program to allow users to input custom groupings for any given dataset with a corresponding VCF file and return a list sites where the

minor allele frequency indicates that a mutation is restricted to a single population or sub-group. Our code is freely available to researchers studying any species who are interested in identifying population-specific alleles.

Primary Poster Presenter: James Heuser

Reference genome assembly of hexaploid chrysanthemum (0300-115)
Hall 2

Chrysanthemum (Chrysanthemum morifolium Ramat.) is an important flower with wide application value around the world. However, the lack of genomic information has severely restricted our research. Previous studies have shown that the cultivated chrysanthemum is a hexaploid species. We found that the chrysanthemum genome has ~8.5 Gb by flow cytometry and Kmer analysis, which is a high-repeat and high-heterozygosity genome. Here, through the integration of Pacbio sequencing, 10 × Genomics and Hi-C data, we assembled a hexaploid chrysanthemum genome at the chromosome level (n=3x=27). The assembly has a total size of 7.69 Gb, representing 90% of the estimated genome size, with the contig N50 reaches 2.03 Mb and the scaffold N50 reaches 262.02 Mb. Using an integrated strategy, a set of 133,718 protein-coding genes was predicted, with 99.3% can be annotated. Through collinearity, gene annotation and other information, we constructed a core gene set (77,320 genes). The data will provide a basis for us to study the origin of the cultivated chrysanthemum and the regulation mechanism of key horticultural traits.

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Primary Poster Presenter: Aiping Song

The pan-genome of Brachypodium distachyon and its implications for polyploid genome evolution (0300-121)
Hall 2

While prokaryotic pan-genomes have been shown to contain many more genes than any individual organism, the prevalence and functional significance of eukaryotic pan-genomes remain poorly understood. Whole-genome de novo assembly and annotation of 54 lines of the grass Brachypodium distachyon yielded a pan-genome containing nearly twice the number of genes found in any individual genome. Genes present in all lines are enriched for essential biological functions, while genes

present in only some lines are enriched for conditionally beneficial functions (e.g. defense and development), display faster evolutionary rates and lie closer to transposable elements. Our data suggest that differentially present genes contribute substantially to phenotypic variation within eukaryotic species. In addition, the pan-genome provides a new lens though which we can examine genome evolution in polyploid species by enabling us to differentiate between polymorphisms that evolved after polyploidization from those that were part of the standing variation in the diploid progenitors. To explore this, we sequenced multiple lines of an allopolyploid B. hybridum and used a pan-genomic approach to study the sub-genome that was derived from B. distachyon (D subgenome). Surprisingly, the vast majority of whole gene presence/absence variation in B. hybridum was part of the standing variation in B. distachyon. Analysis of nuclear SNPs, plastomes and k-mers revealed two independent origins for B. hybridum, ~1.4 and ~0.14 million years ago, creating a natural timecourse of polyploid genome evolution. Our analysis is consistent with a gradual accumulation of genomic changes in the polyploid lineages and an absence of sudden changes in sequence or expression. Significantly, had we compared a single reference genome for each species rather than using a pan-genomic approach, we would have grossly overestimated post-polyploidization evolution.

Primary Poster Presenter: John Vogel

Genes & Genomes: RNA Biology

NAD tagSeq for transcriptome-wide identification and characterization of NAD-capped RNAs (0300-172 (Screen 3))
Hall 2

The 5’ end of a eukaryotic mRNA generally has a methyl guanosine cap (m7G cap) that not only protects the mRNA from decay by 5’-3’ exonucleases, but also plays an essential role in almost all aspects of gene expression. Some RNAs in E. coli, yeast, and mammals were recently found to have NAD+ as a cap. We have developed a new method, termed NAD tagSeq, for transcriptome-wide identification and quantification of NAD+-capped RNAs (NAD-RNAs). The method uses an enzymatic reaction and a click chemistry reaction to label NAD-RNAs with a synthetic RNA tag. The tagged RNA molecules can be enriched and directly sequenced using the Oxford Nanopore sequencing technology. NAD tagSeq not only allows more accurate identification and quantification of NAD-RNAs but can also reveal sequences of whole NAD-RNA transcripts. Using NAD tagSeq, we found that NAD-RNAs in Arabidopsis are mostly produced from a few thousand protein-coding genes. The top 2,000 genes that were found to produce the highest numbers of NAD-RNAs were enriched in the gene ontology terms of responses to stresses, photosynthesis, protein synthesis, and response to cytokinin. For some Arabidopsis genes, over 10% of their transcripts could be NAD-capped. The NAD-RNAs in

Arabidopsis have similar overall sequence structures to their canonical m7G-capped mRNAs. NAD tagSeq has been used to identify NAD-RNAs from maize, rice, and other organisms. The identification and quantification of NAD-RNAs and revealing their sequence features provide essential steps toward understanding functions of NAD-RNAs.

Primary E-Poster Presenter: Yiji Xia

Mechanistic insights of miR167a regulation during temperature stress in Solanum lycopersicum (0300-173 (Screen 2))
Hall 2

MicroRNAs (miRNAs) are transcribed from MIR genes in plants, forming the primary transcript which is processed into the precursor miRNA and subsequent mature form of 20-24 nucleotides, by the Dicer-Like 1 and associated proteins. Therefore, the level of mature miRNAs in a cell may be determined both by transcriptional and processing control. The expression of miR167a was found to be significantly upregulated during cold stress in Solanum lycopersicum with a concomitant decrease in the level of its precursor (pre-MIR167a) hinting towards a substantial enhancement of precursor processing. In-vitro dicer activity assays with protein extracts of cold-stressed plants confirmed increased processing of pre-MIR167a, verifying the above hypothesis. A literature survey was performed to identify the miRNA biogenesis factors regulated by low temperatures and thereby probably involved in MIR167a processing. Of the several trans-factors identified, a transcription factor (TF) SlCDF5 was considered for further analysis since it exhibited significant upregulation during cold stress in both transcriptomics and qPCR analyses. The MIR167a promoter showed enhanced activity upon co-infiltration with SlCDF5, however, primary miR167a level remained unaltered upon transient expression of the TF. Interestingly, the mature miR167a level was increased significantly in the above experiment, indicating that transcriptional augmentation is accompanied by an enhanced rate of primary transcript processing. Heat stress also inflicted similar upregulation of both SlCDF5 and miR167a, suggesting SlCDF5 is involved in a common pathway for miR167a regulation during temperature stresses. Our experiments illustrate the probable role of SlCDF5, in both the transcriptional and processing steps of miR167a regulation and present a unique case of a TF also aiding in the miRNA processing to bring about a concerted effort in temperature stress-mediated upregulation of miR167a.Supported by CSIR and DBT, GoI.

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Cultivated Versus Native Soybean: Revealing Small RNA Expression Patterns (0300-136)
Hall 2

Small RNA is non-coding RNA that regulates diverse processes in plants by transcriptional or posttranscriptional silencing of regulatory genes. It is known there is significant variation in small RNA expression within the same species, but it is unknown how these small RNA are able to specifically regulate plant diversity under varying photoperiod conditions. The goal of this work is to identify the differences in small RNA expression between two closely related species: Glycine soja, the wild antecedent species of Glycine max, the soybean crop we see today. Our objectives are to quantify the changes of these small RNA under varying photoperiod conditions and to characterize variations in small RNA expression between these species. To address these objectives, a small RNA sequencing approach is conducted. First, we harvested leaf samples of Glycine max and Glycine soja that were grown under short day (10 hours light/14 hours dark) and long day (14 hours light/10 hours dark) photoperiod conditions, and isolated the total RNA from these samples. Next, the small RNA from the total RNA is purified using a polyacrylamide gel. cDNA libraries are produced from these small RNA samples that will be sequenced using an Illumina HiSeq 4000. The sequence reads will be aligned to the soybean reference genome and compared to known small RNA. Finally, the identified microRNA (miRNA) and small interfering RNA (siRNA) will be verified and quantified by qRT-PCR analysis. In parallel with sequencing, we are characterizing known small RNA including miRNA156 and miRNA172 in their photoperiodic responses that regulate flowering transition. From these approaches we expect to gather information that will propel our understanding of small RNA in plants forward to create verifiable standards, and increase our knowledge of how this important crop may fair in different climates around the world.

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ELUCIDATING THE FUNCTION OF A NOVEL LONG INTERGENIC NON-CODING RNA DURING LEAF SENESCENCE IN ARABIDO (0300-134)
Hall 2

Long intergenic non-coding RNAs (lincRNAs) are an emerging class of molecules gaining attention for their roles in many biological processes. While over 6,000 lincRNAs are detected in Arabidopsis and several have demonstrated stress responsive expression patterns and functions, the functions of most lincRNAs remain unknown. We previously identified a protein-bound, nuclear lincRNA, CONSERVED IN BRASSICA RAPA 1 (CONBR1), that contains two highly conserved small nucleolar RNAs (snoRNAs).Given its high conservation, we obtained an insertion mutant within the lincRNA that causes a 90-95% reduction in lincRNA levels. Interestingly, mutant plants are significantly smaller throughout development and begin leaf senescence significantly earlier than WT. Leaf

senescence is the final stage of leaf development allowing nutrients to relocate from leaves to developing tissues. As leaf senescence is tightly controlled by genetic programs, understanding the role of CONBR1 in this process will help further elucidate the molecular mechanisms of leaf senescence and its role in crop fitness.To examine the molecular function of this lincRNA, we performed chromatin isolation by RNA purification followed by DNA sequencing (ChIRP-seq) to identify regions throughout the genome that CONBR1 binds. Using biotinylated DNA probes antisense to CONBR1, we specifically isolated the lincRNA and any interacting DNA and identified 94 unique loci that were bound by CONBR1. Interestingly, these loci are enriched for genes that encode proteins involved in cuticle development and phospholipid biosynthesis, which are linked to leaf senescence. Further, CONBR1 interacts with the promoter of LARP1B, which has been shown to lead to early senescence when overexpressed. In fact, expression of LARP1B is significantly increased in mutant plants undergoing early senescence, leading to the hypothesis that CONBR1 binds to LARP1B and regulates its expression, leading to proper development and timing of senescence

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Evolution of DNA-dependent RNA polymerases in plants (0300-138)
Hall 2

Eukaryotic organisms possess three multi-subunit DNA-dependent RNA polymerase complexes (Pol I-III), which are each responsible for transcription of a subset of cellular RNA. Plants encode at least two additional DNA-dependent RNA polymerases (Pol IV and V), which are specialized for RNA-directed DNA methylation. These plant-specific polymerases arose through duplication and re-functionalization of canonical Pol II subunits, creating novel Pol complexes with distinct transcriptional and co-transcriptional functions. The earliest Pol subunit duplication occurred in Streptophytes, the algal sister group to land plants, and this genesis of functionally-distinct Pol complexes might have driven subsequent duplications of additional subunits during land plant diversification. In addition to ancient duplications creating Pol IV and Pol V, duplication of additional Pol subunits indicates that grasses contain a sixth RNA polymerase of unknown function. The evolutionary history of non-canonical RNA polymerases and evidence for a grass-specific Pol VI will be discussed.

Primary Poster Presenter: Rebecca Mosher

Generating a spatial/temporal transcriptome of nodulating Medicago truncatula roots (0300-132)
Hall 2

The legume-rhizobial symbiosis involves a complex signal exchange between the host plant and rhizobia bacteria to initiate the symbiosis, leading to the formation of root nodules in which the bacteria fix nitrogen for the plant. Signal transduction events occur between the host plant cell layers in tissues, organs, and across time. For example, bacterial infection threads pass through the epidermis and outer cortical cells towards the inner cortical cells, while the inner cortex and pericycle cells become mitotically active before the arrival of the infection thread. The vasculature is separated from inner cortical cells by the pericycle and endodermis; signaling passes across these tissues to the infection thread advancing across the cortex. Previous transcriptomic analyses used whole roots to identify host genes involved in nodule development, but this does not capture unique transcription events in specific tissues. To address this, we are performing transcriptome profiling of specific root tissues during nodule development by using laser capture microdissection (LCM) to isolate the tissues for RNA extraction. This is followed by RNA-seq analysis of libraries made from epidermal, vascular, inner and outer cortical cells at 0, 12, 24, 48 and 72 hours post inoculation. We generated enough tissue to make unamplified libraries, but a comparison of unamplified to amplified libraries revealed minimal differences, except for time involved in capturing the tissue. To date we have completed all unfixed controls and five libraries from fixed, inoculated tissue samples. We detected the expression of around 20,000 different transcripts from individual tissues, suggesting adequate coverage depth. Enrichment analysis of the transcripts identified tissue specific genes, demonstrating accurate separation of tissue types by LCM. The data generated will be used to study distinct tissue gene expression profiles during nodule development. This work is supported by NSF IOS ##14444.

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Light triggers the miRNA-biogenetic inconsistency for de-etiolated seedling survivability (0300-128)
Hall 2

The shift of dark-grown seedlings into light causes enormous transcriptome changes followed by a dramatic developmental transition. Here, we show that miRNA biogenesis also undergoes regulatory changes during de-etiolation. Etiolated seedlings maintain low levels of primary-miRNAs (pri-miRNAs) and miRNA processing core proteins, such as Dicer-like 1 (DCL1), SERRATE (SE) and HYPONASTIC LEAVES 1 (HYL1), whereas during de-etiolation, both pri-miRNAs and the processing components accumulated to high levels. However, most miRNA levels did not notably increase in response to light. To reconcile this inconsistency,

we demonstrate that an unknown suppressor decreases miRNA-processing activity and light-induced SMALL RNA DEGRADING NUCLEASE 1 (SDN1) shortens the half-life of several miRNAs in de-etiolated seedlings. Taken together, we suggest a novel mechanism, miRNA-biogenetic inconsistency, which accounts for the intricacy of miRNA biogenesis during de-etiolation. This mechanism is essential for the survival of de-etiolated seedlings after long-term skotomorphogenesis and their optimal adaptation to ever-changing light conditions.

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Primary Poster Presenter: SukWon Choi

Light-induced COP1–HCP complex formation stabilizes HYL1 for miRNA biogenesis (0300-129)
Hall 2

Constitutive photomorphogenic 1 (COP1) is a RING-finger E3 ligase that plays a central role in photomorphogenesis by destabilizing many light-regulated transcription factors and photoreceptors. Previously, we showed a novel function for COP1 E3 ligase in controlling global miRNA biogenesis in Arabidopsis thaliana. In cop1 mutants, the levels of miRNAs are dramatically reduced because of the diminution of HYPONASTIC LEAVES 1 (HYL1), a key component for the precise processing of pri-miRNAs into mature miRNAs. In darkness, HYL1 is destabilized by two newly identified cytoplasmic proteases, HYL1-CLEAVAGE PROTEASES (HCPs) that specifically cleaves the N-terminal region from HYL1, thus neutralizing its function. Besides, under light condition, COP1 E3 ligase to form COP1-HCP complex in the cytoplasm that blocks the destabilization of HYL1. Taken together, we here suggest that COP1-HCP complex is an essential regulatory circuit for light-integrated miRNA biogenesis in plants.

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Missense alleles of the PRP8 and BRR2a splicing factors restore splicing to splice-site mutants (0300-137)
Hall 2

Most plant genes contain introns that are removed through a dynamic multistep process orchestrated by five snRNAs and hundreds of proteins that comprise the

spliceosome. Intron excision requires precise positioning and assembly of the spliceosomal components on the 5' and 3' ends and branchpoint of the intron. The 5' and 3' intron boundaries are characterized by conserved GT and AG sequences respectively. Forward-genetic screens often recover splicing alleles because the essential G residues at splice sites are subject to EMS mutagenesis. For example, out of twelve pex14 alleles recovered from our forward-genetic screens for peroxisome dysfunction, six alleles disrupt splicing. We performed a suppression screen of one of these alleles, pex14-6, to find corrected splicing, and we recovered missense alleles of two splicing factor genes, PRP8 and BRR2a. PRP8 and BRR2a are essential splicing factors critical to pre-mRNA binding and activation of the spliceosome. We are investigating the effect of our prp8 and brr2a alleles on a series of pex14 splice-site alleles to probe splice-site fidelity in Arabidopsis. Additionally, we are using RNA-seq to probe the systemic effects on splicing in both mutants. This research will elucidate mechanisms of spliceosome intron recognition and the genome-wide consequences of reduced splice-site fidelity. (This research is supported by the NIH.)

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Primary Poster Presenter: Roxanna Llinas

Potential Regulation of the Autophagy Response by Calmodulin-like proteins during Low Oxygen Stress (0300-131)
Hall 2

"Regulator of gene silencing" calmodulins (rgsCaMs) represent calmodulin-like protein (CML) calcium sensors that were first identified as potyviral viral targets for suppression of gene silencing (1). Recent evidence suggests that rgsCaM mediates its effects on viral and transgene posttranslational gene silencing through the binding and targeting of Suppressor of gene silencing 3 (SGS3) and RNA-dependent RNA polymerase 6 (RDR6) complex for degradation by selective autophagy (2). Arabidopsis CML38, a member of the rgsCaM subfamily, is a hypoxia-specific calcium sensor protein that is induced by flooding/low oxygen stress, accumulates in cytosolic mRNA granules, and is rapidly turnover upon reoxygenation. Here it is shown that CML38: 1. Binds directly to SGS3 and co-localizes to siRNA body/Stress Granule aggregates; 2. Triggers the turnover of SGS3 both in *Nicotiana benthamiana* as well as in hypoxia challenged Arabidopsis; and 3. Is necessary for a normal Arabidopsis autophagy response upon hypoxia and re-oxygenation recovery. Analogous to previous work with rgsCaM, a potential role for CML38 in autophagic regulation during the low oxygen stress/recovery response is proposed.1. Anandalakshmi et al. (2000) A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. Science 290, 142-4.2. Li et al. (2017) A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in *Nicotiana benthamiana*. PLoS Pathog 13, e1006213.

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Profiling of multi-omics data provides nitrogen starvation-responsive characteristics in rice (0300-130)
Hall 2

Nitrogen (N) is a key macronutrient essential for plant growth, and N availability has a strong influence on crop development. Here, we profiled and characterized transcriptomic responses of annotated genes, newly identified putative long non-coding RNAs (lncRNAs), and microRNAs and their target mRNAs to N starvation in rice using four different transcriptome approaches. Analysis of RNA-Seq, small RNA-Seq, 2P-Seq, and Degradome data provided a comprehensive overview of dynamic gene expression in rice and allowed diverse aspects of rice transcriptomes to be assessed and integrated. The responses of genes, putative lncRNAs, and microRNAs were profiled in response to N starvation in rice roots and shoots, revealing multiple N-responsive transcriptome sets. Comparison of these N-responsive genes with genes responsive to other abiotic stressors such as phosphate starvation allowed the identification of multiple stress-responsive regulatory non-coding RNA pools. The comprehensive, large-scale datasets analyzed in this study expand our knowledge of regulatory pathways in N-starved rice and provide a basis for understanding the molecular mechanisms modulating N homeostasis in rice.This work was supported by the Next-Generation BioGreen 21 Program (No. PJ01332501), Rural Development Administration, Republic of Korea.

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Role of Unique C-terminal Domain in a Plant Aminoacyl-tRNA Trans-editing Protein (0300-133)
Hall 2

Aminoacyl-tRNA synthetases (aaRSs) are responsible for pairing specific amino acids with cognate tRNAs for delivery to the ribosome. To prevent mistranslation due to errors in this process, many aaRSs have evolved quality control mechanisms. Bacterial prolyl-tRNA synthetases (ProRSs) mischarge noncognate Ala onto tRNAPro and possess an insertion domain that can deacylate Ala-tRNAPro. However, some bacteria and all eukaryotes lack this proofreading domain and instead, encode a free-standing trans-editing domain homolog, ProXp-ala. Sequence alignments revealed that all plant ProXp-ala encode a unique, conserved C-terminal domain (CTD) of unknown structure and function. To determine the function of the CTD, we prepared a truncated Arabidopsis thaliana (At) ProXp-ala

variant (dC-ProXp-ala). The in vitro Ala-tRNAPro deacylation rate by dC-ProXp-ala was decreased 16-fold relative to wild-type (WT) ProXp-ala. Preliminary studies suggest that this decrease is primarily due to a tRNA binding defect. In addition, size exclusion chromatography showed that WT At ProXp-ala adopts several oligomeric states while dC-ProXp-ala is exclusively monomeric. The CTD may therefore function to enhance tRNA binding and induce oligomerization in vitro. Preliminary in vivo studies using split-GFP constructs performed to date do not support homo-oligomerization, but do not rule out the possibility that this domain promotes interactions with other binding partners. At ProXp-ala disruption strains showed various growth defects such as lack of germination, reduced rosette size, late flowering, and abnormal fruit development. Trans-complementation experiments are currently being carried out under a variety of growth conditions to better understand the function of the WT enzyme and the role of the CTD in vivo. This work will reveal how plants have uniquely evolved to avoid mistranslation and may lead to the discovery of new ProXp-ala functions.

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Primary Poster Presenter: Jun-Kyu Byun

Transcriptome-wide analysis reveals miRNAs targeting defense genes in pepper (0300-135)
Hall 2

MicroRNAs (miRNAs) play roles in various biological processes in plants including growth, development, and defense. Recent studies revealed that some plant miRNAs produce secondary small interfering RNAs (siRNAs) such as phased, secondary siRNAs (phasiRNAs), and they regulate the cascade of gene expression. We performed a transcriptome-wide analysis of miRNAs and mRNAs in pepper (Capsicum annuum). We further investigated functions of the miRNAs via degradome analysis. Degradome analysis revealed that several miRNAs target many genes encoding nucleotide-binding leucine-rich repeat (NLR) proteins or receptor-like proteins (RLPs), which are known to be major players in defense responses. In addition, resistance-related miRNAs trigger phasiRNA production, indicating amplification of the regulation of disease-resistance gene families. Among these, can-miR-n033a and can-miR-n026, which have specifically evolved in pepper, target many NLRs and RLPs of expanded subgroups in Capsicum species. This study provides an insight into possible co-evolution between miRNAs and their target defense genes in plants.This work was supported by the Next-Generation BioGreen 21 Program (No. PJ01333001), Rural Development Administration, Republic of Korea.

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RNA tagging in plants: live and dead, big and small (0300-174 (Screen 6))
Hall 2

RNAs, including small RNAs, play a variety of important regulatory roles in both plants, including developmental regulation, activation of pathogen defences, and stress responses. In the past, precise, subcellular and even cellular imaging of RNAs in plant tissue was challenging due the thickness of plant tissue and strong autofluorescence. We have developed a suit of imaging tools that span from single-molecule to whole tissue level of RNA imaging in plants, which includes sRNA-FISH, RNA-PAINT, whole mount-FISH, and smFISH. Live-cell imaging of RNAs is even more challenging. Genetically encoded live-cell fluorescent tagging of RNAs is made possible by fusion of a target RNA with a fluorescent RNA aptamer. Using this technique, we created FASTmiR sensors for detection small RNAs in live cells. We also created aptamer sequences that emit fluorescence upon binding of an endogenous fluorophore bliverdin.

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Hormone Biology

In vivo quantitative imaging of auxin and cytokinin responses in soybean roots and nodules (0700-030 (Screen 2))
Hall 2

Legume-Rhizobium symbiosis results in root nodules where rhizobia fix atmospheric nitrogen in to plant usable forms in exchange for plant-derived carbohydrates. Biological nitrogen fixation in legume nodules alleviates the use of energy-intensive, expensive, and environmentally hazardous chemical nitrogen fertilizers. The development of these specialized root organs involves a set of carefully orchestrated plant hormone signaling. In particular, a spatio-temporal balance between auxin and cytokinin appears to be crucial for proper nodule development. We used two photon induced fluorescence microscopy for quantitative 3-dimensional imaging of fluorescent markers to determine cellular level auxin and cytokinin outputs and ratios during root and nodule development in soybean. The relative auxin cytokinin ratios in root tips and lateral roots determined in this study were in agreement with previously reported outputs for each fluorescent reporter individually. The ratiometric method used here is shown to largely compensate for variations in individual outputs due to sample turbidity and scattering, providing

quantitative measures of the relative hormone outputs. Importantly, distinct auxin/cytokinin ratios corresponded to distinct nodule cell types indicating a key role for these hormones in nodule cell type identity. Future applications of the method for time-course imaging of auxin/cytokinin outputs and ratios along the course of nodule development are expected to provide key insights on hormonal control of cell differentiation during nodule development.

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THE HORMOMETER: A SYNTHETIC BIOLOGY TOOLBOX TO STUDY HORMONE INTERACTIONS IN PLANTS (0700-031 (Screen 4))

The interaction between endogenous plant growth regulators is a key process in the integration of environmental and developmental signals. How different plant hormonal pathways talk with one another is, however, poorly understood, and new phenotyping tools that enable simultaneous detection of the activity of multiple pathways are urgently needed. Taking advantage of the GoldenBraid gene multiassembly technology, we are building the hormometer, a multi-hormone sensor that permits detection of the transcriptional output of several growth regulators at once. An ideal hormometer should consist of a single construct comprised of multiple hormone-specific transcriptional reporters for all nine major non-peptide plant hormones arranged in tandem. Each individual reporter would contain five DNA elements (phytobricks): a hormone-specific distal promoter, a synthetic core promoter (+5'UTR), a subcellular localization tag, a fluorescent protein coding sequence, and a synthetic terminator (+3'UTR). A combination of three fluorophores and three subcellular localization tags provides enough multiplexing power to monitor the nine growth regulators simultaneously. Towards this objective, a collection of nearly 120 phytobricks has been generated in our lab, a majority of these parts have been assembled in tester transcriptional units, and to date about a third have been functionally validated in transient assays in tobacco. Using some of the parts from our collection, we have also built two different versions of the ACE hormometer that harbor fluorophore- and localization-tag-compatible reporters for auxin, ethylene and cytokinin, along with a selectable marker, in a single binary vector construct. The upcoming characterization of ACE activity in the resulting Arabidopsis and tomato stable transgenic lines will provide the first proof of concept for our multiplexing approach and offer a new streamlined tool for monitoring the three hormones in parallel.

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Rice qGL3/OsPPKL1 Functions with the GSK3/SHAGGY-Like Kinase OsGSK3 to Modulate Brassinosteroid Sign (0700-032 (Screen 7))
Hall 2

Brassinosteroids (BRs) have been shown to regulate various biological processes in plants including cell elongation, cell division, and cell differentiation. Recent studies have highlighted the importance of BRs in the control of grain yield. Grain size affects yield and we previously cloned qGL3, a major quantitative trait locus regulating grain length in rice (Oryza sativa L.). The japonica variety N411 has extra-large grains and the recessive qGL3 allele from N411 contributes positively to grain length. The indica variety 9311 has relatively smaller grains, qGL39311 had two amino acid changes compared with qGL3N411. qGL3 encodes a putative protein phosphatase with Kelch-like repeat domains (OsPPKL1), an ortholog of Arabidopsis thaliana bri1 SUPPRESSOR1 (BSU1). BSU1 positively regulates brassinosteroid (BR) signaling, while overexpression of qGL3 induced BR loss-of-function phenotypes. Both alleles of qGL3 physically interact with the rice GSK3/SHAGGY-like kinase OsGSK3, an ortholog of Arabidopsis BRASSINOSTEROID INSENSITIVE2 (BIN2). However, qGL39311 dephosphorylates OsGSK3, but qGL3N411 lacks this activity. The osgsk3 mutant showed enhanced lamina joint inclination and increased grain length. In qGL3 transgenic plants, qGL3 levels dictated OsGSK3 protein phosphorylation status and degradation. qGL39311 dephosphorylates OsGSK3 in qGL3 overexpression plants and causes the OsGSK3 accumulation in the cytoplasm but resulted in phenotypes that are consistent with OsGSK3 activation. These results demonstrate that qGL3 suppresses BR signaling by positively regulating OsGSK3 levels, which affect OsBZR1 phosphorylation and subcellular distribution. Our data support the existence of this novel negative qGL3-OsGSK3 regulatory module in modulating BR signaling and grain length, which may help in improving grain yield by enabling precise manipulation of the BR signaling pathway.

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Regulation of Auxin Signaling and Development by Alternative Polyadenylation (0700-033 (Screen 10))
Hall 2

Polyadenylation influences gene expression by affecting mRNA stability, transport, and translatability. Recently, we reported that Cleavage stimulation Factor 77 (AtCstF77), a component of the pre-mRNA 3'-end polyadenylation machinery, affects polyadenylation site (PAS) selection in transcripts of some auxin signaling genes in Arabidopsis (Zeng et al., 2019, Plant Physiology). Disruption of AtCstF77 reduced auxin sensitivity and decreased the expression of the auxin reporter DR5-

GFP. Null mutations of cstf77 caused severe developmental defects, but were not lethal as previously reported. cstf77-2 genetically interacted with transport inhibitor response 1 auxin signaling f-box 2 auxin receptor double mutants, further supporting that polyadenylation affects auxin signaling. AtCstF77 was ubiquitously expressed in embryos, seedlings, and adult plants. We will present our new findings on transcriptional regulation of AtCstF77 by a transcription factor, which directly binds to the promoter of AtCstF77 and repressed its expression. Alteration of the transcription of AtCstF77 caused severe developmental defects. Together these results suggest a model of transcriptional regulation of AtCstF77 and development by auxin in Arabidopsis.

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Primary E-Poster Presenter: Youfa Cheng

Arabidopsis Scaffold Protein RACK1A Regulates Auxin Mediated Lateral Root Developmental Pathway. (0700-034 (Screen 11))
Hall 2

RACK1 (Receptor for Activated C Kinase 1) is a WD-40 type scaffold protein family, conserved in single cell eukaryote yeast to human and plays regulatory roles in diverse signal transduction and stress response pathways. Loss of function mutant in the predominant isoform-RACK1A in Arabidopsis, indicates that it regulates diverse environmental stress resistance through negative regulation of stress hormone ABA and positively regulates Auxin mediated diverse developmental pathways. It is hypothesized that chemical knock-out, as opposed to genetic knock-out, of RACK1A will provide a functional advantage in protecting plants from diverse stress and a small compound stabilizing RACK1 will be useful to promote Auxin regulated developmental pathways. Dozens of small compounds based on our lab derived crystal structure of Arabidopsis RACK1A are isolated and functionally tested as their ability modulate the auxin signaling pathways. These functional modulators of RACK1A appear to regulate the auxin induced lateral root development process. In this pathway, the small compound inhibiting stable RACK1A expression appears to produce hyposensitivity to auxin. On the other hand, a RACK1A stabilizing compound provided hypersensitivity to the Auxin induced lateral root development. The compound augmenting Auxin mediated lateral root development has been found to promote diverse Auxin responsive gene expression as well. Taken together, these results suggested that RACK1A may act as a modulator in the auxin signal pathways. This work may lead to understand the molecular interaction between RACK1A and auxin and the possible application of novel RACK1A modulating small compounds as fertilizers to promote Auxin mediated developmental pathways safely in non- genetically modified crops.

Primary E-Poster Presenter: Shifaa Alshammari

Abscisic acid regulates vitamin E biosynthesis through phytol recycling in sweet cherries (0700-004)
Hall 2

Sweet cherries are non-climacteric fruits highly valued by consumers because of their organoleptic and nutraceutical properties. However, there is little information about accumulation and biosynthesis regulation of important antioxidants for human health like vitamin E in sweet cherries. For this reason, in this study we wanted to determine vitamin E production during on-tree cherry ripening and evaluate if abscisic acid (ABA) could be a potential regulator of vitamin E biosynthesis in sweet cherries. Our results showed an increase in α- and γ-tocopherol after the color change during cherry fruit development on-tree, which achieved maximum values when cherries were fully ripen. This accumulation was parallel to that of ABA and there was a strong correlation between ABA and both forms of tocopherols during cherry ripening. Moreover, there was an increase on both forms of tocopherol content after ABA exogenous application, which also triggered an increase in transcription levels of phytol kinase (VTE5) and phytyl-phosphate kinase (VTE6) genes. Therefore, these results indicate that vitamin E increases during fruit ripening of sweet cherries and the biosynthesis of this antioxidant is mediated by ABA through phytol recycling which, to our knowledge, has not been described for any non-climacteric fruit yet. Keywords: Sweet cherry; vitamin E; ABA; VTE5; VTE6; phytol

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Characterization of Developmental Patterns in Arabidopsis Mutants with Altered States of Immunity (0700-015)
Hall 2

Phytohormones are essential regulators of plant development and response to environmental stresses. Activation of the plant immune system by pathogen attack often results in changes in plant growth, frequently leading to smaller plants with reduced seed set. Previously, we discovered that cytokinin (CK), a hormone known for its role in the regulation of cell division and plant growth, also has an important role in the activation of defense, through synergistic interaction with the defense hormone salicylic acid (SA). Using the model plant species Arabidopsis thaliana, we aim to elucidate the interaction between CK and SA and the crosstalk role on plant growth. Here, we use gene expression data, growth assays, and detailed observation of developmental patterns to advance our understanding of the CK and SA crosstalk, allowing us to further characterize altered phenotypes due to mutations in CK and SA accumulation and/or signaling. Analysis of developmental phenotypes and regulation provides insight into the role of SA and CK on plant growth and development. Further investigation of the complex network of interactions regulating the balance between plant growth and defense may inform

future efforts in synthetic biology to develop advanced crops with increased pathogen resistance and superior plant yield.

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Primary Poster Presenter: Grace Johnston

Characterization of the role of brassinosteroids in the growth of grapevine berries (0700-005)
Hall 2

The agronomic management of grapevines (Vitis vinifera L) include the use of growth regulators to improve different productive parameters such as size, production of color and absence of seeds. The brassinosteroids (BRs) are plant hormones that participate in growth processes and have been associated with the promotion of ripening in fruits such as grapes, strawberries and tomatoes. In the case of grapevine, molecular analysis of the pathway of biosynthesis, perception or signaling of brassinosteroids have not been carried out yet. To understand the role of this hormone in grapevine development,12 different compounds of BRs family were quantified by UHPLC-MS/MS, obtaining a profile associated to different tissues and stages of development. To complement this analysis, the gene expression from the biosynthesis pathway was analyzed. With this result we proposed the predominant synthesis routes and the main intermediate metabolites in different tissues and stages of the development. To clarify the role of BRs in the growth in grapevine berry, treatments were carried out in the field with two commercial compounds in early stages of development. From the morphological analysis it was determined that there is a significant increase in growth in the berries treated with BRs. To understand the mechanism of action of this hormone, in the berries treated with BRs, other plant hormones cytokinins, auxins and gibberellins were measured. We detected changes in the hormonal metabolism in berries that were treated with BRs, which could explain the observed phenotype. In addition, the expression levels of marker genes of these hormonal routes as well as the cellular cycle genes were analyzed, which allows us to propose a molecular mechanism of action. Taken together, the results suggest a complex interaction between hormones to regulate changes in the growth of the grapevine berry in early stages mediated by multiple changes at gene expression level.

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Primary Poster Presenter: Francisca Parada

Crosstalk Between Auxin and Abscisic Acid in Dark-Grown Hypocotyl Elongation (0700-019)
Hall 2

Crosstalk between auxin and abscisic acid (ABA) has been implicated in many plant growth and development processes such as germination, root growth, and root hair elongation. However, many aspects of auxin and ABA crosstalk are still unknown. Using a forward genetics approach, we identified HR12, a mutant that is resistant to the suppressive effects of exogenously applied auxin on dark-grown hypocotyl elongation. Through a whole genome sequencing of bulk segregants approach, we identified a mutation in ABA ALDEHYDE OXIDASE 3 (AAO3), which is causative for the auxin resistance observed in HR12. The AAO3 enzyme carries out the last step in the ABA biosynthetic pathway. Further, all examined ABA biosynthesis and signaling mutants also displayed resistance to the inhibitory effects of auxin on dark-grown hypocotyl elongation. Our results suggest that ABA biosynthesis and signaling act downstream of auxin in inhibition of dark-grown hypocotyl elongation. Because ABA acts in stress signaling, this suggests a potential mechanism by which ABA can restrict pre-emergence growth under stress conditions.

Primary Poster Presenter: Ryan Emenecker

Cytokinin modulates microtubules dynamics, a mechanism regulating cell division and differentiation (0700-007)
Hall 2

In plants, the root pericycle cells represents a unique tissue with conditional meristematic activity, and its tight control determines initiation of lateral organs. Pericycle meristematic activity is constrained by the interaction with the adjacent endodermis. The elimination of endodermal cells by single-cell ablation allows the pericycle cells to re-enter the cell cycle. However, the phytohormone auxin is indispensable to steer the cell division plane orientation of new organ-defining divisions, and for releasing the endodermis constriction. Furthermore, another important phytohormone, cytokinin, has also an important antagonistic role in this process. However, how auxin and cytokinin define the correct cell division orientation during the lateral root initiation remains elusive. We aim to dissect mechanisms that coordinate proper orientation of pericycle cell divisions and set up lateral root organogenesis. For that, we focused on auxin and cytokinin interaction with microtubules cytoskeleton as one of the important molecular components decisive for the cell division plane orientation. Using epidermal root cells we observed that cytokinin induces microtubules reorientation and reduces microtubules dynamics, slowing down the plus-end microtubule speed growth in cells that requires high microtubules dynamics, mimicking with the microtubules orientation and dynamics of differentiated cells. These results suggest that cytokinin induces microtubules stabilization, and therefore, cell differentiation. We also observed that a specific cytokinin receptor, CRE1/AHK4, is necessary for this effect. How cytokinin signaling transduces the signal to microtubules and its potential role in the orientation of the cell division during the lateral root formation is an exciting and unknown field that we are currently exploring.

Co-author(s): Eva Benkova

Primary Poster Presenter: Juan Carlos Montesinos López

Cytokinin Signaling in Rice (0700-022)
Hall 2

Cytokinin plays a key role in many aspects of plant development, including root architecture, nutrient uptake, and grain yield. Most of the work on understanding the cytokinin signaling pathway has been done in the dicot Arabidopsis thaliana. As cytokinin impacts many important agronomic traits and many major crop species are monocots, it is critical to understand cytokinin signaling in monocots. We are using rice as a model system to study cytokinin signaling in monocots. Cytokinin signals through a modified phosphorelay comprised of histidine kinases, functioning as cytokinin receptors, and histidine phosphotransfer proteins that shuttle a phosphate from these receptors to nuclear-localized type-A and type-B response regulators that modulate the output of the signaling pathway. We have used the CRISPR/Cas9 system to create mutations in nearly all the rice genes encoding cytokinin signaling elements. Investigation of these mutants has revealed both commonalities and fundamental differences between the cytokinin function in Arabidopsis and rice. The most striking differences occur in the histidine kinase (hk) mutants. In Arabidopsis, mutations in all three of the HKs are required to produce significant phenotypes. In rice, single hk mutants have substantial developmental phenotypes, including defects in apical meristem activity, floral development, and lateral roots. Our results suggest that rice uses the canonical cytokinin signaling pathway for novel developmental roles.

Primary Poster Presenter: Christian Burr

Cytokinin-N-glucosides have unique physiological and transcriptional roles in Arabidopsis thaliana (0700-018)
Hall 2

Cytokinin is a hormone indispensable for proper plant growth and development. The word “cytokinin,” however, is an umbrella term encompassing dozens of compounds found in plant tissues. Over the last several decades, biologists studying cytokinins have focused almost exclusively on “active” cytokinin bases, which comprise only a small subset of these compounds. Recent developments in analytical chemistry have allowed researchers to accurately measure cytokinin compounds, and reveal that the most abundant cytokinins in Arabidopsis thaliana are the cytokinin-N-glucosides, which are composed of a cytokinin base irreversibly conjugated to a glucose molecule. Since their discovery over four decades ago, cytokinin-N-glucosides have been largely ignored and labelled as inactive compounds, despite some published data suggesting the compounds have biological activity. In the work presented, we demonstrate that some cytokinin-N-glucosides have physiological activity when exogenously applied to Arabidopsis tissue, particularly in delaying senescence in detached leaves. Transcriptome profiling has revealed cytokinin-N-glucosides have transcriptional effects that differ from known “active” cytokinin bases. Analysis of Arabidopsis mutants either producing no

cytokinin-N-glucosides or producing them in excess indicates these compounds are involved in plant development, specifically in longevity. We conclude that cytokinin-N-glucosides are capable of altering Arabidopsis physiology in a manner similar to, but distinct from, active cytokinin bases, suggesting these long-overlooked compounds merit further investigation.

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Primary Poster Presenter: Tucker Hallmark

Elucidating Hormonal Crosstalk During Tomato Development and Disease
(0700-009)
Hall 2

Hormonal crosstalk is essential to plant survival through regulation of growth and responses to abiotic and biotic stresses. While classic plant hormones have been investigated for a long time, additional hormones have recently been identified. Considerable progress has been made in recent years in defining the molecular mechanisms involved in plant hormone recognition and signaling using the model species Arabidopsis. However, basic plant hormone signaling research would benefit considerably from a broader approach that includes crop species and emerging model systems. Here, we have used the tomato cultivar MicroTom, which has considerable genetic resources, to simultaneously quantify hormones with ultrapure liquid chromatography (UPLC) coupled with selected reaction monitoring tandem quadrupole mass spectrometry (SRM-TQ MS) throughout plant and fruit development. Above- and below-ground tissues were collected at different stages of development for a comprehensive panel to establish the hormone atlas. Collected data will be incorporated with biological illustration to create a visual representation of the scientific findings and will be made available as a resource to the scientific community through online sources such as the Bio-Array Resource and the Solanaceae Genomics Network. To further understand hormonal role during development and defense, MicroTom mutant and transgenic lines of each major hormone have been crossed to establish a panel of double mutants. MicroTom single and double mutants will be used to address hormonal changes in response to the bacterial pathogen Pseudomonas syringae pv. tomato to address and evaluate hormone interactions critical to disease response. Investigation into hormonal crosstalk throughout tomato plant and fruit development and pathogen infection will allow for a comprehensive map of hormonal networks.

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Primary Poster Presenter: Hannah Berry

Examine the Gene Expression and Functions of Two SLEEPY-1 Homologs in Soybean (0700-020)
Hall 2

Giberellic acid (GA) is an important plant hormone that regulates growth, cell division, seed development. Sleepy 1 (SLY1) positively regulates GA signaling in Arabidopsis. SLY1 is the F-box subunit of an SCF E3 ubiquitin ligase that regulates the GA response by degrading DELLA proteins, RGA and GAI, that inhibit GA signaling. We have identified GmSLY1a and GmSLY1b, two orthologs of SLY1 in soybean whereas Arabidopsis only have one Sly1 gene. Both putative orthologs contain an F-Box motif as well as several other domains found in the Arabidopsis SLY1 protein. However, it has not yet been determined whether these orthologs maintain the same function as SLY1. We measured the relative expression of GmSLY1a and GmSLY1b in different soybean tissues and compare them to Arabidopsis in order to gain insight to the functionality of the two genes. We also transformed the sly1 mutants with these two genes. The sly1 mutant in Arabidopsis has a dwarf phenotype and show variable seed dormancy. We were able to partially rescue the phenotype of Arabidopsis sly by over-expressing GmSLY1a and GmSLY1b separately. Our results suggest that both genes are the SLY1 orthologs in soybean. Though GmSLY1a expresses more in most soybean tissues, GmSLY1b may be more important in regulating flowering and seed germination.

Co-author(s): Sam Henson

Primary Poster Presenter: Pei-Lan Tsou

Extrapolating a Synthetic, Orthogonal Auxin-TIR1 Receptor Pair in Solanum Lycopersicum (0700-024)
Hall 2

Auxins are a class of bioregulatory hormones which impact nearly every aspect of plant growth and development. Indole-3-acetic acid (IAA) and one of its corresponding receptors (TIR1) have been shown to be involved in seed germination, lateral root formation, stem elongation, fruit set and development, along with numerous other developmental processes. In an effort to more effectively study auxin-dependent pathways, Torii et al. developed a synthetic version of the IAA/TIR1 receptor pair which was shown to act independent of the endogenous pair in Arabidopsis. Here, I extrapolate our synthetically engineered auxin-receptor pair into a model system with agricultural and environmental implications (e.g. Solanum lycopersicum, tomato) and aim to test whether we are able to 'hijack' endogenous auxin signaling in tomato to precisely modulate auxin-dependent, spatiotemporal developmental outcomes. These findings could shed some light onto the vastly complex, auxin-dependent developmental process in Solanum lycopersicum and provide a tool to study auxin's specific role in varying developmental processes such as stomatal formation, leaf morphology, temporal flowering and fruit setting.

Primary Poster Presenter: Antonio Chaparro

Histone modification and transcriptional repression regulation in ethylene response (0700-014)
Hall 2

Ethylene is an important plant hormone that regulates plant growth, in which the master transcription activator EIN3 (Ethylene Insensitive 3)-mediated transcriptional activation plays vital roles. But the transcriptional repression in ethylene-mediated growth regulation is unknown. We report here that a Transcriptional Repressor of EIN3-dependent Ethylene-response 1 (TREE1) interacts with EIN3 to regulate transcriptional repression that leads to an inhibition of shoot growth in response to ethylene. Tissue-specific transcriptome analysis showed that most of the genes are down-regulated by ethylene in shoot, and a DNA binding motif was identified that is important for this transcriptional repression. TREE1 binds to the DNA motif to repress gene expression in an EIN3-dependent manner. Genetic validation demonstrated that repression of TREE1-targeted genes leads to an inhibition of shoot growth. Overall, this work establishes a mechanism by which transcriptional repressor TREE1 interacts with EIN3 to inhibit shoot growth via transcriptional repression in response to ethylene.

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Primary Poster Presenter: hong qiao

Identification and Characterization of Gibberellin 2-Oxidase Genes in Apple (0700-017)
Hall 2

Gibberellin 2-oxidases (GA2oxs) are a family of enzymes that catalyze the deactivation of gibberellins (GAs) through 2β-hydroxylation reaction. While they have been genetically modified in many plant species with the aim of developing new cultivars with desirable architecture, stress resistance, biomass, and flowering traits, GA2OX genes are yet to be studied and exploited in the economically important fruit crop apple (Malus x domestica Borkh.). Using Illumina-based transcriptional sequence data and a newly available, high-quality apple genome sequence, we identified a total of 14 canonical GA2OX-like genes in the apple genome. A phylogenetic analysis of MdGA2OX genes suggests they represent seven duplicated genes, consistent with their annotated chromosomal locations within the apple genome and syntenic relationship among apple chromosomes. To characterize the developmental regulation of MdGA2OX genes, we documented their diurnal expression, and their expression in various spur and seedling structures. We found that two of them were ubiquitously expressed in all apple structures studied, suggesting their potential involvement in a variety of GA-mediated processes. In addition, one MdGA2OX gene was consistently and preferentially expressed in both juvenile and adult shoot apices, suggesting its potential role in regulating GA levels in the shoot apex. By treating adult apple trees and young seedlings with bioactive GAs, we found that a small subset of MdGA2OX genes were under tight genetic

control in response to GA, which is known as GA feedforward regulation. Our primary findings paved the way for exploiting GA2ox in apple breeding. To further identify genetic loci that may be important for the development of new apple cultivars with desirable traits, we are now identifying specific components in the mechanism linking GA perception with the response of GA2ox, using the positive regulation of the GA2ox gene family by GA in model plant Arabidopsis.

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Primary Poster Presenter: Songwen Zhang

Initial Characterization of an Arabidopsis mutant partially resistant to the growth factor AtRALF1 (0700-023)
Hall 2

The Rapid Alkalinization Factor (RALF) family of signaling peptides has been shown to play crucial roles in plant growth, development, and stress responses. RALFs induce alkalinization of the apoplast, and with their receptors initiate a signaling cascade that inhibits growth through mechanisms that are not fully understood. In this project, a forward-genetics screen was used to identify EMS-mutagenized lines of Arabidopsis thaliana (Ler-0) that were resistant to AtRALF1-induced root growth arrest (RRMs). Here, we focus on the initial characterization of one of those mutants RRM30.We used data from microarray experiments to compare genes that are up-regulated 30 minutes after a 10 μM AtRALF1 treatment with those up-regulated by other hormones/elicitors. We uncovered significant overlap with the AtRALF1 transcriptional response and the response to a 2 nM flg22 treatment. We therefore hypothesized that some of the RRMs might also be flg22 response mutants. RRM30 was tested for resistance to flg22-induced root growth inhibition. This was done to gain insight into the AtRALF1 signal transduction pathway and to evaluate whether flg22 could serve as a lower cost substitute for AtRALF1 to screen for mapping population recombinants. Seedlings were grown on culture plates containing 2 μM flg22 peptide, and initial experiments suggested that RRM30 was partially resistant to flg22-induced root growth inhibition and surprisingly resistant to shoot growth inhibition. A dose-response assessment was conducted to determine the range of concentrations at which RRM30 is resistant. The results of this assessment indicate RRM30 is more resistant to flg22 at concentrations above 0.5 μM. In addition to flg22, we are characterizing RRM30's response to ethylene, JA, auxin, and other non-signaling peptides. Our microarray data and mutant analysis add to a body of work that suggests flg22 and AtRALF1 probably converge on a common signaling pathway to influence plant growth.

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Primary Poster Presenter: Jonathan Gilkerson

Investigating the stability of ABF transcription factors in Arabidopsis

thaliana (0700-002)
Hall 2

The ABA Responsive Element Binding Factor (ABF) family of transcription factors plays an important role in abscisic acid (ABA) signaling during vegetative growth. Under conditions of elevated ABA, ABF proteins induce transcription of ABA-responsive genes to enhance a plant's ability to respond to abiotic stresses. We are interested in how ABA leads to an increase in ABF activity. Previous studies in our lab showed that the degradation of ABF1 and ABF3 proteins is slowed after seedlings are treated with ABA (1). However, the link between ABF stability and changes in activity has yet to be established. To better understand how ABA activates ABFs and whether protein stability plays a role, we are studying the degradation and post-translational regulation of ABF proteins in Arabidopsis thaliana using both in vitro and in vivo methods. It has been shown that ABFs are phosphorylated at five conserved serines or threonines in an ABA-dependent manner, and four of these phosphorylations are important for ABF activity (2). Our current experiments focus on determining whether these phosphorylations play a role in ABF protein stability. Using transgenic plants expressing ABFs with substitutions at these sites, we are measuring ABF degradation under normal conditions and after ABA treatment. Results from these experiments will provide insight into the mechanisms by which ABA induces ABF activity, and the role of proteolysis in ABF activation. Supported in part by NSF-IOS 1557760.

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Primary Poster Presenter: Katrina Linden

KARRIKIN-UPREGULATED F-BOX1 regulates KARRIKIN-INSENSITIVE2 signaling by a negative feedback loop (0700-012)
Hall 2

Karrikins (KARs) are plant growth regulators found in smoke that promote seed germination and influence seedling growth. KAR responses require KARRIKIN-INSENSITIVE 2 (KAI2), a receptor that is also thought to be activated by an endogenous, undiscovered molecule called KAI2 ligand (KL). Recent evidence suggests that KARs are metabolised before perception by KAI2. KAI2 works with the F-box protein MAX2 to promote KAR/KL responses by targeting SUPPRESSOR OF MORE AXILLARY GROWTH2 1 (SMAX1) for degradation. In a search for additional components of KAR/KL responses in Arabidopsis thaliana, we characterized the function of KARRIKIN-UPREGULATED F-BOX PROTEIN1 (KUF1), a reliable transcriptional marker for karrikin signaling. We used an egg-cell promoter-driven CRISPR-Cas9 system to create a loss-of-function mutation in KUF1. We found that kuf1 mutant phenotypes are consistent with enhanced KAI2 activity. Interestingly, kuf1 plants are hypersensitive to KAR1, but not to other molecules that stimulate KAI2. This indicates that KUF1 influences the ability of KAI2 to recognize specific signals. We hypothesize that KUF1 inhibits KAR/KL responses by

regulating KAR1/KL metabolism, and that it may be involved in a negative feedback loop with KAI2.

Primary Poster Presenter: Michael Guzman

Mapping of the wheat ABA hypersensitive mutant ERA8 identified TaMKK3 as a candidate gene (0700-003)
Hall 2

Preharvest sprouting (PHS), the initiation of mature grain germination on the mother plant when rainy conditionsoccur before harvest, can result in serious economic losses for wheat farmers. PHS tolerance is associated withhigher grain dormancy, the inability to germinate even under favorable conditions. The plant hormone abscisic acid(ABA) induces dormancy during seed maturation, and maintains dormancy in mature seeds. The ABAhypersensitive mutant of wheat, ERA8 (Enhanced Response to ABA8), resulted in increased seed dormancy andPHS tolerance. The EMS-induced ERA8 mutation was mapped in a backcross population by conducting bulked-segregant analysis (BSA) of wild-type versus ERA8 exome-sequence. Fine mapping localized ERA8 to a 4.6 Mbregion of chromosome 4A containing 70 genes. Within this region, ERA8 was most tightly linked to TaMKK3-A, aknown PHS-related gene (16.5 LOD score). ERA8 was associated with a missense mutation in the MKK3 NTF2domain, whereas the previously published PHS tolerance allele was associated with a single nucleotidepolymorphism in the kinase domain. The ERA8 interval was confirmed by QTL analysis of an Otis/ZakERA8population but not in a Louise/ZakERA8 population. This is likely because Louise carries the original PHS tolerantallele of TaMKK3-A whereas Otis carried the PHS susceptible allele of TaMKK3. This inadvertent complementationtest suggests that the ERA8 phenotype results from the TaMKK3-A-G1093A mutation, a new PHS tolerance allele.To identify possible regulatory DNA mutations which could have been missed by the exome-capture approach, wealso performed RNA-Seq of imbibed seeds from wild-type Zak and ERA8. None of these 70 genes showed thedifferential gene expression which expected from a promoter mutation. The ERA8 flanking KASP markers identifiedin this study can be used for marker-assisted selection or rapid genomic selection for PHS tolerance in wheatbreeding programs.

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Primary Poster Presenter: Camille Steber

PBS3 is the missing link in plant-specific isochorismate-derived salicylic acid biosynthesis (0700-006)
Hall 2

The phytohormone salicylic acid (SA) is a central regulator of plant immunity. Despite this functional importance, our knowledge of SA biosynthesis is incomplete.

Previous work showed that salicylates are synthesized from chorismic acid in plastids. The bulk of pathogen-induced SA derives from isochorismate generated by the catalytic activity of ISOCHORISMATE SYNTHASE1 (ICS1). How and in which cellular compartment isochorismate is converted to SA is unknown. We show that the pathway downstream of isochorismate requires only two additional proteins: the plastidial isochorismate exporter ENHANCED DISEASE SUSCEPTIBILITY5 (EDS5) and the cytosolic amido-transferase AvrPphB SUSCEPTIBLE3 (PBS3). PBS3 catalyzes the conjugation of glutamate to isochorismate. The reaction product Isochorismate-9-glutamate spontaneously decomposes into enolpyruvyl-N-glutamate and SA. This previously unknown reaction mechanism appears to be conserved throughout the plant kingdom.

Co-author(s): Ivo Feussner

Primary Poster Presenter: Dmitrij Rekhter

Phytohormones in the light of plant evolution: Towards the understanding cytokinin phylogenetic patt (0700-013)
Hall 2

Evolutionary physiology represents an explicit fusion of two approaches, evolution and physiology. We address an important question regarding physiological evolution: What phylogenetic patterns are reflected in the metabolism of phytohormones cytokinins during plant evolution? In our comprehensive screening throughout the plant kingdom and fungi, CK-N7- and CK-N9-glucosides were found ubiquitous in vascular plants differing among main evolutionary groups. Contrarily, their only rather low levels or a total absence was shown in non-vascular plants, algae and mosses. Surprisingly, fungi representatives showed similar CK spectra with a lack of CK-N-glucosides and a prevalence of cis-zeatin types as lower plants. It is possible that the absent or sparse N-glucosyltransferase pathway is substituted here by other down-regulating mechanisms, e.g. by enhanced formation of cis-zeatin derivatives, as considerably higher cis/trans-zeatin ratios were revealed for most of non-vascular than vascular plants. Moreover, comparison of CK distribution in angiosperms revealed prevalence of tZ7G and tZ9G from all CK-N-glucosides. This study represents a preliminary background for study of male gametophyte in the evolutionary context.The phylogenetic analysis of ZOG gene (zeatin O-glucosyltransferase) defining two distinct groups generally agreed with monocot and dicot evolutionary plant lineages. Within these groups, no phylogenetic clades corresponding to exact ZOG gene families were found. Rather tree structure showing different orders in separate branches. They were confirmed by the presence of the PSPG motif with typical position 41 (His) and 42 (Ser) corresponding to the UGT subfamily O and classified into 19 main groups (7 in monocots and 12 in dicots). BLAST and PSPG domain analyses showed that ZOG gene seems to be unique in angiosperms, however, being absent in Arabidopsis thaliana and other members of order Brassicales. Supported by the Czech Science Foundation (19-02699S).

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Primary Poster Presenter: Lenka Zaveska Drabkova

PUB40-mediated root-specific degradation of Brassinazole Resistant 1 in Arabidopsis (0700-021)
Hall 2

The steroid hormone brassinosteroid (BR) regulates a wide range of physiological responses through activation of Brassinazole Resistant 1 (BZR1). BZR1 activity in plant tissues is tightly controlled by its phosphorylation status and degradation. Although BZR1 appeared to be degraded in distinct ways under differential hormonal or environmental cues, little is known how BR signaling regulates BZR1 degradation. Here we show that BR-regulated PUB40 mediates proteasomal degradation of BZR1 in a root-specific manner. Notably, the bzr1-1D gain-of-function mutation reduces the interaction with PUB40, which suppresses PUB40-mediated BZR1 degradation in the roots. Moreover, we demonstrate that cell layer-specific expression of PUB40 in the root tip contributes to induce selective BZR1 accumulation in the epidermal layer. Both BR treatment and PUB40 loss-of-function expanded BZR1 accumulation to most of the cell-layers. The triple mutant for PUB40 and its homologs, pub39 pub40 pub41, displayed significantly increased BR sensitivity in roots but not hypocotyls. In addition, resistance to low inorganic phosphate availability shown in the bzr1-1D mutant was also observed in the triple mutant roots. We demonstrate that BIN2 interacts with and phosphorylates PUB40, leading to elevated interaction with BZR1 as well as PUB40 stability. Our results suggest a molecular mechanism of root-specific BZR1 degradation regulated by BR signaling.

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Primary Poster Presenter: Tae-Wuk Kim

The Arabidopsis BRIZ ubiquitin E3 ligase functions in abscisic acid response (0700-001)
Hall 2

The ubiquitin system is essential in multiple hormone signaling pathways in plants. Here we characterize further the loss of an Arabidopsis thaliana RING E3 ligase called BRIZ and identify a pathway in which BRIZ participates. BRIZ is a heteromeric ligase proposed to consist minimally of both BRIZ1 and BRIZ2 proteins. On growth media, both briz1 and briz2 homozygous mutant seeds either fail to germinate or seedlings emerge later than wild type with little cotyledon expansion or greening and no root elongation. However, viability stainings indicate briz1 or briz2 embryos are alive at least 30 days post-plating. Growth of briz1 or briz2 seedlings is stimulated by addition of the carotenoid biosynthetic inhibitor

fluridone, but the briz phenotype returns with addition of low levels of abscisic acid (ABA). Endogenous ABA is not higher in briz2 seeds compared to wild type after 24 hr imbibition and ABA does not regulate BRIZ mRNA abundance in 6-day-old seedlings. These data suggest briz mutants are hypersensitive to ABA. In either the abi5-7 or gin1-3 (low ABA) background, a higher percentage of homozygous briz2 seeds germinate and develop green cotyledons, true leaves and inflorescences. ABI5 is a major transcription factor during seed maturation and seed germination. The nature of BRIZ's relationship to ABI5 action is currently under investigation. From these data, we propose a model in which the BRIZ E3 ligase functions as a negative regulator of ABA responses only at a specific development stage either during late seed maturation or early during germination. Supported in part by NSF-IOS 1557760.

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Primary Poster Presenter: Katrina Linden

The key amino acids involved in the enzymatic activity of rice GA2oxidases
(0700-008)
Hall 2

GA2-oxidases (GA2oxs) are enzymes involved in phytohormone Gibberellin (GA) catabolism, which inactive GA by 2β-hydroxylation to regulate plant growth and development. Three classes of rice GA2oxs were classified based on protein sequence similarity. Among them, class III GA2oxs are able to inactive C20-GAs, while class-I and class-II GA2oxs are able to inactive C19-GAs. The transgenic plants overexpressing any of these GA2oxs display severe (70-90%) inhibition effects on rice growth except OsGA2ox7- a class II GA2ox that revealed low (~30%) growth inhibition. Further phylogenetic analysis of class-II GA2oxs from Poaceae showed OsGA2ox7 and its GA2ox7 orthologs forming a separated clade from other GA2oxs (GA2ox3, GA2ox4, GA2ox8), and orthologous genes BdGA2ox3、BdGA2ox4 and BdGA2ox7 from Brachypodium distachyon were functional identified to show the same inhibition effects as their counterparts in rice. Through multiple sequence alignment of all class-II GA2oxs, and protein structure simulation study, two amino acids, E227 and F283, located around the putative substrate binding site were considered as the key amino acids that affect their enzymatic activities. Substitution of these two key amino acids between OsGA2ox3 and OsGA2ox7 by point mutation and domain swapping demonstrated their important roles in affecting the enzymatic activity of GA2oxidases. Biochemical and CRISPR/cas9 approaches will be performed to confirm their effects on enzymatic activities and growth inhibition.

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Primary Poster Presenter: Liang-Jwu Chen

The molecular basis for enhanced responses to karrikins, a class of germination stimulants in smoke (0700-010)
Hall 2

Many fire-following plant species require fire for the germination of their seeds. Some of these plants germinate after fires because they have adapted to sense molecules found in smoke called karrikins (KARs). Responses to KARs require the receptor KARRIKIN INSENSITIVE2 (KAI2), which is found in all land plants. The evolutionary process behind the increased KAR sensitivity in fire-followers is currently unknown. We hypothesize that fire-followers have KAI2 proteins that are more sensitive to KARs. To investigate the basis of high sensitivity to KARs, we examined the function of KAI2 genes in lettuce (Lactuca sativa), which responds to nanomolar concentrations of KARs. We hypothesized that one of the two genes in L. sativa has evolved an increased sensitivity to KARs. To test this hypothesis, we introduced L. sativa KAI2 genes into Arabidopsis thaliana. We found that L. sativa KAI2B is more responsive to KAR1 than L. sativa KAI2A. A comparison of the protein sequences of the two L. sativa KAI2 and other KAI2 with differential responses to KARs revealed four candidate residues of interest associated with the ligand binding pocket. To analyze the contribution of these residues to KAR sensing, we created a series of KAI2 mutants and tested their function in transgenic Arabidopsis plants. Our analysis shows that these residues are important for determining KAR specificity. In future work we will survey KAI2 sequences in fire-followers to investigate whether convergent evolution at these positions has led to increased sensitivity to KARs.

Co-author(s): David Nelson

Primary Poster Presenter: Stephanie Martinez

Understanding KAI2-SMAX1 interaction site (0700-011)
Hall 2

The karrikin and strigolactone signaling pathways in plants are two homologous pathways that affect many different plant processes such as germination and branching, respectively. While karrikins and strigolactones are both butenolide compounds, karrikins are small molecules released in smoke and strigolactones are a class of endogenous plant hormones. In Arabidopsis thaliana, the receptor in the karrikin pathway is known as KARRIKIN INSENSITIVE2 (KAI2) and the receptor for strigolactonesis DWARF14 (D14). KAI2 and D14 are alpha/beta hydrolases that have a high degree of similarity at the sequence and structural levels due to their shared ancestry. D14, which emerged in the gymnosperms following a KAI2 duplication, targets a clade of D53-type proteins (SMXL6/7/8) in the SMAX1-LIKE (SMXL) family for degradation upon activation by strigolactones. KAI2 likely targets proteins in a separate clade (SMAX1 and SMXL2) for degradation. The major aim of

this project is to understand the specificity determining residues in the interaction site of KAI2 with SMAX1, and how this differs from the D14-SMXL7 interaction site. We identified conserved amino acids on the surface of KAI2 that have the potential to confer protein binding specificity. At the corresponding positions on D14, these amino acids are highly conserved as different residues. Using yeast two-hybrid, we have found substitutions that change the affinity of KAI2 for SMAX1 and allows it to interact with SMXL7. The findings from this project helps establish the molecular basis for receptor-target specificity in karrikin and strigolactone signaling pathways, enabling us to investigate the evolutionary basis of their diversification. This project also deepens the understanding of how karrikin and strigolactone signaling can be rewired.

Primary Poster Presenter: alexandra white

WRKY71 transcription factor mediates crosstalk of abscisic acid and gibberellin signaling in rice (0700-016)
Hall 2

Abscisic acid (ABA) and gibberellins (GAs) are phytohormones widely recognized to play mostly antagonistic roles in controlling several plant developmental processes including seed maturation, dormancy, and germination. Our previous studies via particle bombardment-mediated transient expression indicate that WRKY71 functions as a negative regulator of GA signaling in aleurone cells. Herein, we present genetic evidence to show that wrky71 mutant rice lines have higher α-amylase activities in aleurone cells compared to wild-type. Exogenous GA3 treatments induced over 10 times more α-amylase activity in the mutants compared to wild-type. In contrast, inhibition by exogenous ABA treatments on GA-induced α-amylase activities was approximately 3 times more in the mutants compared to wildtype. Quantitative RT-PCR analyses revealed the α-amylase genes whose responses to GA and GA plus ABA are altered in the mutants. Together, these data suggest that the mutants are hypersensitive to GA induction but hyposensitive to ABA repression of GA induction in terms of α-amylase gene expression. RNA-seq was carried out to address the molecular foundation of the hypersensitivity to GA induction and hyposensitivity to ABA repression and to reveal the direct and indirect targets of the WRKY71 transcription factor. Overall, our data firmly established that WRKY71 is a negative regulator of GA signaling in aleurone cells; it mediates the crosstalk of GA and ABA signaling by targeting key GA and ABA signaling pathways and regulating the expression of hundreds of genes.

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Primary Poster Presenter: Santiago Bataller

A simple and sensitive SYBR Gold-based assay to quantify DNA-protein

interactions (0700-035 (Screen 4))
Hall 2

Auxin is one of the key plant hormones and regulates several developmental processes. Auxin inducible gene expression is mediated primarily by Auxin Response Factor (ARF) transcription factors binding as dimers to Auxin Response Elements (AuxREs) in the promoters of auxin-inducible genes. Detecting DNA-protein interactions (AuxRE-ARF) is a first step towards understanding their function in plants. We developed a simple and sensitive SYBR Gold- based assay to detect and quantify DNA-protein interactions. The assay is based on the versatility of SYBR Gold to bind to double stranded nucleic acids in a dose dependent manner. 6xHis-tagged recombinant protein were combined with dsDNA (AuxREs) and incubated to facilitate binding. The protein DNA complex is then captured using Ni-NTA sepharose resin. DNA protein complex is then eluted using elution buffer containing imidazole and the DNA is stained with SYBR Gold and quantified. Quantification of fluorescence from SYBR-DNA complex was detected using a plate reader, Odyssey imager and UV transilluminator to test its utility in various research and undergraduate labs. The assay was validated using AtARF5 for which binding affinities were reported earlier. We tested both AtARF5 and GmARF8a, activator ARFs from Arabidopsis and soybean respectively for their binding affinity to different AuxREs. Both the ARFs showed significantly higher binding to AuxREs tested (ER7-TGTCTC/ER7GG-TGTCGG) compared to control. AtARF5 showed greater affinity for ER7GG, while GmARF8a showed its affinity for canonical ER7, suggesting the preference ARF exhibit for different AuxREs.

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Primary E-Poster Presenter: Bhanu Petla

Signal Transduction

Arabidopsis ACYL-COA-BINDING PROTEIN1 regulates sterol-mediated signaling by protein–protein interac (0600-020 (Screen 8))
Hall 2

Sterols are isoprenoid derivatives and membrane constituents. Whereas animals and fungi primarily produce cholesterol and ergosterol, respectively, plants synthesize a diversity of sterols and sterol biosynthetic intermediates that can function as signaling molecules. However, the regulation of plant sterol synthesis and signaling remain little understood. Our studies showed that Arabidopsis ACYL-COA-BINDING PROTEIN1 (ACBP1) modulates a rate-limiting step in the sterol pathway by interacting with STEROL C4-METHYL OXIDASE1 (SMO1-1 & SMO1-2).

The ACBP1–SMO1 interaction is developmentally important considering the lethality of SMO1-1/SMO1-2 knockdowns in acbp1 and the aberrant fatty acid and sterol composition resulting from manipulation of SMO1 and/or ACBP1 expression. As fatty acids and sterols are acetyl-CoA derivatives, our studies provide critical insights into the metabolic crosstalk between these two lipid classes. The ligand-binding status of ACBP1 may reflect the cellular levels of certain acyl-CoA or phospholipid species, thereby influencing SMO1 in generating sterol signals. SMO1-2 silencing in hemizygous acbp1 mis-regulated genes encoding homeodomain-leucine zipper IV transcription factors (e.g. GLABRA2), which potentially bind phospholipids/sterols. GLABRA2 targets were also affected, leading to glabra2-like trichome, seed coat mucilage and high-oil phenotypes. Collectively, we assign a new role for ACBP1 in cellular signaling. Reference: (1) Lung et al. 2017. Acyl-CoA-binding protein ACBP1 modulates sterol synthesis during embryogenesis. Plant Physiol 174:1420–35 (2) Lung et al. 2018. Arabidopsis ACYL-COA-BINDING PROTEIN1 interacts with STEROL C4-METHYL OXIDASE1-2 to modulate gene expression of homeodomain-leucine zipper IV transcription factors. New Phytol 218: 183–200 (3) Lung & Chye. 2019. Arabidopsis acyl-CoA-binding proteins regulate the synthesis of lipid signals. New Phytol, in press [Funded by Hong Kong Research Grants Council (AoE/M-403/16)]

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Primary E-Poster Presenter: Shiu-Cheung Lung

A molecular network for plants acclimation to warm temperature (0600-022 (Screen 12))
Hall 2

Temperature is a key signal for plant growth and development. To face the challenges of crop yields imposed by global warming, we need to understand how plants acclimate to the elevated temperature and apply these knowledge to generate thermo-tolerant crops. Warm ambient temperatures, below the heat-stress range, cause plant morphological and architectural changes, which is called thermo-morphogenesis. Arabidopsis acclimates to warm temperature through promoting stem elongation, which moves the sensitive meristematic and photosynthetically active tissues away from heat-absorbing soil and promotes cooling by allowing better access to moving air. Despite the important role of thermo-morphogenesis on plants, the underlying signaling pathways are not studied comprehensively. A transcription factor named phytochrome-interacting factor 4 (PIF4) has been identified as a major regulator of thermo-morphogenesis in Arabidopsis. Here our studies reveal that thermo-responsive stem elongation is regulated by a transcription module which consists not only PIF4, but also BRASSINAZOLE RESISTANT 1(BZR1) and AUXIN RESPONSE FACTORS (ARFs), which are the transcription factors of the phytohormone brassinosteroid (BR) and

auxin signaling pathways. Genetic analysis indicates that the transcription module integrates hormonal and environmental signals in response to warm temperature. In addition, a GSK3-like kinase BIN2 phosphorylates and inactivates PIF4 under the light. Our previous studies have demonstrated that KIB1 inactivates BIN2 in the presence of BR. Here we uncover the post-translation regulation of PIF4 protein levels through KIB1. The genetic and biochemical analysis show that warm temperature induces KIB1 protein accumulation and promotes BIN2 degradation, contributing to the dephosphorylation and accumulation of PIF4. Our studies reveal KIB1 promotes thermo-response growth as a molecular link between BR and warm temperature signaling pathways.

Co-author(s): Zhi-Yong Wang

Primary E-Poster Presenter: Jiaying Zhu

A NAC domain transcription factor XVP regulates vascular stem cell function

Vascular stem cells proliferate, and then progeny cells differentiate into xylem and phloem tissues that transport water, nutrients and other molecules vital for plant growth. Plant stem cell maintenance, mostly derived from studies of SAM and RAM, involves a feedback loop that comprises a CLE peptide, a leucine rich repeat receptor like kinase (LRR-RLK) and a downstream WOX transcription factor. Vascular stem cells are also regulated by a CLE peptide signaling, but no feedback regulatory mechanism has been identified. We report that a novel NAC domain transcription factor XVP function as a negative regulator of TDIF peptide signaling in vascular meristem. The XVP protein is localized on plasma membrane and forms a complex with TDIF co-receptors PXY-BAK1. Simultaneous mutations of XVP and its close homologs enhance TDIF signaling. XVP activates the TDIF coding gene CLE44, while over-expression of TDIF represses XVP. Therefore, XVP negatively regulates the TDIF-PXY module, and fine-tunes TDIF signaling in vascular development through a feedback loop. Genetics analysis also indicated that XVP promotes xylem differentiation through master regulator VND6. These results enhanced our understanding of the peptide signaling in vascular meristem, and shed new light on the regulation of stem cell maintenance.

Chair and Concurrent Symposium Speaker: Shuo Yang, Liying Qi, Qian Du, Jung Hyun Yang, Kwang-Hee Lee

Primary Poster Presenter: Huanzhong Wang

A receptor-like kinase phosphorylates Gα protein to control signaling during soybean nodulation (0600-004)

Hall 2

Heterotrimeric G-proteins, comprised of Gα, Gβ and Gγ subunits regulate signaling in all eukaryotes. While in non-plant systems G-proteins are activated by GPCR-mediated GDP to GTP exchange on Gα, the roles of receptors in regulating plant G-proteins remains equivocal. Mounting evidence points to the involvement of receptor like kinases in G-protein mediated pathways in plants. We have previously shown that during soybean nodulation, Gα and its regulatory RGS proteins interact with the nod factor receptors (NFR1) to control signaling. NFR1 phosphorylates RGS to enhance deactivation of Gα, which act as negative regulators of nodulation. We now demonstrate direct, phosphorylation-based regulation of Gα by symbiosis receptor kinase (SymRK). SymRKs interact with and phosphorylate Gα at multiple sites, including two in its GTP-binding region, which abolishes GTP-binding. Phospho-mimic Gα is also unable to interact with Gβγ, potentially allowing for constitutive signaling by the freed Gβγ. Overexpression of the phospho-mimic Gα results in phenotypes similar to the overexpression of Gβγ. These results identify a novel mechanism of the regulation of G-protein cycle in plants where receptor-mediated phosphorylation of the Gα protein not only affects its activity, but also influences the availability of its signaling partners, exerting a two-pronged control on signaling by G-proteins.

Co-author(s): Swarup Roy Choudhury

Primary Poster Presenter: Sona Pandey

Arabidopsis VPS13a supports rapid pollen germination during pollen rehydration on the stigma (0600-007)
Hall 2

Pollination is an important step in flowering plant reproduction. Pollen receives water from the stigma, becomes hydrated, then germinates and grows a pollen tube into the female tissues to deliver sperm cells for fertilization. A forward genetic screen using novel pollen compatibility assay revealed that Arabidopsis thaliana Vacuolar protein sorting-associated protein 13a (AtVPS13a) was important for pollen germination on the stigma. The hydration of vps13a pollen on stigma was intact, however, the mutation affected pollen germination ability resulting in a reduced seed set of the mutant. GUS expression analysis and reciprocal cross experiment showed that VPS13a expressed gametophytically in pollen implicated in fertilization success of the pollen grain. VPS13a contains a predicted Ca2+-dependent lipid binding C2 domain suggesting its involvement with Ca2+ signaling. Live-imaging of pollen expressing the calcium-biosensor, Yellow Cameleon 3.6, showed that vps13a pollen calcium dynamic after pollination was not different from that of wild type pollen grains. We propose that VPS13a senses Ca2+ dynamic in the pollen grain after compatible pollination and mediates cellular response(s) essential for rapid pollen germination on the stigma which leads to successful fertilization of Arabidopsis.

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Primary Poster Presenter: Surachat Tangpranomkorn

BSU1 family phosphatases mediate Flagellin-FLS2 signaling through specific phosphocodes. (0600-005)
Hall 2

There are hundreds of receptor-like kinases (RLKs) in plants and how they induce specific cellular responses remains an outstanding question. The brassinosteroid (BR) hormone receptor kinase BRI1 and immunity receptor kinase flagellin sensing 2 (FLS2) share a common co-receptor kinase and some of their kinase substrates, but lead to growth and immunity through inactivation of the growth-inhibiting GSK3-like kinases and activation of the immunity-promoting MAP kinases, respectively. Here we show that the BSU1 family of phosphatases, known to mediate BR inactivation of GSK3s, also mediate FLS2 signaling to the MAP kinases, through different phosphocodes. The bsu quadruple mutant showed defects in pathogen-associated molecular pattern (PAMP)-triggered immune responses such as MAP kinase activation, flagellin-responsive gene expression and flagellin-induced bacterial growth inhibition. BSU1 family of phosphatases are phosphorylated upon flagellin elicitation. The Botrytis-induced kinase 1 (BIK1), a substrate of FLS2, phosphorylated the N-terminal kelch-repeat domain of BSU1 at serine-251 (S251) in a flagellin-dependent manner. Mutation of S251 to alanine reduced BIK1 phosphorylation and abolished BSU1's ability to restore flagellin-induced MAP kinase activation of the bsu quadruple mutant, without affecting its ability to activate BR-dependent growth. Our results demonstrate that BRI1 and FLS2 transduce hormonal and pathogen signals to specific growth and immunity responses through shared downstream components, the BSU1 family phosphatases, by using different phosphorylation sites. Our results suggest that many RLKs may share downstream components and maintain signaling specificity through phosphocodes in higher plants.

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Primary Poster Presenter: Chan-Ho Park

Characterization of Arabidopsis Early Gravity Response Genes (0600-010)
Hall 2

Gravity signaling and response are vital to plant growth and architecture. Despite its importance, little is known of the earliest aspects of this signaling. Gravity Persistence Signal (GPS) treatment was used to isolate early signaling transduction events. For this treatment, Arabidopsis plants were placed on their side at 4° C. At

2, 4, 10, and 30 minutes after reorientation in the cold, the inflorescence stems were collected, flash frozen in liquid nitrogen, and RNA extracted for a gene expression microarray. At the 2 minute time point, the genes PP2-A13, ARL, and NRT1.12 were found to be differentially expressed. PP2-A13 was upregulated with a log2 fold (LF2) change of 1.34, NRT1.12 with a LF2 change of 1.29, and ARL with a LF2 change of 1.08. Mutant lines were bred to homozygosity for PP2-A13, ARL, and NRT1.12, with homozygosity confirmed via PCR and knock-out confirmed via RT-PCR. Phenotypic analysis revealed PP2-A13 to have a significant decrease in stem and root curvature as compared to WT. ARL expressed a significant increase in stem curvature. RT-PCR for NRT1.12 revealed the SALK line's T-DNA insert to be spliced out as the result of occurring in an antisense gene inside of NRT1.12, potentially causing over expression of NRT1.12 rather than disrupting it. Phenotypic analysis of NRT1.12 showed a significantly reduced angle of root skewing compared to WT. These phenotypic data suggest that PP2-A13, ARL, and NRT1.12 likely take part in the early gravitropic response. Partial funding for this project was provided by NSF (IOS #1147087) to SEW.

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Primary Poster Presenter: Ava Heller

Characterizing the Function of VICTR-like Genes and Their Role in the Regulation of ABA in Response (0600-001)
Hall 2

The hormone abscisic acid (ABA) triggers signal transduction that mediates drought resistance. A synthetic small molecule "DFPM" ([5-(3,4-dichlorophenyl) furan-2-yl]-piperidine-1- ylmethanethione) has been shown to negatively regulate ABA signaling and stimulate plant defense related genes. The Variation In Compound Triggered Root growth response (VICTR) locus is required for DFPM-mediated root growth arrest in Arabidopsis thaliana. VICTR encodes a Toll-Interleukin1 Receptor–nucleotide binding–Leucine-rich repeat (TIR-NB-NLR) protein. To determine whether VICTR and its homologous tandem genes play a role in DFPM inhibition of ABA signal transduction we utilized a Near Isogenic Line (NIL) NIL-Bu-5 of Arabidopsis with accessions of the Bu-5 ecotype crossed into the background of Columbia-0 (Col-0). NIL-Col-0 which contains functional VICTR and its homologous genes and NIL-Bu-5 which lacks all four tandem VICTR homologs were used in this study. We investigated whether VICTR and the VICTR-like genes contribute to the regulation of ABA signal transduction. We first grew seedlings of Col-0, NIL-Col-0, and NIL-Bu-5 for two weeks, exposed plants to various chemical conditions including ABA, DFPM, and solvent control for 5 hours followed by RNA extraction, complementary DNA synthesis, and reverse transcriptase-quantitative PCR to analyze expression of ABA reporter genes RAB18, RD29A, and ERD10. We analyzed fluorescence intensity of the pRAB18::GFP ABA signaling reporter after chemical treatments and compared Mitogen-Activated Protein Kinase (MAP Kinase) activity between genotypes after DFPM treatment under various exposure times. DFPM-mediated

MAP Kinase activation was not disrupted when VICTR and its homologs are lacking. The roles of VICTR and its homologs are being studied with respect to ABA signal transduction under ABA and DFPM chemical treatments, further analysis will allow us to understand the function of VICTR and its homologous genes in DFPM signal transduction.

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Primary Poster Presenter: Eduardo Ramirez

Developmental Programming of Thermonastic Leaf Movement (0600-014)
Hall 2

Plants exhibit diverse polar behaviors in response to directional and non-directional environmental signals, termed tropic and nastic movements, respectively. The ways in which plants incorporate directional information into tropic behaviors is well understood, but it is less well understood how non-directional stimuli, such as ambient temperatures, specify the polarity of nastic behaviors. Here, we demonstrate that a developmentally programmed polarity of auxin flow underlies thermo-induced leaf hyponasty in Arabidopsis (Arabidopsis thaliana). In warm environments, PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) stimulates auxin production in the leaf. This results in the accumulation of auxin in leaf petioles, where PIF4 directly activates a gene encoding the PINOID (PID) protein kinase. PID is involved in polarization of the auxin transporter PIN-FORMED 3 (PIN3) to the outer membranes of petiole cells. Notably, the leaf polarity-determining ASYMMETRIC LEAVES 1 (AS1) directs the induction of PID to occur predominantly in the abaxial petiole region. These observations indicate that the integration of PIF4-mediated auxin biosynthesis and polar transport, and the AS1-mediated developmental shaping of polar auxin flow, coordinate leaf thermonasty, which facilitates leaf cooling in warm environments. We believe that leaf thermonasty is a suitable model system for studying the developmental programming of environmental adaptation in plants.

Co-author(s): Chung-Mo Park

Primary Poster Presenter: Young-Joon Park

Dynamic Plasticity of the Arabidopsis Circadian Oscillator in Response to Sugar Signals (0600-002)
Hall 2

The defining characteristic of circadian rhythms is that they have a period of about 24 h in constant conditions. However, circadian period is not fixed, it is variable. Many signals regulate the speed of the circadian clock in a reversible manner, with the effect dependent on the time of the day that the stimulus is experienced in a process we have called dynamic plasticity (Webb et al., 2019 Nature Comms 10, 550). We have been investigating the mechanism and purpose of the dynamic

plasticity of the circadian oscillator to sugar signals. We have previously demonstrated that sugars can speed up the circadian oscillator and identified three signalling pathways by which sugars regulate the circadian oscillator, including one dependent on the regulation of the expression of the circadian clock gene PSEUDO-RESPONSE REGULATOR 7 (PRR7) by the energy sensitive transcription factor bZIP63 (Frank et al., 2018 Current Biol. 28, 2597-2609). We are now investigating why the circadian oscillator responds to sugar signals. We will describe new data that demonstrates that the circadian oscillator responds to endogenous changes in sugars that affect the entrainment of the circadian oscillator to light intensity and photoperiod dependent on the correct functioning of PRR7. Experimentation and mathematical modelling demonstrate that responses of the circadian oscillator to responses to moderate changes in light intensity can be explained in terms of changes in sugar signalling associated with the management of transient starch reserves in the leaf. Our data suggest that response the circadian oscillator to endogenous sugar signals is required for the correct timing of internal events with respect to the environment.

Primary Poster Presenter: Alex Webb

GSK3-like kinases modulate cortical microtubule stability following abiotic stress through interacti (0600-012)
Hall 2

Cellulose is one of the most abundant biopolymers on the planet, and synthesis of this paracrystalline polysaccharide is mediated by the cellulose synthase complex (CSC). CSCs contain multiple non-redundant Cellulose Synthase A (CESA) subunits as well as multiple accessory subunits, including Cellulose Synthase Interactive 1 (CSI1), the KORRIGAN endoglucanase, and the recently identified Companion of Cellulose Synthase (CC) proteins. While CSC composition is becoming increasingly clear, the regulatory mechanisms controlling CSC activity are poorly understood. Large-scale phosphoproteomic surveys indicate that CSC subunits contain multiple spectrally supported phosphorylation sites, however, the mechanisms of CSC phosphoregulation remain to be fully elucidated. In Arabidopsis thaliana, cortical microtubules (MTs) depopulate the plasma membrane following abiotic stress, promoting CSC sequestration into Small CESA Compartments (SmaCCs). CC proteins promote cortical microtubule stability under normal growth conditions and the cc1;cc2 double mutant displays decreased cortical microtubule stability following salt stress. Additionally, phosphoproteomic data indicates that CC1 is phosphorylated at multiple sites throughout its N-terminal domain, suggesting that phosphorylation may regulate CC1 function. Here, we show that CC1 is post-translationally regulated by Brassinosteroid signaling through phosphorylation by the BRASSINOSTEROID INSENSITIVE 2 (BIN2) kinase. CC1 is polyphosphorylated at its N-terminus by BIN2, and these phosphorylation events negatively regulate the tubulin stabilization activity of CC1. Additionally, CC1 phosphorylation by BIN2 has virtually no effect on the MT-binding activity of CC1. These data suggest a mechanism by which brassinosteroid signaling controls CSC dynamics during

development and subsequent BIN2 activity causes CC1 to promote depolymerization of cortical microtubules.

Co-author(s): Staffan Persson

Primary Poster Presenter: Bret Hart

Mapping Proteome-Wide Targets of Protein Kinases in Plant Stress Responses (0600-017)
Hall 2

Eukaryotic protein kinases are major regulatory components for various cellular functions. By adding a phosphate group to substrate proteins, protein kinases control the activity, localization, association with other proteins, and overall function of many proteins, and thereby regulate almost all cellular processes. Despite the importance of phosphorylation-dependent signaling for cellular physiology, the identification of kinase substrates, which is essential for understanding the role of the kinases in signaling pathways, is still very challenging. Here we developed a new kinase assay linked phosphoproteomics approach to allow for a high throughput identification of kinase substrates, using one single mass spectrometry analysis for each kinase. This new strategy is based on isotope-labeled in vitro phosphorylation reactions using in vivo phosphorylated peptides as kinase substrate pools, and our use of enriched phosphoproteomes substantially reduces the complexity of substrate pools. Moreover, stable isotope labeled-ATP is used as ATP donor to avoid the interference from background phosphorylation, and a two-step phosphopeptide-enrichment workflow is used to increase sensitivity. We demonstrate the simplicity, sensitivity, and reproducibility of this approach by identifying MAPK substrates as a proof of concept, and further applied this approach to profile the putative substrates of several plant kinases that play critical roles in the regulation of plant responses to environmental stresses. Using this method, 5,075 putative targets of 9 protein kinases were identified. Several of these substrates were validated by traditional in vitro assays using recombinant proteins, confirming the reliability of this approach. These results provide comprehensive information on the role of these protein kinases in controlling cellular activities, and advance our understanding of plant responses to biotic and abiotic stresses.

Primary Poster Presenter: Pengcheng Wang

Molecular mechanism involved in the regulation of chrysanthemum CmMLO17 to the infection of Alternar (0600-013)
Hall 2

Chrysanthemum（Chrysanthemum morifolium）is one of the ten traditional famous flower and one of the four important cut flowers all over the word, which is of high ornamental and economic values. The black leaf spot disease caused by the fungi Alternaria tenuissima, is one of the most severe diseases during chrysanthemum production. We have previously screened candidate CmMLO17 from full transcripts

generated from chrysanthemum infected by Alternaria tenuissima, and get the stably over-expressed and RNAi of CmMLO17 in chrysanthemum. When CmMLO17 was stably over-expressed in chrysanthemum, the plants showed an increased sensitivity to A. tenuissima, and the RNAi of CmMLO17 in chrysanthemum lead to resistant to A. tenuissima. Then we identified its function by analyzing the expression pattern of the relevant CmMLO17 and its subcellular localization; and we also screened and verified genes regulated downstream by CmMLO17 by transcriptomic analysis; by using the yeast-two-hybrid，we screened the interacting proteins with CmMLO17, and by BiFC assays, we validate the interation proteins in vitro. Above all, we identified the function and clarify the mechanism of CmMLO17 involved in the response of chrysanthemum to the infection of A. tenuissima. Our study enriched molecular mechanisms of chrysanthemum in response to pathogen infection, which meanwhile will provide a theoretical basis for the control of leaf spot disease caused by A. tenuissima and the disease resistant breeding of Chrysanthemum.

Primary Poster Presenter: ye liu

Nitrogen-responsive CLAVATA signaling modulates root architecture through altered auxin transport (0600-016)
Hall 2

Root system architecture (RSA) exhibits considerable plasticity depending on the availability of nutrients in the surrounding environment. These changes in root development may be partially modulated by nutrient-responsive small signaling peptide pathways. For example, severe nitrogen (N) limitation induces the expression of the CLAVATA3/ESR RELATED 3 (CLE3) peptide-coding gene in Arabidopsis thaliana. The CLE3 peptide binds to the leucine-rich repeat receptor kinase CLAVATA1 (CLV1) to inhibit the development of lateral roots, a mechanism proposed to prevent root outgrowth into the N-poor soil environment. Based on analysis of gene expression profiles of clv1 mutants and CLE3-overexpressing lines, PHOSPHOLIPID-BINDING PROTEIN 1 (PLBP1) was identified as a potential downstream target of the CLE3-CLV1 pathway. While the plbp1 mutant shows a moderate increase in lateral root density, it also exhibits significantly weakened root gravitropic response. PLBP1 appears to modulate the flow of auxin, a key phytohormone regulating root gravitropic response and other aspects of RSA, as the plbp1 mutant has significant reduction in the amount of PIN3, an auxin efflux transporter, localized to the columella cells of the primary root tip. Analysis of the effect of N supply on this signaling pathway indicates that PLBP1 transcript expression is repressed by both ammonium (NH4+) and nitrate (NO3-) supplies. Particularly, NH4+ application leads to a reduction in gravitropic response rate in wild-type seedlings concomitantly with a decrease in both PLBP1 and PIN3 expression levels. In line with these observations, the clv1 mutant shows weakened root gravitropic response when grown under minimal NO3- supply, suggesting that CLE3-CLV1-PLBP1 signaling mainly influences gravitropic responses when N is limited. We propose this signaling pathway regulates RSA in response to N

availability to simultaneously inhibit lateral root growth outward and promote growth to deeper soil environments.

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Primary Poster Presenter: Katerina Lay-Pruitt

Regulation of translation in response to reactive oxygen species by the protein kinase GCN2 (0600-003)
Hall 2

The translation of cytosolic mRNAs is subject to global and mRNA-specific control mechanisms. Phosphorylation of the essential translation initiation factor eIF2alpha anchors a reversible switch that represses translation globally. The stress-responsive GCN2 kinase is the only known kinase for eIF2alpha in Arabidopsis. Here we show that conditions that generate reactive oxygen species (ROS) in the chloroplast, such as dark-light transitions, high light, cold, salt and the herbicide paraquat all rapidly activated the GCN2 kinase, as visualized by eIF2alpha phosphorylation, whereas mitochondrial and ER stress did not. Treatment with hydrogen peroxide activated GCN2 kinase in darkness; otherwise, GCN2 activation was light dependent. It was also suppressed by inhibitors of photosynthetic electron transport as well as ROS quenchers. Gcn2 mutants were more sensitive to continuous high light, cold and salt stress as compared to wild type in their root growth and seedling development, implicating the GCN2-eIF2alpha pathway in responses to stress associated with chloroplast ROS. The GCN2 kinase suppressed the ribosome loading of mRNAs for functions such as mitochondrial ATP synthesis, vesicle trafficking, and translation. However, the global polyribosome profile of the gcn2 mutant was normal under herbicide stress conditions. The transcriptome of gcn2 was hypersensitive to herbicide, specifically in functions related to abiotic stresses including oxidative stress, as well as innate immune responses. In conclusion, we provide evidence that GCN2-mediated eIF2alpha phosphorylation is a missing link in a retrograde signaling pathway whereby the status of the photosynthetic machinery feeds back to the cytosolic protein synthesis apparatus. Supported by the US National Science Foundation.

Primary Poster Presenter: Ansul Lokdarshi

The Carboxy-terminus region of LRR-RLKs regulates kinase activity in Arabidopsis thaliana and Brassi (0600-011)
Hall 2

Protein post-translational modification by phosphorylation is essential for the activity and stability of proteins in higher plants and underlies their responses to diverse stimuli. There are more than 300 leucine-rich repeat receptor-like kinases (LRR-RLKs), a major group of receptor-like kinases (RLKs) that plays an important

role in growth, development, and biotic stress responses in higher plants. To analyze auto- and transphosphorylation patterns and kinase activities in vitro, 43 full-length complementary DNA (cDNA) sequences was cloned from genes encoding LRR-RLKs. Autophosphorylation activity was found in the cytoplasmic domains (CDs) of 18 LRR-RLKs; 13 of these LRR-RLKs with autophosphorylation activity showed transphosphorylation in E. coli. BRI1-Associated Receptor Kinase (BAK1), which is critically involved in the brassinosteroid and plant innate immunity signal transduction pathways, showed strong auto- and transphosphorylation with multi-specific kinase activity within 2 h of induction of Brassica oleraceae BAK1-CD (BoBAK1-CD) in E. coli; moreover, the carboxy-terminus of LRR-RLKs regulated phosphorylation and kinase activity in Arabidopsis thaliana and vegetative crops.

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Primary Poster Presenter: Man-Ho Oh

The compatibility factor BnGLO1 in A. thaliana may aid in the break down self-pollen rejection. (0600-008)
Hall 2

Many groups of flowering plants have developed self-incompatibility systems to prevent inbreeding and encourage genetic diversity through outcrossing. In the mustard family of plants, which includes Arabidopsis thaliana and Brassica napus (canola), the default in these species is have a self-incompatibility system. One of the major exceptions, is A. thaliana, having lost its self-incompatibility system including the pollen ligand SCR/SP11, the stigma receptor, SRK, and the downstream signaling component ARC1. The self-incompatibility pathway works by inhibiting the basal pollen response and compatible pollen factors in Brassica species that facilitate pollen acceptance. Several of these compatible pollen factors in B. napus have been shown to be targeted by the E3 ubiquitin ligase ARC1, such as EXO70A1, BnGLO1 and PLDa1. BnGLO1 has been shown to be important in the basal pollen response to detoxify methyl glyoxalase (MG), a toxic product of glycolysis. To determine if BnGLO1's role is conserved in the self-incompatibility response in Arabidopsis, its role was examined using self-incompatible A. thaliana expressing AlSRK+AlSCR+AlARC1. With the addition of BnGLO1 under the control of a stigma specific promoter, it should result in excess levels of BnGLO1 in the stigma when combined with the endogenous activity of the A. thaliana GLX1. To determine if additional glyoxalase activity is able to break down self-incompatibility in A. thaliana, the pollination responses in plants expressing AlSRK+AlSCR+AlARC1+BnGLO1 were observed. These experiments looked at short-term responses like Aniline Blue stains and pollen grain adherence and a long-term response, seed set. To measure the changes to MG levels, we are currently probing stigma protein extracts for the impact of BnGLO1 when compared to self-incompatible stigmas. These experiments will allow for the role of BnGLO1 to be defined in self-incompatibility in Arabidopsis as was observed in B. napus.

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Primary Poster Presenter: Emily Indriolo

The Missing Plant β–arrestin; A Key Adaptor for Clathrin-Mediated Endocytosis of 7-Transmembrane Pro (0600-006)
Hall 2

In animals, 7-transmembrane G-protein coupled receptors (GPCRs) at the plasma membrane that activate G-protein coupled signaling become phosphorylated at their cytoplasmic C-terminal tail in a ligand-dependent manner by specific cytoplasmic kinases. This leads to decoupling of the phosphorylated GPCR from its cognate G protein complex resulting in de-sensitization toward that ligand. The well-studied adaptor called β–arrestin recognizes the phosphorylated tail and recruits the clathrin complex to initiate endocytosis of this GPCR. Instead of GPCRs, most plants have a 7 –transmembrane protein (AtRGS1 in Arabidopsis) that modulates the active state of a self-activating G protein complex. Analogous to the animal GPCR paradigm, endocytosis of AtRGS1 is phosphorylated at its C terminal tail by receptor-like kinases such as BAK1 and this leads to endocytosis. However, plants lack canonical β–arrestins. Therefore, we mined structures of plant proteins for homology to animal β–arrestins and discovered AtVPS26, a component of the retromer well known for trafficking vesicles from the endosomes to the trans Golgi network. In vivo analysis shows AtVPS26A and AtVPS26B form dimers and interact with AtRGS1, promoting clathrin-mediated endocytosis. We conclude that AtVPS26 and AtVPS26B serve as arrestin-like proteins in plants that are crucial for AtRGS1 internalization and subsequent trafficking to endosomal compartments.

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Primary Poster Presenter: Justin Watkins

Spatiotemporal analysis of phosphatidic acid formation, during osmotic stress in Arabidopsis thalian (0600-023 (Screen 7))
Hall 2

Phosphatidic acid (PA), a membrane phospholipid has been shown to act as a second messenger in plants' response to osmotic stress (Munnik, T et al.,2001), yet the spatio-temporal formation of PA in response to osmotic stress has not been elucidated. We are developing a PA biosensor using dimerization-dependent fluorescent proteins. The assay system, previously reported (Ding Y et al., 2014), involves two monomers of quenched fluorescent proteins that fluoresce upon the formation of a heterodimer with an activator protein. We are using this assay system to develop a PA biosensor where fluorescent proteins are tagged to a PA binding protein. Transgenic Arabidopsis thaliana expressing the PA biosensor were

generated. In our preliminary results, PA production increases at the plasma membrane on the application of 115mM of sodium chloride, while it remains constant in cytosol on the same treatment. This suggests that PA produced at the plasma membrane and enzymes related to its formation are involved in signal transduction during salt stress. On the other hand, PA production in cytosolic membranes is not involved in signal transduction during salt stress.

Co-author(s): Naohiro Kato

Primary E-Poster Presenter: Ruth Ndathe

Nitric Oxide Takes Center-stage in Gravitropic Response in Arabidopsis
(0600-024 (Screen 10))
Hall 2

In 2015, the Space X Falcon 9 carried 22 petri dishes of bulk plated A. thaliana (Col-0) seeds to the International Space Station where they germinated, then grew for three days prior to fixation. This mission was remarkable in its coupling of deep RNA sequencing with the most comprehensive proteomic analysis of spaceflown plant material to date (membrane and soluble fractionation). As the initial signaling molecules are altered within seconds of gravistimulation, the response must initially be governed by post-translational mechanisms. Protein LC-MS/MS revealed alterations not observed at the RNA level highlighting NO as a central component of adaptation to microgravity. Further, we identify systematic alteration of components regulating not only NO signaling but also ethylene signaling. Ethylene and NO synthesis have mutually inhibitory interactions where NO inhibits ACC Oxidase activity and ethylene inhibits Nitrate Reductase activity. Guided by the BRIC-20 ‘omics data, we have constructed an evidence-based signaling schematic for understanding gravitropic signaling and beyond. This schematic provides a mechanism for the polarity of gravity response where the interaction of NO, calcium, ROS, IP3 and beyond unify previous theories of signaling in gravity response. These same components, which ubiquitously govern signaling, are involved in multiple pathways and the evidence for this schematic has been synthesized from pathogen, salt, gravity, light, and forward/reverse genetic experiments. These findings argue for a center-stage role for NO in gravity response and provides an essential framework for understanding how individual processes utilize a shared network of signaling components. The proposed signaling schematic takes us beyond the “grey cloud” of gravity signaling to an integrated framework for understanding and building hypotheses to genetically dissect signaling cascades.

Co-author(s): Sarah Wyatt

Primary Poster Presenter: Colin Kruse

Systems, Synthetic, and Computational Biology

5:00 PM - 5:30 PM

Simulation of Plant Metabolism in DOE Systems Biology KnowledgeBase
(0400-020 (Screen 4))

The Department of Energy Systems Biology Knowledgebase (KBase; http://kbase.us) is a knowledge creation and discovery environment designed for both biologists and bioinformaticians. KBase integrates a variety of data and analysis tools, from DOE and other public services, into an easy-to-use platform that leverages scalable computing infrastructure to perform sophisticated systems biology analyses. KBase provides an extensible range of integrated biological data types and associated analytical tools (Apps) presently including gene expression and transcriptomics analysis, comparative genomics, genome annotation, metabolic simulation, and visualization. KBase has a rich set of computational methods and curated datasets for gene expression analysis based on RNA-seq, including a selection of preprocessed high-quality reference genomes and a variety of Apps supporting the Tuxedo tool suites, allowing users to generate an expression matrix of reads based on plant genomes. Apps for downstream analysis include clustering of expression profiles, reconstruction of primary metabolism, integration of multiple metabolic networks and simulation of metabolic pathways. We demonstrate some Apps via two means: 1) We utilize the integration of RNA-seq data sampled from the roots of several species grown on different sources of nitrogen; specifically we highlight several key metabolic subsystems which exhibit a range of fluxes; 2) We explore the nitrogen cycling in a mutualistic plant-microbe interaction between a diazotroph and peat moss and we highlight the levels of light required for the supply of nitrogen fixed by the diazotroph. The data and Apps are available from within an interactive notebook that supports the creation of dynamic workflow documents called Narratives, enabling experimental and computational biologists to work together to share and publish their data, approaches, workflows, and conclusions, leading to transparent and reproducible computational experiments.

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Primary E-Poster Presenter: Sam Seaver

Arabidopsis thaliana floral transition gene regulatory network model.
(0400-022 (Screen 12))
Hall 2

In plants, organs develop post-embryonically from the meristems, small groups of cells that retain the ability to proliferate and whose ultimate fate remains undetermined. After a period of vegetative growth, plants respond to a combination

of intrinsic and extrinsic signals, such as photoperiod, temperature, hormones and age, that trigger the reproductive phase. This transition is accompanied by molecular changes at the shoot apical meristem (SAM). In the flowering transition of the model plant Arabidopsis thaliana, the SAM changes its identity from a Vegetative Meristem (VM) to an Inflorescence Meristem (IM) when the proper endogenous and exogenous signals are perceived. Once the SAM becomes an IM, it starts to form floral meristems (FM) at its flanks. Finally, the floral organs (sepals, petals, stamens and carpels) develop from the FM. We constructed a boolean model of the hormonal and genetic network involved in the floral transition of A. thaliana to understand how endogenous and environmental cues integrate to establish reproductive development. We integrated different "flowering pathways" in a single network and found that it is highly connected and redundant. The dynamic analysis recovers the expression patterns found in the VM, IM, FM and the floral organs. Furthermore, in silico single mutant analysis supports that the network dynamics is robust. We highlight that a systemic perspective may be essential to fully understand the floral transition in A. thaliana and other developmental processes.

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Primary E-Poster Presenter: Elva Carolina Chávez Hernández

Predicting subcellular localization of metabolic reactions using metabolic networks (0400-021 (Screen 5))
Hall 2

Metabolic processes in eukaryotic cells are highly compartmentalized into organelles. In plants, unique reactions localized in organelles carry out specific metabolic functions, such as photosynthesis and starch metabolism in the chloroplast and oxidative phosphorylation in the mitochondria. Databases such as SUBA4 and CropPAL contains protein compartmentalization data from thousands of publications on Arabidopsis thaliana and several commodity crop species, which enabled training of classifiers to predict protein subcellular localization that can guide further research. Many classifiers have been built using various machine learning algorithms, but these methods rely on protein sequences as input. Although sequence-derived features, including target signals, have been successfully identified and utilized in sequence-based classification methods, recent studies have shown that many well-accepted prediction algorithms perform poorly for chloroplast and mitochondria-targeted proteins, possibly due to limitations in sequence-derived data. Considering that each organelle can perform specific metabolic functions, our study explored the possibility of using metabolic networks to infer subcellular localization of enzymatic reactions. Our algorithm, LocPred, utilizes graph mining techniques to train and predict reaction subcellular locations with features extracted from metabolic networks. LocPred showed surprisingly high performance, with average AUC-ROC in cross-validation around 0.79, and F1,

precision, and recall, that doubled the baseline. Results from LocPred indicate that metabolic network are useful in inferring subcellular localization of reactions. Based on its high accuracy in predicting mitochondrial and plastidic reactions, 0.8 and 0.81 AUC-ROC respectively, LocPred may be a novel solution to circumvent the challenges of sequence-based methods.

Co-author(s): Seung Rhee,
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Primary E-Poster Presenter: Jiun Yen

A Modified Reverse TCA Cycle Functionally Increases Carbon Fixation in Camelina sativa (0400-003)
Hall 2

Plants employ the Calvin-Benson cycle to fix atmospheric CO2 for the production of biomass. Toward the development of sustainable agriculture and biofuels, increasing the efficiency and productivity of photosynthesis is crucial. Under current conditions, the flux of carbon through the Calvin cycle is limited by the activity and selectivity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Attempts to engineer RuBisCO kinetics more favorable to agriculture have led to only moderate success. In nature, alternative, RuBisCO-independent pathways to fix CO2 exist but occur only in bacteria or archaea. The purpose of this study is to implement such a carbon fixation pathway for the use in green plants. This pathway is a carbon-fixation cycle inspired by the metabolisms of bacterial autrotrophs: a condensed, reverse TriCarboxylic Acid (crTCA) cycle. The crTCA cycle operates with five bacterial enzymes that utilize endogenous plant metabolites as carboxylation substrates while using 20% less energy per CO2 capture compared to the Calvin cycle. The crTCA cycle functions in vitro under aerobic conditions, successively incorporating carbon to generate product while re-generating substrate. crTCA enzymes also have been demonstrated to retain activity when transiently expressed in plant systems. We use stable, chloroplast-localized expression of the crTCA cycle in Camelina sativa to assess changes in photosynthetic parameters. Transgenic crTCA lines have increases in CO2 assimilation rates, greater efficiency in electron usage, and differences in morphology compared to WT plants. The focus of this work is to establish that supplementing endogenous photoassimilation with synthetic pathways can be a viable approach to increasing plant productivity. Future work will develop an understanding of how this engineered pathway contributes to endogenous plant metabolism. This research is funded by the Department of Energy (ARPAe AR-0000207 & BER DE-SC0018269).

Primary Poster Presenter: Nathan Wilson

A nondestructive, machine learning based method for monitoring

anthocyanin accumulation (0400-005)
Hall 2

When plants are exposed to stress conditions for an extended period, irreversible damage can occur, and yields will be negatively impacted. Therefore, it is important to detect stress symptoms of plants as early as possible to ensure crop yields. One stress symptom common across many higher plants is the accumulation of anthocyanin, a pigment which may act as a neutralizing agent for reactive oxygen species. In this study, we investigated a machine learning based method to predict the accumulation of anthocyanin in Arabidopsis thaliana plants based on digital image data. 19 regression methods were trained using color data from 5 color spaces, resulting in a total of 95 trained regressions. Of the 95 regressions, we found that a Rational Quadratic Gaussian Process Regression trained with data in the YIQ color space was able to most accurately predict actual anthocyanin levels ($r^2 = 0.957$). Using this regression, we were able to noninvasively monitor the spatial and temporal accumulation of anthocyanin in Arabidopsis leaves exposed to different stress conditions. Because anthocyanin accumulation is a stress symptom shared by many economically valuable plants (e.g. maize, tomato, cotton), applying a similar methodology to these crops could lead to the development of low-cost, noninvasive digital imaging based systems for monitoring plant health.

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Primary Poster Presenter: Bryce Askey

Automated 3D root phenotyping in the field may paves the way to increased carbon sequestration (0400-013)
Hall 2

The earth is at risk to enter "Hothouse Earth" conditions - a climate stabilizing at a global average of 4-5°C higher than pre-industrial temperatures with a sea level 10-60 m higher than today. Avoiding a "Hothouse Earth" requires not only reduction of carbon dioxide but also enhancement biological carbon stores. One good solution is deeper maize root systems that converts atmospheric carbon to carbon stores into the soil. For example, the 91 million acres of maize grown in US provide an estimated one gigaton of stored carbon into the soil every year. The key to deeper maize root systems is its traits hidden underground. However, genetic studies on maize root traits are hampered by the difficulty to measure the dense and occluded root architecture. Therefore, the discovery of genes associated with deeper maize rooting requires advanced root phenotyping methods. We developed an optical 3D root phenotyping system that can automatically scan the maize roots in 6 minutes Our software can reconstruct 3D root model and measure root traits, such as root system density, distributions of diameters, number of whorls, crown roots, brace roots, 1st order lateral roots, and individual root traits like root curvatures, angles and lengths can also be obtained.We validate our method on 16 maize genotypes with six replicates for each genotype. Our system paves a promising way to access

previously inaccessible traits that directly relate to deeper rooting. It contributes to increase soil carbon sequestration and carbon stores, and it will help to avoid our earth entering to "Hothouse Earth" conditions.

Co-author(s): Alexander Bucksch

Primary Poster Presenter: Suxing Liu

1:30 PM - 3:00 PM

Characterizing key regulators of miRNAs involved in root development (0400-002)
Hall 2

Roots are essential plant organs that provide structural support and are responsible for acquisition of water and certain mineral nutrients. MicroRNA (miRNAs) can post-transcriptionally repress their targets and affect root morphogenesis, patterning and lateral root development. They are also involved in plant adaptation to nutritional stress. The miR167, miR169 and miR393 families participate in the response to nitrogen (N) starvation by altering root architecture and modulating N uptake and transport. To improve our understanding of the genes controlling root development, we are using a gene centered approach to characterize upstream regulators. We have used a nearly complete root transcription factor (TF) library in an enhanced Yeast 1 Hybrid screen of promoters of these miRNAs and their targets. The result is a regulatory network of protein-DNA interactions, between transcription factors and the promoters of miRNAs and their down-stream targets. We have combined this with known and predicted miRNA targets and publicly available genome wide protein-DNA interaction data, thus forming a more complete Gene Regulatory Network (GRN). We further utilize extensive high-resolution spatial and temporal gene expression data with models to infer significant interactions and predict key regulators of root development. This information was used to evaluate 50 genetically perturbed lines for their root developmental phenotype and their response to N-limiting conditions.

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Primary Poster Presenter: Lifang Zhang

Computer-aided Engineering of Biomass Production under Drought in Sorghum and Setaria (0400-001)
Hall 2

This project aims to leverage Setaria viridis as a model system to develop novel technologies and methodologies to redesign the bioenergy feedstock Sorghum bicolor to enhance water use and photosynthetic efficiencies. In this study, we performed comparative metabolic network analysis under well-watered and water deficient conditions, using Flux Balance Analysis (FBA) to investigate how plants allocate metabolic resources for biomass production in response to drought. First,

we collected biomass composition data for Setaria and sorghum shoot and root under well-watered and water-limiting conditions at multiple time points, using 13C solid state NMR and near-infrared (NIR) spectroscopy. Second, we generated genome-scale metabolic models of Setaria italica, Setaria viridis, and Sorghum bicolor by an automated computational pipeline, converting pathway genome databases to metabolic network reconstructions. The biomass composition will be used to describe the objective function in the S. bicolor model. We will constrain the models by the transcriptome data generated from the consortium to further adjust the upper and lower bounds of each reaction. We will perform Flux Variability Analysis for determining the maximum range of flux that every reaction can possibly take on while the network is optimized for biomass production to identify the key reactions that limit biomass production under drought conditions.

Co-author(s): Seung Rhee

Primary Poster Presenter: cheng zhao

Computing on phenotypic descriptions enables candidate gene prediction

Natural language descriptions of plant phenotypes are a rich source of information for genetics and genomics research. We computationally translated descriptions of plant phenotypes into structured representations that can be analyzed to identify biologically meaningful associations. These representations include the EQ (Entity-Quality) formalism, which uses terms from biological ontologies to represent phenotypes in a standardized, semantically-rich format, as well as numerical vector representations generated using Natural Language Processing (NLP) methods (such as the bag-of-words approach and document embedding). We compared resulting phenotype similarity measures to those derived from manually curated data to determine the performance of each method. Computationally derived EQ and vector representations were comparably successful in recapitulating biological truth to representations created through manual EQ statement curation. Moreover, NLP methods for generating vector representations of phenotypes are scalable to large quantities of text because they require no human input. These results indicate that it is now possible to computationally and automatically produce and populate large-scale information resources that enable researchers to query phenotypic descriptions directly.

Chair and Concurrent Symposium Speaker: Carolyn Lawrence-Dill

Primary Poster Presenter: Ian Braun

Developing a modular framework for orthogonal control of gene expression in plants (0400-012)
Hall 2

As part of increasing utility of synthetic biology methods for biological engineering, a wide range of toolkits have begun to be developed that allow the modular construction of circuits in a wide variety of organisms. Here inspired by the yeast

toolkit, we have developed a highly optimized framework to enable the easy construction of variety of plant expression vectors in a high throughput fashion. Using this highly modular and programmable assembly framework we have engineered synthetic promoters that essentially serves as an orthogonal control. Our core promoter elements are based on the minimal 35S promoter with synthetic binding sites dictated by gRNAs and Artificial Transcription Factors (ATFs) that rely on dCas9:VP64 (a mutant form of Cas9 without endonuclease activity) and corresponding guide RNAs binding for promoter activation. To date, we have constructed 3 functional artificial gRNA-ATF promoter pairs that can be used in the tool kit, and this number will be further increased by continuing to identify gRNA binding sites that are unlikely to be normally found in plants. Apart from using pol III (U6) promoter to drive gRNA expression, we have inserted hammerhead and human Hepatitis-δ ribozymes into the gRNA expression cassette, which in turn allows functional expression and processing via Pol II promoters, greatly expanding options for engineering gene regulation. As a proof of concept, we have generated several orthogonal control regulatory circuits using this system where gRNA expression is induced by ethylene and the corresponding ATF responsive promoter activates downstream YFP expression. We also demonstrated ratiometric induction of YFP upon ethylene treatment while level of RFP and BFP which are under orthogonal control and driven by mutually exclusive gRNAs in the same circuit, remains constant. Thus this programmable platform will enable construction of orthogonal control system to understand and manipulate novel pathways and phenotypes in plants.

Co-author(s): Sibum Sung

Primary Poster Presenter: Yogendra Bordiya

Engineering modular plant-to-plant communication (0400-007)
Hall 2

The ability to engineer plants using the tools of synthetic biology is of increasing importance for solving agricultural problems, and for adapting plants to novel uses. A collaboration between five labs has begun to engineer plant-to-plant communication using volatile organic compounds (VOCs). By modifying and enhancing natural hormone pathways in plants we have been able to demonstrate synthetic communication. In particular, we have engineered a modular ethylene sensor, and identified modular promoter structures that are responsive to methyl salicylate and methyl jasmonate. When these are cloned adjacent to reporter genes, gas-dependent production of signals can be observed. In parallel, we have determined that carbon flux through the normal pathway for the production of the volatile ethylene may be limited, and have been able to generate transgenic plants with enhanced ethylene production. When ethylene 'senders' are aligned with ethylene 'receivers,' plant-to-plant communication can be observed. Along the way, we have developed an extensive new tool kit that allows for the establishment of an Orthogonal Control System (OCS) in plants that operates on top of extant plant regulatory and metabolic systems. We have for the first time created wholly

orthogonal transcription factors using dCas9:VP64 as a transcription factor, and shown that these can activate completely artificial promoters. While these demonstrations have so far been shown in model plant species (Nicotiana, Arabidopsis), in parallel we have undertaken an effort to demonstrate that constructs developed can be transported into new species, and to this end have made great progress in 'taming' a non-model plant, common dandelion (Taraxacum). Ultimately, by funneling engineered sensor 'inputs' through VOC communication channel to appropriate reporter 'outputs' it should be possible to allow fields of plants to better serve as self-sentinels against pests and other environmental incursions.

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Primary Poster Presenter: Kasia Dinkeloo

Epigenomic Landscape of Arabidopsis thaliana Metabolism Reveals Bivalent Chromatin on Specialized Me (0400-009)
Hall 2

Plant metabolism synthesizes a plethora of compounds, many of which are used as food, feed, fuel, and pharmaceuticals. However, little is known about how metabolic genes and pathways are regulated in eukaryotes, especially at the epigenetic level. Here, we sought to discover general rules of metabolic regulation in Arabidopsis thaliana based on 16 high-resolution epigenomic profiles, including histone variants, DNA methylation, and histone modifications. To see if there are any interactions between epigenetic modifications across metabolic genes, we computed pairwise Pearson's correlation coefficients between 16 epigenetic modifications based on their relative abundance at each metabolic gene region and identified four groups of epigenetic regulons. Moreover, we discovered predominant epigenetic marks associated with various types of metabolic genes. Genes involved in energy metabolism were enriched with activation marks, such as histone 3 lysine 4 trimethylation (H3K4me3) and H3K36me3, and depleted of a repression mark, H3K27me3, which might be important in maintaining active expression of genes involved in energy metabolism. In contrast, specialized metabolic genes, often

involved in defense, were predominantly regulated by two modifications that have opposite effects on gene expression, H3K27me3 (repressing) and H3K18ac (activating). Using sequential ChIP-qPCR on camalexin biosynthesis genes as an example, we confirmed that these two modifications were co-localized to form bivalent chromatin. Mutants defective in H3K27m3 and H3K18ac modifications showed that both modifications were required to determine the normal transcriptional kinetics of these genes upon stress stimuli. Our results indicate that this type of bivalent chromatin controls the precise timing of gene expression upon stress stimuli. In summary, this study advances our current understanding of biological function of bivalent chromatin and reveals a novel mechanism of precise control of expression.

Co-author(s): Seung Rhee,
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Primary Poster Presenter: Kangmei Zhao

Evolution of CBP60 protein family, which includes major regulators of SA-mediated immune response (0400-006)
Hall 2

We are interested in evolution of the plant immune signaling network and its components. CBP60 protein family in Arabidopsis consists of eight members: five prototypical (AtCBP60b-f) and three (AtCBP60a, AtCBP60g, AtSARD1) which are important immune regulators. AtCBP60a is a negative, while AtCBP60g and AtSARD1 are positive regulators of plant immunity. All three are involved in regulation of SA-signaling, and calmodulin (CaM) binding ability has been show to be essential for immune-related functions of AtCBP60a and AtCBP60g, while AtSARD1 lacks CaM-binding ability. To investigate evolution of protein families in land plants, we have created a protein database from 327 currently sequenced plant species, ranging from Liverwort to Eudicots. Subsequently we have established a pipeline for family member search and phylogenetic tree construction. We have applied the pipeline to the CBP60 family to infer family evolution. Our analysis shows that divergence of prototypical and immune-related clades occurred before divergence of ferns from the land plant lineage. Diversification of the three immune subfamilies within immune-related clade was completed before divergence of basal angiosperms. We have further analyzed all detected proteins by predicting their CaM-binding ability, binding site position and additional sequence features. CaM-binding ability seems to be present in majority of prototypical members. After divergence, this ability is gradually lost in members of immune-related subfamilies. We have identified two major drivers of this loss: (i) deletion of C-terminal CaM-binding domain and (ii) mutations at C-terminus that decrease alpha-helical propensity of the region. Surprisingly, CaM-binding ability seems not to be essential for immune-related function of CBP60 proteins in most Angiosperms, and could therefore be sacrificed while accumulating mutations in evasion of pathogen effectors. This study was supported by NSF grants MCB-1518058 and IOS-1645460.

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Primary Poster Presenter: Kristina Majsec

Interrogating transcription factor complex DNA binding specificity through DIMR, a novel yeast synth (0400-011)
Hall 2

Transcription factors (TFs) are fundamental components of biological regulation, facilitating the basal and differential gene expression necessary for life. TFs exert transcriptional regulation through interactions with both DNA and other TFs, ultimately influencing the action of RNA polymerase at a genomic locus. Current approaches are proficient at identification of binding site requirements for individual TFs, but few methods have been adapted to study oligomeric TF complexes. Further, many approaches that have been turned toward understanding DNA binding of TF complexes, such as electrophoretic mobility shift assays, require protein purification steps that can be burdensome or scope-limiting when considering more exhaustive experimental design. In order to address these shortfalls and to facilitate a more streamlined approach to understanding DNA binding by TF complexes, we developed the DIMR (Dynamic, Interdependent TF binding Molecular Reporter) system, a modular, synthetic yeast-based transcriptional activity reporter. As a proof of concept, we focused on the NUCLEAR FACTOR-Y (NF-Y) family of obligate heterotrimeric TFs. The DIMR system was able to reproduce the strict DNA-binding requirements of the NF-Y complex with high fidelity, including recapitulation of previously-characterized mutations in complex subunits that break either subunit interactions or DNA binding. With this model firmly established, we can directly test the effects DNA and amino acid changes have on NF-Y complex function, and have begun to address the DNA-binding impacts of an atypical conserved linker domain within a particular NF-YA subunit of Arabidopsis thaliana. The DIMR system provides a powerful, easy-to-use approach to address these and similar types of questions concerned with the binding of both monomeric and oligomeric TFs to DNA.

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Primary Poster Presenter: Zachary Myers

Optimizing the use of gene expression data to predict metabolic pathway memberships with machine lea (0400-008)
Hall 2

Plants produce diverse metabolites in metabolic pathways important for not only plant survival but also human nutrition and medicine. Some genes in the same pathway can be identified based on their correlated expression profiles. However, pathway gene co-expression may be only under specific spatiotemporal and conditional contexts, and the coexpression relations can be non-linear. Here, we

develop a supervised machine learning approach to maximize the utility of gene expression data for predicting pathway gene memberships by considering 656 combinations of datasets, and linear/non-linear co-expression measures, using tomato as a model. With each combination dataset, we established a multi-class model to predict whether a gene belongs to one of the 85 pathways (classes) and evaluated model performance with the F1 score (range from 0 to 1, where 1 indicates perfect predictions). Among 656 models, the best overall model (i.e. have highest average F1 across 85 pathways) has significantly improved performance in predicting pathway memberships (average F1=0.34) compared to random guess (F1=0.008). By identifying which of the 656 models has the best membership prediction for each pathway, we uncovered optimal pathway models that have an even higher average F1 of 0.83, where 26% pathways are predicted perfectly. The optimal pathway models also have much better performance compared to those based on the best cluster (from unsupervised learning) that maximize F1 for each of 85 pathways (average F1=0.45), indicating the importance of modeling training with supervised learning. Our study highlights the need to extensively explore expression features to build models that can maximize the utility of expression data for pinpointing pathway membership. Through this detailed exploration, novel connections between pathways and biological processes can also be identified based the optimal expression dataset used, improving our mechanistic understanding of the metabolic network.

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Primary Poster Presenter: Shin-Han Shiu

Simulation of Plant Metabolism in DOE Systems Biology KnowledgeBase
(0400-014)

The Department of Energy Systems Biology Knowledgebase (KBase; http://kbase.us) is a knowledge creation and discovery environment designed for both biologists and bioinformaticians. KBase integrates a variety of data and analysis tools, from DOE and other public services, into an easy-to-use platform that leverages scalable computing infrastructure to perform sophisticated systems biology analyses. KBase provides an extensible range of integrated biological data types and associated analytical tools (Apps) presently including gene expression and transcriptomics analysis, comparative genomics, genome annotation, metabolic simulation, and visualization. KBase has a rich set of computational methods and curated datasets for gene expression analysis based on RNA-seq, including a selection of preprocessed high-quality reference genomes and a variety of Apps supporting the Tuxedo tool suites, allowing users to generate an expression matrix of reads based on plant genomes. Apps for downstream analysis include clustering of expression profiles, reconstruction of primary metabolism, integration of multiple metabolic networks and simulation of metabolic pathways. We demonstrate some

Apps via two means: 1) We utilize the integration of RNA-seq data sampled from the roots of several species grown on different sources of nitrogen; specifically we highlight several key metabolic subsystems which exhibit a range of fluxes; 2) We explore the nitrogen cycling in a mutualistic plant-microbe interaction between a diazotroph and peat moss and we highlight the levels of light required for the supply of nitrogen fixed by the diazotroph. The data and Apps are available from within an interactive notebook that supports the creation of dynamic workflow documents called Narratives, enabling experimental and computational biologists to work together to share and publish their data, approaches, workflows, and conclusions, leading to transparent and reproducible computational experiments.

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Primary Poster Presenter: Sam Seaver

Towards an advanced model of Crassulacean Acid Metabolism (CAM) to explain stomatal sensitivity (0400-004)
Hall 2

Despite the remarkable characteristic of nocturnal primary CO2-fixation and acid accumulation in plants that exhibit Crassulacean Acid Metabolism (CAM), the controls over stomata that permit such plastic responses are not yet fully understood. The complex and highly dynamic CAM system had previously been captured using a modeling approach with a simplified stomatal module (Owen and Griffiths, 2013). In this study, the original CAM systems dynamic model was transformed onto the MATLAB platform, which is more favourable for standardised model development, has a greater capacity for reducing the numerical-integration error and allows for stomatal sensitivity refinement. Here, we show that the CAM systems dynamic model on MATLAB platform also has predictive power beyond the original descriptive function from simulations of atmospheric CO2 manipulations and internal perturbation (PEPcarboxylase activity); both interventions influence the concentration of intercellular CO2 (Ci), hypothesised to be the key stimulus for the distinct CAM stomatal behaviour. Simulations of the perturbed CAM systems matched well with experimental gas-exchange data, and provide an explanation for the differential CO2-sensitivity between two closely-related CAM species with different degree of succulence (K. daigremontiana and K. pinnata). At this stage, the CAM systems dynamic model has provided insight into CAM stomatal responsiveness to intercellular CO2 (Ci) and the reformulation in MATLAB will permit more widespread model development in the future. The ultimate goal is to dissect the relative contribution between biochemical versus circadian controls in governing CAM bahaviour using the system dynamic model. These insights will provide the basis for future experimental design, which will use the model as an

analytical tool to interpret experimental outputs and enhance the predictive power in the long run.

Co-author(s): Howard Griffiths

Primary Poster Presenter: Methawi Chomthong

Versatile High Throughput In Planta Genetic Screening (0400-010)
Hall 2

Traditional functional genetic studies in crops are time-consuming, complicated and cannot be readily scaled up. The reason is that, for most crops, mutant or stably transformed plants need to be generated to study the effect of particular gene modifications on specific traits of interest. In addition, many crop species exhibit complex genomes and long generation times. As a result, usually several months to over a year are needed to obtain desired mutants or transgenic plants. This represents a significant bottleneck in the development of new crop varieties, tailored to meet future agricultural needs in an ever changing environment.To overcome this major issue we are currently establishing a world's first versatile in planta genetic screening platform, amenable to high throughput screening for a wide range of future output traits in almost any crop species, with a unique workflow. The platform we are developing combines transformation of expression libraries in protoplasts, followed by fluorescence activated cell sorting and single cell sequencing. This workflow will allow us to screen complex genetic libraries for a specific agricultural trait, in a single experiment, in a matter of days, as opposed to several years that are needed by conventional means.As a proof-of-concept we chose to firstly focus on the highly valuable traits that are storage lipid and storage protein accumulation and develop strategies using our new workflow to identify unreported genes or genes combinations that can improve these traits in plants.Here we will present two of these strategies and latest results. One involves successful enrichment of high-lipid-accumulating plant cells by using a fluorescent lipid stain. The second one is based on the use of a synthetic reporting construct with a storage protein promoter driving the expression of a fluorescent protein.

Primary Poster Presenter: Benjamin Pouvreau

Joint reconstruction of multiple gene regulatory networks with spatial data (0400-023 (Screen 13))
Hall 2

Joint reconstruction of multiple gene regulatory networks (GRNs) using gene expression data from multiple tissues, developmental stages or environmental conditions is very important for understanding common and tissue/stage/condition-specific regulation. However, there are currently no computational models and methods available for directly constructing multiple GRNs that not only share some common hub genes but also possess tissue/stage/condition-specific regulatory edges. To solve this challenge. we developed a new graphic Gaussian model for

joint reconstruction of multiple gene regulatory networks (JRmGRNs), which highlighted hub genes, using gene expression data from several tissues, developmental stages or environmental conditions. Under the framework of Gaussian graphical model, JRmGRN method constructs multiple GRNs through maximizing a penalized log likelihood function. We formulated it as a convex optimization problem, and then solved it with an alternating direction method of multipliers (ADMM) algorithm. The performance of the method was first evaluated with synthetic data and the results showed that it outperformed several other methods for reconstruction of GRNs. We also applied our method to real Arabidopsis thaliana RNA-seq data from two light regime conditions in comparison with other methods, and both common hub genes and some conditions-specific hub genes were identified with higher accuracy and precision. The method is instrumental for identifying both hub genes and tissue/stage/condition-specific regulation from multiple spatial data sets.

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Primary E-Poster Presenter: Hairong Wei

Whole-Plant: Environmental and Ecophysiological

Accumulation and effects of perfluoroalkyl substances (PFASs) in three Salix L. species (1200-035 (Screen 3))
Hall 2

Over the past decade, there has been an alarming increase in environmental pollution by organic and inorganic compounds, like antibiotics and Per- and Poly fluoroalkyl Substances (PFASs). SDZ, belonging to the class of sulfonamides is one of the most prevalent antibiotics detected in the agricultural soil. Here, the effect of SDZ on the growth, changes in antioxidant metabolite content and enzyme activities related to oxidative stress in Arabidopsis thaliana were analyzed. Further, the proteome level alterations in roots was examined by a iTRAQ-LC-MS/MS approach. A dose-dependent decrease in leaf biomass and root length was evidenced in response to SDZ. Increased malondialdehyde content at higher concentration (2 µM) of SDZ, indicated increased lipid peroxidation and suggest the induction of oxidative stress. Glutathione levels were significantly higher compared to control and there was no increase in ascorbate content or the enzyme activities of glutathione metabolism, even at higher concentrations. In total, 48 differentially abundant proteins related to stress/stimuli response, transcription and translation, metabolism, transport and other functions were identified. Proteins related to

oxidative, dehydration, salinity and heavy metal stresses were represented. Upregulation of peroxidases was validated with total peroxidase activity. Pathway analysis provided an indication of phenylpropanoid biosynthesis. The Greenpeace 2016, mentioned about PFASs pollution, the most prominent contaminants in four different locations around the world. The chemical companies manufacture PFASs including Teflon in the USA, Netherlands and Veneto region in Italy and China. Effects of different concentrations of PFASs on A. thaliana, Zea mays and Salix were examined using hydroponic system. Comprehensively, PFAS adversely affected the overall growth, specifically the root length. Interestingly, there was a slight increase in photosynthetic parameters compared to untreated plants.

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Primary E-Poster Presenter: NISHA SHARMA

Patterns of circadian clock gene expression at different temperatures relate to Nothofagus species d (1200-037 (Screen 11))
Hall 2

Circadian clock increases organisms´ fitness by providing a mechanism to anticipate events such as sunrise and adjust their transcriptional programs. Due to its ability to maintain 24h-rhythms over a wide range of temperatures (property of compensation by temperature), circadian clocks have been proposed to contribute to thermal adaptation and plasticity in plants. However, consequences of clock performance on plant behavior in natural ecosystems are scarcely known. Here we show that circadian clock of Nothofagus obliqua and N. pumilio, two emblematic tree species of the sub-Antarctic Patagonian forests, lose their property of compensation by temperature at 34°C. These species constitute examples of extremes of adaptation to altitude, inhabiting non-overlapping thermal niches. Moreover, at 31°C, daily oscillation in the expression of the homolog clock gene NoTOC1 is maintained in N. obliqua, which inhabits warmer and lower altitudes in the mountain but is lost in N. pumilio, which inhabits higher and colder habitats. In the latter, warm temperatures exert a strong effect on its transcriptome. Experiments in an old-growth temperate forest show that the performance of the clock of N. pumilio, measured as the expression of NpTOC1, is affected in warmer and lower environments out of its distribution range, indicating that clock functioning is susceptible to warm temperatures, and this is associated with reduced accumulation of dry weight, chlorophylls and survival of the seedlings. This behavior is not evident in N. obliqua seedlings grown in colder environments in

higher environments out of its natural ecological range. Taken together, our results provide the first evidences in favor that differences between plant species in the influence of temperature on the performance of their circadian clocks are the bases for physiological adaptation to the local thermic environment and therefore for differences in altitudinal distribution.

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Rootstock and irrigation have distinct effects on 'Chambourcin' vines, berries, and wine (1200-036 (Screen 10))
Hall 2

Grafting is a horticultural practice that joins the roots (rootstock) of one plant to the shoot (scion) of another. Grafting is most commonly used in woody perennial crops. Grapevine (Vitis spp.) is an excellent model for understanding how rootstocks can impact shoot systems phenotypes due to the available genomic resources and ability to grow across diverse environments. We examined an experimental vineyard in Mount Vernon, Missouri which includes a locally important scion ('Chambourcin') growing ungrafted as well as grafted onto three different rootstocks ('SO4', '1103P' and '3309C'). The vineyard also includes 3 different irrigation treatments. From 2013-2016, we assessed different kinds of phenotypic variation including leaf ion concentrations, viticulture measurements such as pruning weight, and GC-MS using a targeted panel of metabolites in berries and wine. We also examined rootstock-induced changes in gene expression in the scion using RNA-seq. Each phenotype was studied for 1 to 3 years. We found distinct and significant effects of rootstock and irrigation on the phenotypes examined. Current work underway expands sampling to include additional phenotypes, samples, and time points across three years.

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Isoprene emission affects growth-defense tradeoffs in plants (1200-038 (Screen 9))
Hall 2

Some, but not all, plants make isoprene, which is lost from the plants and causes substantial effects on atmospheric chemistry. Plants making isoprene, or exposed to isoprene, are better able to tolerate some stresses, especially high temperature, but tolerance of other stresses is not affected. Tolerance of chilling stress appears to be reduced by isoprene and isoprene emission is reduced at low temperature. Gene expression changes are consistent with a role of isoprene in preparing plants to tolerate stress. Genes involved in synthesis of jasmonic acid and abscisic acid are expressed at higher levels when isoprene is present although salicylic acid related genes are not affected. Isoprene also affects plant growth. In some cases, growth is enhanced and in some cases it is suppressed. Expression of transcription factors likely to affect DELLA and PIF proteins, which are related to growth, is altered by isoprene when growth is stimulated. Development is also stimulated and expression of genes related to cytokinins is found to be altered by isoprene. It appears that isoprene can cause widespread changes in gene expression that alters growth/defense tradeoffs and results in plants better prepared for warm season stresses. This is consistent with the observations of very large temperature effects on isoprene emission capacity.

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Concurrent Symposium Speaker: Thomas Sharkey

Phylloxera meets drought: increased risk for grapevines under climate change? (1200-039 (Screen 13))
Hall 2

Climate changes exert impacts on agro-economic activities. Viticulture is further threatened by worldwide reported outbreaks of phylloxera, i.e a sucking insect obligate biotroph of Vitis species. Knowledge on how the pest influences plants' physiology and growth is limited, in particular when the infection is coupled to water scarcity. In the light of forecasted drought periods, it is fundamental to understand and predict eventual cumulative effects of combined biotic-abiotic

stress.We monitored water and carbon metabolism of Teleki 5C rootstock (with or without scion-Riesling) subjected to drought (D) and/or phylloxera (P) stress. P vines were root inoculated with insect's eggs collected from a field population. A subset of plants was drought stressed (D), while the other was maintained well-watered (W). Growth, biomass, and root infestation (abundance of nodosities) were also investigated.Drought significantly impacted vines' water status and growth, with the two genotypes adopting a different strategy to cope with stress. Significantly higher root infestation was observed in D plants compared to W, suggesting that drought favors insect's fitness. Phylloxera influenced plants water and carbon metabolism and enforced the sink strength of the roots by stimulating carbon translocation in pest favor. Furthermore, phylloxera reprogramed vine growth and prevented biomass compensation. The synergic effect of biotic-abiotic stress could be detected in several physiological and morpho-anatomical traits. We conclude that root infestation imposes a considerable stress to the plants exacerbating the negative effects of drought. Teleky 5C supported large insect populations efficiently buffering negative effects on plant growth. The overall more marked response of grafted Riesling compared to own-rooted 5C, indicates a higher sensitivity to phylloxera of the former genotype and demonstrates that the scion-rootstock interaction may influence the whole-plant physiology.

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EFFECTS OF DIFFERENT SOWING DEPTHS ON GERMINATION AND GROWTH OF ARACHIS HYPOGEA L. (GROUNDNUT) (1200-040 (Screen 7))
Hall 2

A split plot experiment in a randomized complete block design with four replicates was used to study the effect of four (3cm, 6cm, 9cm and 12cm) sowing depths on the germination and growth of Arachis hypogea L. at Imo State University Agricultural Farm, Owerri, Imo State, Nigeria. Plant height, number of leaves, leaf area, root length, number of nodes, number of seeds per plant, and dry weights were the parameters obtained. 9cm and 3cm had significantly the highest and least germination rates respectively. Seeds sown at 9cm and 12cm depths germinated faster than seeds sown at 3cm and 6cm depths. Seeds sown at 9cm depth had significantly the highest germination percentages. At the end of the experiment, there was no significant difference found between the plant height, the number of leaves, leaf area, number of nodes, number of seeds and dry weights in relation to sowing depth. Difference was found in root length (9cm and 12cm) but it is not statistically significant. The study also revealed that seeds of 9cm and 12cm are larger in size and attractive. In other words, the plant obtained more roots at the deeper end than at the shallow end.

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Primary E-Poster Presenter: Nkiru Umeoka

A new dawn for plant water potential measurements (1200-041 (Screen 1))
Hall 2

For over 50 years, since the pioneering work of Boyer set the methodologies that are still in use today, plant physiologists and physiological ecologists have demonstrated how plant water potential controls a myriad processes in plants, from metabolism to cell expansion. In the field, the most robust and widely used measurement of leaf water potential involves excising a leaf and placing it into a pressure chamber, often called a pressure bomb. This method is destructive, cumbersome, and generally collected pre-dawn (for the least negative values) and mid-day (for the most negative values). The pre-dawn measurements have been the bane of nearly all students whom have worked on plant water relations in the field for the past half-century, and the mid-day measurements are merely an approximation of the maximum stress as the precise timing is untested. In an effort to solve the problems of destructive harvesting, cumbersome equipment, inconvenient measurement timing, and uncertain peak stress assessments, we have developed a passive electrical system for continuous monitoring of plant water potentials using microneedles that can occupy as little as a square millimeter on a single leaf surface. We have validated our data with pressure chamber and leaf psychrometer measurements, and have tested them in a field setting where they capture the expected diurnal patterns and longer-term responses to drying soil. The sensors work in all plant types tested, including herbaceous monocots, dicots, and woody species. They also work in multiple tissue types, illustrating differences between vascular tissues and less hydraulically connected tissues a few millimeters away. We will present these data to demonstrate how our system will end need for pre-dawn pressure chamber measurements, thereby lessening the suffering of students working on plant water relations, as well as illustrating some new applications.

Primary E-Poster Presenter: David Hanson

Determining Mechanisms for Shifting Flowering Time in Response to Global Change (1200-042 (Screen 9))
Hall 2

Background/Question/MethodsFor this study, we used two closely related genotypes of Arabidopsis (SG and CG). SG was selected for high seed number at elevated [CO2] (700 ppm) over multiple generations. CG is a random, non-selected control. SG has shown an increased affinity toward high seed output at E[CO2], producing twice as many seeds following selection. In previous work, it has been shown that FLOWERING LOCUS C (FLC) has strong repressive effects on floral initiation, and there appears to be at least a correlative effect of E[CO2] that enhances FLC

expression in the SG genotype. Interestingly, overexpression of FLC correlated with delayed flowering at E[CO2] . Direct manipulation of FLC under E[CO2] has not been conducted to determine a causal effect of FLC driving the late flowering phenotype at E[CO2]. Is altered flowering time at E[CO2] driven by variation in FLC expression in Arabidopsis? As FLC is known to be repressed by cold-winter temperatures (vernalization) to allow flowering to occur only in the spring, we used vernalization as a tool to explore the influence of E[CO2] on FLC and flowering. After several trial runs to improve experimental methods, we have a protocol to test our Arabidopsis lines under vernalization to determine the effects of flowering time under conditions where FLC expression is reduced, as likely occurs with vernalization.Preliminary DataAs expected, non-vernalized SG plants grown at E[CO2] to flowered at a significantly higher leaf number compared to ambient [CO2] levels as had been shown previously shown (Springer et al., 2008). Vernalized SG grown at E[CO2] flowered at a similar leaf number of SG plants grown at A[CO2]. Therefore, we presume that a knock-down of FLC expression due to vernalization recovered earlier flowering times at E[CO2] that are similar to A[CO2]. Confirmation of FLC reduction through the vernalization treatment is underway.

Primary E-Poster Presenter: Aleah Henderson-Carter

The molecular and environmental control of growth rate variation in Brachypodium (1200-043 (Screen 8))
Hall 2

Plant growth rate is a complex trait that reflects a balance between resource acquisition and allocation. Here, we present results from a series of experiments investigating the dynamic control of growth rate by genetic and environmental factors in the model grass genus Brachypodium. First, we fit models of growth rate using natural diversity panels of both annual and perennial Brachypodium species. We observe significant genetic diversity in whole-plant growth rate as well as the relative biomass partitioning to roots, leaves, and stems. We next identify genetic correlations between these growth rate coefficients and eigengenes estimated from root and leaf RNASequencing libraries; significantly correlated eigengenes from this analysis represent candidates for the molecular control of growth rate variation in Brachypodium. We manipulated carbon source-sink relationships two annual and two perennial species of Brachypodium grown at three atmospheric CO2 concentrations in a second experiment. We relate measures of the acquisition of carbon, its partitioning to respiration, storage, and growth, and the response of eigengenes – identified above – to the varying carbon source strengths. To support this latter experiment we also briefly present the de novo sequencing and annotation of new Brachypodium genomes which will facilitate additional functional genomic analysis in the genus. Our work offers the first glimpse at the molecular control of growth rate variation in annual and perennial species and sets the stage for future work on the evolution of life histories in grasses.

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Primary E-Poster Presenter: David Des Marais

A multi-pronged investigation into thermoadaptation of photosynthesis in an extremophilic plant Ariz (1200-009)
Hall 2

In the 1970s, Carnegie scientists studying C4 plant Tidestromia oblongifolia in Death Valley discovered that it is highly adapted to high temperatures and has optimal photosynthetic rate at 47ºC. CO2 assimilation at this temperature is comparable to that in crop plants in their most favorable conditions. This remarkable thermoadaptation of photosynthesis remains largely uninvestigated. Here, we aim to combine complementary approaches in genomics, ecophysiology, biochemistry, anatomy, cell biology, and modeling to decipher the mechanisms underlying this thermoadaptation at multiple scales and determine which innovations increase photosynthetic rate at high temperatures in T. oblongifolia. We have successfully recreated Death Valley summer and winter conditions in our lab and will compare T. oblongifolia accessions from Death Valley and Dos Palmas, which have different maximum temperatures. We will also compare T. oblongifolia with T. lanuginosa from Arizona and three different accessions of Amaranthus hypochondriacus (Nebraska, India, and Mexico). A. hypochondriacus is a pseudo-cereal crop with high protein content and tolerance to abiotic stresses, but still heat sensitive to extreme conditions of Death Valley. The aim of these comparisons is to underscore the complexity of how life copes with environmental and ecological stressors and to view organisms in the context of their ever-changing environment. This is especially of interest as the environment is changing ever more rapidly and drastically than initially predicted. Outcomes of this project may lead to the development of industrial biocatalysts, improvement of thermoadaptation in crops, and new ways of approaching conservation of plants in the context of climate change. In this talk, I will present the models that allowed us to mimic the climate of Death Valley in the lab and the ecophysiological measurements that represent the basis of the project for further investigation.

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Primary Poster Presenter: Karine Prado

Allelopathy in weedy rice: Stair-step screening and root system architecture (1200-011)
Hall 2

Rice supplies a significant portion of the daily diet of millions worldwide, and demand for rice production is projected to increase significantly in coming years

due to population growth. Unfortunately, weed competition is a limiting factor in rice production, and there is need to identify supplemental weed management strategy. Weedy rice is of the same species as cultivated rice, Oryza, and is a common rice weed exhibiting allopathic characteristics. Allelopathy is defined as the release of chemical compounds from one plant into the environment that inhibit the growth and development of neighboring species. Identification and incorporation of allelopathic traits from weedy rice into cultivated rice lines may have a positive impact on rice yield. The overall objective of this study is to characterize weedy rice accessions based on allelopathic potential to suppress prevalent weeds like barnyardgrass, and to identify root system architectural changes associated with allelopathic phenotypes. In this study 10 weedy rice accessions, 2 non allelopathic rice cultivars (REX CL163), and 3 allelopathic rice cultivars (RONDO CL777 CL046) were screened using a stair step technique. Allelopathic potential was calculated and categorized based on percent inhibition of barnyardgrass. B2 and B8 weedy rice accessions were identified as highly allelopathic as it reduced the biomass and height of barnyardgrass by 20-35% and 0-35%, respectively. These two allelopathic accessions had longer roots and larger root area, 30 and 15%, respectively, compared to non-allelopathic weedy and cultivated rice. Allelopathic weedy rice, however, had 13% lesser number of secondary roots than their non-allelopathic counterparts. Allelopathic genes found in weedy rice, being the same species as rice, can easily be used in rice breeding programs. These accessions may thus serve as a genetic resource for the development of weed-suppressive rice cultivars.

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Primary Poster Presenter: Te Ming Tseng

An assessment of phenotypic plasticity of Chenopodium quinoa (1200-024)
Hall 2

Quinoa is a highly nutritious crop that can grow under a wide range of environments due to high tolerances to challenging environmental conditions such as drought, salinity and low temperature. The genetic richness and resilience of the crop has not been fully explored, since the primary locations for cultivation are still the countries that have a tradition of growing quinoa like Bolivia, Peru, Ecuador, Argentina and Chile. Expansion of the cultivation into other countries to upscale the production of the crop to meet the increasing demand of the market and reduce transportation costs requires information on the performance of the varieties in a range of environmental conditions. We are studying the phenotypic plasticity of ~1000 accessions of Chenopodium quinoa using parameters such as height, yield, panicle size, growth habit, etc., recorded from large-scale field trials at two field sites in China and a field site in Australia, conducted during May to October 2018 and May to August/September 2018, respectively. Among the many phenotypic

differences, we observed a trend of taller plants as well as a prolonged growth period to reaching maturity in China, compared to Australia. This is likely to be due to the differences in day length between the two sites (with plants growing through winter in Australia and summer in China). With the genotypic data available to date, from the cooperative effort of our lab and our collaborators, with a total of ~25 million SNPs over 312 accessions, a GWAS analysis was performed and will be discussed. Our study has implications for the process of further domesticating quinoa, through identification genes and their alleles associated with an insensitivity to daylength, as well as providing information that can be used in the selection process for varieties suitable for different environmental conditions. This is beneficial for future genomics-informed breeding programs.

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Primary Poster Presenter: Clara Stanschewski

Carbon assimilation and fast fluorescence kinetics between cotton thinner fiber mutant, im, and its (1200-022)
Hall 2

Cotton fiber thickness influences both price of the raw material and quality of the finished product in textile industry. The recessive immature fiber (im) gene reduces the fiber thickness in allotetraploid Gossypium hirsutum. Given that how cotton fiber thickness is regulated is largely unknown, im and its near isogenic wildtype, TM-1, offer a valuable comparative model to understand the genetic and physiological basis of cotton fiber maturity. The im phenotype was recently found to be linked to a 22-bp deletion in a pentatricopeptide repeat gene. However, how the im gene affects carbon assimilatory properties in cotton plants is unknown. Our preliminary investigations showed that im maintained a higher stomatal conductance in the field and greenhouse conditions where the environmental conditions were not tightly controlled. Thus, the objective of this study was to compare the two genotypes under more controlled growth chamber conditions to understand the effect of the mutation on the photosynthetic physiology without the effects of the environmental changes. Leaf gas exchange characteristics including net photosynthesis, stomatal conductance, intercellular [CO2] and transpiration were similar in the two genotypes. However, the JIP test of fast fluorescence kinetics showed that several key parameters related to electron flux within the intermembrane system and potential of these electrons reducing the PSI acceptor side were greater in im compared with TM-1. These energy conserving mechanisms provided a greater total performance index (PItot) in im. These findings suggest that the mutation of the im gene causing thinner fiber may also affect photosynthesis through changes in electron use within the thylakoid. These findings are potentially useful in understanding the association between fiber maturity and photosynthetic physiology in cotton.

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Primary Poster Presenter: Evans Marrero

1:30 PM - 3:00 PM

Comparisons of the Pollination Service Provided by Honeybees Vs Wild Bees in Summer Squash Varieties

Honeybee colonies have been on a significant decline due to a combination of diseases, nutrition, stress, pesticides, and nesting site degradation. When crops lack the presence of honeybees, native bees can sustain the pollinating process. The objective of this study is to compare the pollination activity of honeybee versus wild bees in summer squash varieties. Four summer squash varieties (Desert, Dunja, Yellowfin, and Tempest) were planted in two separate fields four miles apart. One beehive placed in the first squash field (Field 1) during the blooming stage of the squash plants. It is always recommended to put one beehive per acre of any cucurbit plants (Squash, watermelon…etc.). In the second field (Field 2), insectary flowering plants (Zinnia, sunflower, and other flowering plants) were planted adjacent to the squash plants, and in the third field (Field 3) neither insectary flowering plants were sown nor beehive placed in the field (control). Planting dates were adjusted to have both the insectary flowering plants and squash plants to bloom at the same time. Similar agronomic practice performed in all squash fields. Data on the number of pollinator visiting and yield of squash plants collected. There was no significant difference in total yield among the fields with the beehive and insectary flowering plants, except in some varieties. High marketable yield recorded in Desert and Dunja varieties grew in field 2. This is maybe due to the presence of several predators such as soldier beetles, hoverflies, ladybug beetles…etc. The abundance of and diversity of wild bees was high in the insectary flowering plant plots when compared with beehive and control plots. It seems growing insectary flowering plants near crop fields might enhance yield potential of some pollinator-dependent crops. This study might help farmers to avoid renting expensive beehives for pollination purposes.

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Primary Poster Presenter: Simon Zebelo

Controlling Salvinia molesta through allelopathy (1200-012)
Hall 2

Salvinia molesta is an invasive aquatic fern originally from Brazil that causes ecological and economic damage to freshwater ecosystems in Louisiana and the SW United States. The fern outcompetes other plant species and causes anoxia, leading to mortality of numerous species in aquatic ecosystems. It also has economic impacts via hunting and fishing. The species has been difficult to control despite

numerous attempts with biological, mechanical, and chemical methods. The goal of this study was to assess whether other plant species commonly found in freshwater ecosystems have allelopathic effects on S. molesta, whereby they produce chemicals that harm S. molesta. It was hypothesized that S. molesta growth would be reduced and health negatively affected by extracts of other plants. Plants were collected at Wallace Lake, Louisiana, and extracts were created, filtered, and used in growth experiments. S. molesta growth was not affected by extracts; however, the health of S. molesta was significantly affected in a negative way compared to controls. Leaves had a greater impact than roots on S. molesta. There are was a positive correlation between health and final root mass, suggesting that extracts harmed the roots. In the future, higher concentrations of effective extracts will be tested.

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Primary Poster Presenter: Amy Erickson

Developmental changes in leaf physiology and its implications for environmental adaptation (1200-019)
Hall 2

Plants transition through distinct stages as they develop, the timing of which, significantly impacts large-scale ecological and evolutionary processes. Vegetative phase change (VPC), the developmental transition from juvenile to adult vegetative growth, has been well studied at the molecular level however, little is known about its importance for plant performance and physiological functioning. In this study, variation in physiological and morphological characteristics between juvenile and adult leaves of four diverse species, Zea mays, Passiflora edulis, Populus tremula x alba, and Arabidopsis thaliana, were analyzed. Mutants of miR156, the master regulator of VPC, were used to modulate the timing of VPC in all four species to investigate differences in photosynthetic traits and leaf morphology associated with vegetative development. Further, these mutants were used to determine whether variation in traits between juvenile and adult leaves translate into variation in plant performance under environmental stress. Through this research we found significant variation in photosynthetic properties between juvenile and adult leaves in all species including maximum photosynthetic rates and rates of photosynthetic light induction. Additionally, juvenile and adult leaves showed differences in morphology including specific leaf area, stomatal densities and venation, traits with major implications for foliar function. Lastly, when both mutants and natural genotypes with variation in the timing of VPC were subjected to the environmental stresses of heat, drought and low light, there were significant relationship between

plant performance under some of these stresses and the timing of VPC. This research begins to uncover the role of vegetative development in plant physiology, potential mechanisms to be utilized in plant breeding programs and insight into the underpinnings that may have led to the evolutionary conservation of VPC and its master regulator miR156.

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Primary Poster Presenter: Erica Lawrence

Dynamic Relationship Between Sweet Corn Quality and Canopy Microclimate in Semi-Tropical Climate (1200-017)
Hall 2

The unstable quality among different planting season has brought considerable economic loss on sweet corn production in Taiwan. In order to excavate the dynamic relationship between sweet corn quality and canopy microclimate. Focus on sweet corn with shrunken-2 (sh2) allele, field data including ear physical property and kernel sugar content, which covered different planting seasons were collected from six different regions. Additionally, canopy microclimate sensors were deployed in each sampling field, including temperature, humidity, precipitation and photosynthesically active radiation (PAR). In our experiment, for canopy microclimate, more extreme canopy daily maximum and minimum temperature were observed, strengthen the urgent need to monitoring canopy environment through field sensors. From the relationship between each quality aspect, ear weight and ear width shared significant positive correlation with sucrose content, while shared significant negative correlation with glucose and fructose content, suggesting the limitation of ear development may reduce sucrose synthesis from glucose and fructose. Based on the dynamic relationship between quality and canopy microclimate, we propose the optimal climate for sweet corn production, with 20 – 22 ℃ average temperature, 15-18 MJ/m2 average PAR and accumulate precipitation under 200 mm. Taken the result together, we report the dynamic relationship between quality and each canopy microclimate factors; propose sweet corn quality prediction model considering canopy microclimate; and provide reference to define suitable area for sweet corn production under semi-tropical climate.

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Primary Poster Presenter: Huu-Sheng Lur

Ecophysiolomics of tapping for Frankincense in Boswellia sacra tree (1200-025)
Hall 2

The outer bark of Boswellia sacra tree is tapped to obtain oleo-gum resin, known as frankincense. Unsustainable tapping hinders tree growth and impairs the regeneration capacity of its population. The healing processes that are triggered to promote tree growth after wounding remain incompletely understood. Herein, we used de novo site-specific transcriptomic, physio-hormonal and mRNA expression analyses to understand the tapping responses in frankincense tree, and known plant growth regulators (PGRs; jasmonic acid, salicylic acid and gibberellic acids) were applied to assess the wound-healing process. Site-specific differential gene expression revealed the participation of 619 key signaling networks related to terpenoid biosyntehsis, phytohormonal regulation, cellular transport and cell-wall synthesis. Exogenous jasmonic acid (JA) resulted in significant recovery of epidermis cell-wall integrity during 30min and or 3days after tapping than other PGRs (salicylic acid - SA and gibberellic acids - GA) and control samples. Endogenous SA and JA were significantly activated by exo-JA and SA compared to controls. This was in concordance with gene expression patterns of terpenoid, cell-wall and wounding-stress signaling cascades, which showed significantly higher transcript levels after 30min compared with 3days of tapping. The findings elucidates for the first time a detailed transcriptome of site-specific tapping in one of the ecologically, economically and medicinally important frankincense producing tree species. In addition, this has not been done for any of the species at genus and Family Burseraceae levels. Thus, this transcript dataset would also work as references for more studies on this and related species. In conclusion, our study showed that tapping immediately activated several cell development and regeneration processes along with defense-induced terpenoid and phytohormonal metabolism to heal damaged tissues in the epidermis.

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EFFECTS OF SPENT ENGINE OIL ON THE GERMINATION AND GROWTH OF SOLANUM MELONGENA L. (GARDEN EGG) (1200-027)
Hall 2

The effect of spent engine oil pollution on soil properties and germination, growth and development of Solanum melongena was investigated using six treatments (0ml, 20ml, 40ml, 60ml 8oml and 100ml) of the spent engine oil were applied to 20kg of soil in perforated barco bags and the experiment was arranged in randomized complete block design with four replicates. Soil analysis showed that spent engine oil had no effect on the pH and texture of the soil and there is significant difference in the levels of heavy metals (Cu, Mn, Pb, Zn, Cd) in both the

polluted and unpolluted soil samples. The result further revealed that the percentage germination decreased as the concentration of spent engine oil increased. Also, the result revealed that plant height, leaf length, leaf area and number of leaves decreases as the concentration of spent engine oil increases.

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Primary Poster Presenter: Evaristus Nwagbara

Genetic and genomic studies of climate adaptation and genotype-by-environment interaction in switchg (1200-021)
Hall 2

Plants live in an ever-changing and unpredictable environment. As sessile organisms, they must cope with perturbations to their particular microhabitat in space and time. Which environments matter most? What physiological or metabolic mechanisms buffer responses to the environment and climatic change? How are these responses encoded in genomes and how do they evolve? Genome-enabled research has characterized the myriad expression and metabolite responses of many species to common stresses including drought, temperature extremes, light stress and salinity. The challenge now is to disentangle evolved and adaptive responses of plants to stress from the deleterious results of stress. A promising avenue is the use of locally adapted natural variation to winnow the beneficial responses from the maladaptive consequences of stress. Switchgrass (Panicum virgatum) is a polyploid C4 perennial grass that is native to North America and has been championed as a promising biofuel feedstock. It is a common member of most native prairie communities and exhibits extensive phenotypic variability and adaptation across its range, especially related to latitude and precipitation gradients. Much of this variability is associated with evolved lowland and upland ecotypes. Here, I report on the development of genetic and genomic resources for switchgrass, as well as present results from field experiments aimed at understanding upland/lowland ecotype divergence and local adaptation. In particular, I present preliminary results from QTL studies aimed at detecting gene-by-environment interactions for a variety of traits utilizing collaborative common garden experiments across the species latitudinal/climatic range.

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Genetic structure analysis of Phalaris arundinacea identifies native Minnesota vs exotic populations (1200-023)

Hall 2

Reed canary grass (RCG, Phalaris arundinacea) is a major invader of Minnesota wetlands. It forms large, monospecific stands and it is highly competitive, especially in disturbed areas. Native vs. exotic status of RCG is not clear and past research suggested the existence of native RCG stands in N. America. The purpose of this study was to examine RCG populations to aid land managers in identifying native vs. exotic types. Herbarium specimens native in origin to N. America, as well as extant wild RCG collections from Minnesota and the Czech Republic were used as a benchmark to determine current RCG populations' genetic background. Genetic variation among and within RGC populations were assessed by DArTseqLD1.0 with use of 2,889 SNPs. This is the first use of this technology in RGC. Minnesota wild and Czech wild RCG collections are genetically distinct. The RCG populations from six major MN rivers are panmictic. Both Minnesota and Czech Republic collections show that most genetic variation is within populations, 98.8 and 94.2, respectively. Fst values for Minnesota (0.003 - 0.037) and Czech Republic (0.97 - 0.141) indicate low genetic divergence among each river population, with Czech Republic populations being more genetically diverged than Minnesota. Minnesota herbarium specimens cluster with extant samples, indicating the persistence of potentially native RCG genotypes in Minnesota. Extant specimens from the locations of previous herbarium samples are similar to wild MN river populations. In addition, 'Palaton' and 'Venture' (forage cultivars), are distinct from MN wild river populations except for the Roseau River. We conclude that the RCG in MN river populations are most likely native to N. America. These findings have profound implications for managing this native wetland invader.

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Heterologous expression of serine hydroxymethyltransferase3 from rice confers tolerance to salinity (1200-020)
Hall 2

Among abiotic stresses, salt stress adversely affects growth and development in rice. Contrasting salt tolerant (CSR27), and salt sensitive (MI48) rice varieties provided information on an array of genes that may contribute for salt tolerance of rice. Earlier studies on transcriptome and proteome profiling led to the identification of salt stress-induced serine hydroxyl methyltransferase-3 (SHMT3) gene. In the present study, SHMT3 gene was isolated from salt-tolerant (CSR27) rice. The functional characterization of OsSHMT3 from salt-tolerant OsSHMT3 exhibited salinity-stress induced accentuated and differential expression levels in different tissues of rice. OsSHMT3 was overexpressed in E. coli and assayed for enzymatic

activity and modelling protein structure. Further, Arabidopsis transgenic plants overexpressing OsSHMT3 exhibited tolerance towards salt stress. Comparative analyses of OsSHMT3 vis a vis wild type by ionomic, transcriptomic and metabolic profiling, protein expression and analysis of various agronomic traits revealed a pivotal role of OsSHMT3 in conferring tolerance towards salt stress. The gene can further be used in developing gene based markers for salt stress to be employed in marker assisted breeding programs.

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1:30 PM - 3:00 PM

Improving cadmium tolerance in rice: Insight into vanillic acid-induced upregulation of antioxidant (1200-004)
Hall 2

Cadmium (Cd) is a serious environmental threat because it accumulates in plants from soil and is subsequently transported into the food cycle. Increased Cd uptake in plants disrupts plant metabolism and hampers crop growth and development. Therefore, remediation of Cd from the soil and enhancing plant tolerance to metal toxicity is vital. In the present study, we investigated the effect of vanillic acid (VA) on Cd toxicity in terms of metal accumulation and stress tolerance in rice (Oryza sativa L. cv. BRRI dhan54). Thirteen-day-old rice seedlings were treated with Cd (1.0 and 2.0 mM Cadmium chloride) alone and in combination with VA (50 µM) in a hydroponic medium for 3 d. Increasing the Cd concentration led to reduced growth, biomass, water status, and chlorophyll (Chl) content resulting from increased oxidative damage (elevated malondialdehyde content; hydrogen peroxide and superoxide radical, generation; lipoxygenase activity; and methylglyoxal content) and downregulation of the major enzymes of the antioxidant defense and glyoxalase systems. Under either dose of Cd stress, VA improved the growth of the plants by enhancing leaf relative water content and Chl content; reducing oxidative damage; enhancing the pool of ascorbate and glutathione and the activities of the antioxidant enzymes, improving the performance of the glyoxalase system; and increasing the phytochelatin content. Therefore, our findings suggest that VA plays an important role in rice seedlings by enhancing stress tolerance through increasing metal chelation and sequestration as well as upregulating the antioxidant defense and glyoxalase systems.

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Increased Soil Respiration and Arbuscular Mycorrhizal Fungi Under Elevated [CO2] and Soil Water Defi (1200-015)
Hall 2

Predicted increases in atmospheric [CO2], decreased availability of irrigation water and high ambient temperatures are challenges that will impact food production in coming decades. Peanut is an important food crop cultivated in semi-arid region and its symbiotic capability with both mycorrhizal fungi and N2 fixing bacteria make it a model crop for understanding the soil microbial community dynamic. This study investigated the effect of elevated [CO2] (650 ppm) and induced soil water deficit on the soil microbial component during two growing seasons in the Texas High Plains peanut production system consisted of Amarillo fine sandy loam soil. Microbial community size and structure were evaluated via ester-linked fatty acid methyl ester (EL-FAME) profiling. Soil metabolic activity was examined by measuring soil respiration with the LI-6400 (LI-COR Biosciences, Lincoln, NE) and ß-glucosidase activity, which is involved in cellulose degradation. Regardless of the soil-water deficit or plant growth stage, elevated [CO2] shifted the microbial community composition towards 46% more arbuscular mycorrhizae (AMF) compared to the ambient [CO2]. Elevated [CO2] did not significantly change ß-glucosidase activity; however, soil respiration increased by 82% during periods of soil-water deficit. Further, elevated [CO2] increased soil total carbon and nitrogen content (measured with LECO TruSpec CN) under limited soil water conditions, in agreement with the increase in AMF. Our results suggested that elevated [CO2] is most likely to increase AMF populations, soil total carbon and respiration. Further ecological studies are needed for understanding the long-term impact of these findings in a changing climate.

Primary Poster Presenter: HAYDEE LAZA

Movement of Metal Nanoparticles in Arabidopsis (1200-016)
Hall 2

One of the important basic information for developing nanoparticles as a functional materials in biology is to discover the movement of nanoparticles in organisms. Research in the field of plant nanoparticle tracking is still in its infancy. In Arabidopsis, we traced nanoparticles in two different ways. First, Arabidopsis was exposed to CdSe/CdZnS QDs with three different coatings, anionic, cationic, and relatively neutral, and nanoparticle movement was dramatically changed from the particle absorption into the plant root cell to the particle localization in plant leaves. T. ni caterpillars that fed on Arabidopsis exposed to QDs had reduced performance, and QD fluorescence was detected in both T. ni bodies and frass, demonstrating trophic transfer of intact QDs from plants to insects. Second, gold nanoparticle was applied to test the biological responses to photothermal effects of nanoparticles. The uptake of gold nanoparticle through Arabidopsis roots and translocation to leaves are reported through the photoacoustic signal detection. Furthermore, Arabidopsis leaves harboring GNPs and exposed to continuous laser or noncoherent light show elevated temperatures across the leaf surface and induced expression of

heat-shock regulated genes. Overall, these results demonstrate that metal based nanoparticles are strong candidate as a substance carrier for manipulating plant physiology.

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Primary Poster Presenter: Yeonjong Koo

Ozone effects on soybean roots (1200-002)
Hall 2

High concentrations of tropospheric ozone (O3) during the growing season leads to significant reductions in global soybean yields. Research on the impact of O3 on soybean has generally focused on above-ground tissues, not on roots, which are major tissues supporting plant fitness. Mandarin (Ottawa) (O3-sensitive) and Fiskeby III (O3-tolerent) soybean genotypes provide contrasting materials with which to investigate how O3 alters root morphology. In this study, we analyzed root initiation, biomass, and diameter respectively within root classes of 16-day-old (approximately V2 stage) Mandarin (Ottawa) and Fiskeby III grown in greenhouse with charcoal-filtered (CF) air followed by treatment with CF or 75 ppbv O3 for 7 h/day in continuously stirred-tank reactors (CSTR) for 0, 4, and 7 days. The results showed that O3 significantly decreased the biomass of rapidly developing basal roots in Mandarin (Ottawa) during the O3 treatment; root initiation rates were not affected. Biomass accumulation was not significantly affected in either basal or tap roots present before the O3 treatment began. However, root diameter was reduced by O3 treatment in Mandarin (Ottawa) with a reduction of 0.0726 mm in the developing basal roots and 0.0181 mm in lateral roots of developing basal roots, basal roots, and tap roots. There were no impacts of O3 on the root biomass or root diameter of O3-tolerant Fiskeby III. Our study provides robust evidence that O3 alters root architecture complexity in O3-sensitive soybeans by decreasing the biomass and root diameter of developing basal roots along with a general reduction in root diameter of all lateral roots. Our findings uncover a morphological impact of O3 on below-ground tissues and potentially identifies O3-tolerent root trait assessments for soybean breeders.

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Physiological changes associated with heat stress acclimation for developing apple fruit (1200-001)
Hall 2

Physiological changes associated with heat stress acclimation for developing apple fruit

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Plant Diversity, Canopy Cover and Water Quality in a Pterocarpus Forest after Hurricane Maria (1200-008)
Hall 2

Wetlands serve as buffer zones, flood barriers, and habitat for wildlife. The Pterocarpus Forest at Palmas del Mar (PFPM) in Puerto Rico is a wetland forest surrounded by an urban landscape. PFPM was devastated by Hurricanes Irma and Maria in September 2017. The goal of this course-based group project was to conduct a plant survey, report plant diversity, estimate canopy cover using photographs, and evaluate water quality in 15 5 x 5m plots distributed throughout three zones of PFPM (entrance, exit, center) two months after Hurricane Maria. Diversity and evenness were calculated, pictures of the forest canopy were taken with a cell phone camera and analyzed with ImageJ, and water quality was evaluated by measuring salinity, pH, electrical conductivity, total dissolved solids, dissolved oxygen, and colony forming units. A total of 198 plants were encountered with zone 1 having the most individuals (80/198). Most plants encountered in the survey were Pterocarpus officinalis (67.2%) and Acrostichum aureum (16.2%). Diversity ranged from 0.75 in zone 1 to 1.32 in zone 3. The average percent canopy cover in November 2017 ranged from 12.5% in zone 3 to 27% in zone 2 and the total average canopy cover including all data was 21.4%. In March 2018 the total average canopy cover was 37% and ranged from 14.8% in zone 3 to 52.7% in zone 1. Standing water in zone 2, nearest to accumulated vegetative matter from post-hurricane clean-up efforts, had significantly greater total dissolved solids, electrical conductivity, and viable CFU's than samples from other zones. This study reports plant diversity, canopy cover, and water quality at PFPM in PR two months after the impact of Hurricane Maria. Furthermore, this course-based project served to teach 11 graduate students about environmental science, plant diversity, wetland ecosystems, and the effects of hurricanes.

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Quercetin mediated salt tolerance in tomato by escalating plant antioxidant defense and glyoxalase s (1200-003)
Hall 2

Quercetin (Qu) is a strong antioxidant among phenolic compounds by having physiological and biochemical roles in alleviating abiotic stress in plants. Hence, we had studied the Qu-induced protection against salinity (150 mM NaCl) in tomato (Solanum lycopersicon L.). Salinity caused ionic stress by increasing Na+ toxicity along with the depletion of K+, Ca2+ and Mg2+. Osmotic stress was detected by higher free proline (Pro) content and lower leaf relative water content (RWC) in salt-stressed seedlings. Salt stress also caused excess H2O2 generation, MDA production and LOX activity as a sign of oxidative stress. Tomato seedlings were suffered from higher MG toxicity, degradation of Chl along with lower biomass accumulation and growth by saline exposure. However, Qu application in salt-treated plants showed lower Na+/K+, higher RWC, increased Pro, and reduction of H2O2, MDA, LOX activity which indicated alleviation of ionic, osmotic and oxidative stresses, respectively. Quercetin caused lessening of oxidative stress through strengthening of both enzymatic and non-enzymatic antioxidants of plant antioxidant defense system. Importantly, Qu increased GST activity in salt affected seedlings which might be stimulated ROS scavenging activities along with higher GSH content. While, MG detoxification was ensured in Qu supplemented salt treated seedlings by increasing both Gly I and Gly II activities. Consequently, Qu insisted better plant growth and photosynthetic pigments synthesis. So, exogenous applied Qu can be an important actor to confer salt tolerance in glycophytes.

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Reducing water use in ornamentals: acetic acid as a low cost anti-transpirant (1200-007)
Hall 2

Maintaining adequate water to potted ornamental plants throughout the transition from the production nursery to planting in consumer yards creates a logistical challenge for both transportation and retailer. A cost effective anti-transpirant, which both reduces water demand and protects salability in response to drought, could have value to the nursery industry. The application of low concentrations (10-40mM) of acetic acid have been observed to increase survival in response to drought in Arabidopsis, corn, wheat, rice and canola under controlled conditions (Kim et al. 2017 Nature Plants 3:17097). This year, an acetic acid-induced increase in leaf temperature, associated with reduced transpiration, was reported in greenhouse grown cassava (Utsumi et al. 2019 Frontiers in Plant Science 10:521).

Here we test the anti-transpirant performance of 10-40 mM of acetic acid in the water supply of commercial ornamental Begonia × hybrida 'Dragon Wing', growing outdoors under both well-watered and drought-stressed conditions. We observed significant reductions in pot water use, that was extended for weeks after acetic acid was applied, and related this to changes in leaf temperature and relative water content. Using retail prices, this vinegar treatment would add only 0.3% to the cost of a plant. Finally, we explored whether this promising phenotype had commercial potential through increased flower retention and survival under drought stress.

Co-author(s): Megan Allen

Primary Poster Presenter: Damian Allen

Soybean drought tolerance mechanisms: testing a growth-chamber based hypothesis in the field (1200-006)
Hall 2

Soybean breeders have identified several drought tolerant genotypes in the field by assessing the degree of leaf wilting during periods of low rainfall. This phenotype could result from multiple potential mechanisms of drought tolerance. Extensive work with potted plants in growth chambers identified transpiration limitation at high vapor pressure deficit to be a probable drought tolerance mechanism in many slow-wilting soybean genotypes. However, this hypothesis had not been tested in the field, and the potential loss of photosynthesis due to stomatal restriction during otherwise favorable conditions had not been measured. N06-7194, a drought tolerant soybean genotype that was expected to limit transpiration at high vapor pressure deficit, was grown in +/- irrigation plots in the field alongside a drought sensitive soybean genotype. Fast-draining, sandy soil allowed for water stress to rapidly develop in the absence of irrigation during periods without rainfall in 2017 and 2018. Although the drought tolerant genotype maintained higher rates of photosynthesis during periods of moderate to severe drought, stomatal responses did not indicate a greater restriction of transpiration at high vapor pressure deficits than in the drought sensitive genotype. These results suggest that stomatal responses to vapor pressure deficit in growth chambers may not fully capture plant responses in the field, and the mechanism underlying N06-7194's drought tolerance remains unclear. A third year of field experimentation is underway.

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Primary Poster Presenter: Anna Locke

Spatially resolved CO2 assimilation: gas exchange combined with thermal and chlorophyll fluorescence (1200-010)
Hall 2

To date, photosynthetic gas exchange data is collected one dimensionally, integrating across entire leaf areas in a cuvette. Spatial heterogeneity across the leaf surface could bias conclusions based on gas exchange data when determining

leaf structure-function relationships, especially during stress. Previously, thermal and chlorophyll fluorescence imaging have been used to understand spatial variation in stomatal conductance, however this approach has not been used to spatially partition gas exchange under ambient O2. Here we use a leaf energy balance model to map variation in transpiration across the leaf surface with the thermal imaging platform to infer stomatal conductance across the leaf surface. We then map photosynthetic electron transport obtained through chlorophyll fluorescence, derive a net CO2 assimilation to electron transport relationship based on average values across the whole leaf. We map net CO2 assimilation and intercellular CO2 concentrations across the leaf surface under normal and low O2 conditions. We focus on spatial heterogeneity in steady state CO2 and light responses due to their importance in understanding and modeling carbon assimilation. Our new approach opens a new area of investigation for understanding how leaf physiology is coordinated across the leaf surface.

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STUDIES ON THE EFFECT OF Chromolaena odorata (L.) King & H.E. Robins ON THE GROWTH PERFORMANCES OF T (1200-028)
Hall 2

Green house studies were conducted to assess the efficacy of Chromolaena odorata on the growth performance of three varieties of Abelmoschus esculentus, namely HIRE, SAHARI F1 and KIRIKOU F1. The experiments were laid out on Completely Randomised Design (CRD) in Split Plot Design with six treatments and each treatment having four replicates. The effects of these treatments on the germination percentage, plant height, number of leaves, leaf area, fruits weight, seeds count, number of roots, length of longest root, fresh weight, dry weight, moisture content and physicochemical properties of the soil. The data was analysed using Analysis of Variance (ANOVA) to test for the significance difference at ($p \leq 0.05$). The study indicated there was amendment in soil chemical properties after the application of Chromolaena odorata, compost achieving optimal fertility for Abelmoschus esculentus. Chromolaena odorata had higher stimulating effect on the growth performances of variety SAHARI F1 followed by KIRIKOU F1 and HIRE. This study therefore recommend the use of Chromolaena odorata in farming practices considering its economic important as a green manure in Nigeria.

Primary Poster Presenter: Sunday Sam

The identification of the gene encoding for isoprene synthase (IspS) in dominant Missouri oaks (1200-014)
Hall 2

Oak species are the dominant woody species in North America with 68% coverage consisting of 191 million acres. Despite their abundance and the high scientific interest in oak genomes, to date only the pedunculate oak's (Quercus robur) genome has been sequenced, but these sequences have not been yet annotated, limiting gene discovery applications. The goal of the current study was to identify for the first time and characterize the gene encoding for the isoprene synthase (IspS) from a diverse group of oaks (Quercus spp.), dominant in Missouri, such as pin oak (Q. palustris), swamp white oak (Q. bicolor), post oak (Q. palustris), white oak (Q. alba) and chinquapin oak (Q. muehlenbergii), to facilitate further genomic studies. The IspS gene encodes for the terminal enzyme responsible for converting dimethylallyl pyrophosphate (DMAPP), and its isomer, isopentenyl pyrophosphate (IPP), to isoprene. Isoprene is a volatile hydrocarbon released from the leaves of many, but not all plant species. The capacity to synthesize isoprene has been shown to provide a variety of benefits to emitter species, protecting membranes from damages induced by high temperature and oxidative stress. In the troposphere, isoprene reactions may contribute to a positive feedback on a warming climate. The IspS genes identified in this study will enable gene expression studies and the investigation of the evolution of isoprene synthesis and emission in oaks, unexplored to date.

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Primary Poster Presenter: Csengele Barta

The older plant gets the sun: age-related changes in perennial grass phenology, and implications for (1200-018)
Hall 2

Many biomass crops are perennial, but the models we use to assess them often overlook the influence of plant age on seasonal plant phenology. For example,

sapling trees leaf out earlier than conspecific adults to capture early season sun before the older stand closes canopy over them. Little information on age and phenology dynamics exists for perennial warm season grasses. We used a novel REplicated PLAnting Year (REPLAY) experimental design to study age-related phenology changes during the establishment phase (first three years) of Miscanthus × giganteus. We also considered the interactive effects of nitrogen (N) fertilization on phenology since N pools could be diluted in older, larger individuals. We found that two- and three-year-old (mature) stands produced ~30% more stems, with ~20% more leaves and nodes than one-year-old (young) stands. Faster developmental rates were usually seen in young stands, but they did not lead to more advanced developmental stages. Normalized over thermal time (growing degree days), older stands emerged ~3 months earlier than newly planted rhizomes. Nitrogen fertilization partially overrode age-related changes in emergence and senescence, and delayed flowering in mature stands thereby extending the growing season at least 10 days. We then used the process-based ecosystem model Agro-IBIS to understand how observed age-related phenology changes could influence ecosystem performance over the life time of a M. × giganteus stand. Over a 20-year crop lifespan, we found growing season length, leaf area, biomass production, and associated water cycling to be sensitive to the effects of plant age and nitrogen on grass phenology, and will discuss implications for crop and ecosystem assessment.

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Primary Poster Presenter: Emily Heaton

Weed-response genes of corn open opportunities for creating weed-tolerant varieties (1200-026)
Hall 2

Weeds reduce crop yield, but not because they compete for resources. Rather, weeds are perceived by crops early in the season before competition for resources are manifested, and that perception leads to developmental changes and stress responses that ultimately reduce yield. Weed-responsive genes are needed to identify signals and pathways involved in crop responses to weeds. We investigated transcriptome changes in corn caused by weeds in two different experiments each with three biological replicates per experiment under both field and greenhouse conditions. We identified salicylic acid (SA) and phytochrome signaling as generally up-regulated, and identified 8 genes that were consistently differentially expressed under all conditions when weeds were present- regardless of the weed species tested. Up-regulated genes encoded a polyol sugar transporter, a nucleoredoxin and downregulated genes encoded a ribosomal protein S21 family protein, a tetratricopeptide repeat (TPR)-like superfamily protein, a chloroplast beta-amylase, a regulator of chromosome condensation (RCC1) family protein, and two genes of unknown function. No obvious common regulatory sequences were observed in the promoters of these genes, although phylogentic foot-printing provided some

possible targets. We are developing a reporter system using promoter sequences from the two weed-induced genes to test the hypothesis that promoter sequences drive the weed-inducible expression of these genes. If the promoters prove effective, they could be used to drive expression of visible markers for remote early weed detection, and to begin dissecting the regulatory pathways involved in weed detection by corn. They will also be used to drive expression of NahG to reduce SA signaling to make the corn more weed-tolerant and hopefully reduce herbicide use.

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Primary Poster Presenter: David Horvath

Ecophysiolomics of tapping for Frankincense in Boswellia sacra tree (1200-045 (Screen 9))
Hall 2

The outer bark of Boswellia sacra tree is tapped to obtain oleo-gum resin, known as frankincense. Unsustainable tapping hinders tree growth and impairs the regeneration capacity of its population. The healing processes that are triggered to promote tree growth after wounding remain incompletely understood. Herein, we used de novo site-specific transcriptomic, physio-hormonal and mRNA expression analyses to understand the tapping responses in frankincense tree, and known plant growth regulators (PGRs; jasmonic acid, salicylic acid and gibberellic acids) were applied to assess the wound-healing process. Site-specific differential gene expression revealed the participation of 619 key signaling networks related to terpenoid biosyntehsis, phytohormonal regulation, cellular transport and cell-wall synthesis. Exogenous jasmonic acid (JA) resulted in significant recovery of epidermis cell-wall integrity during 30min and or 3days after tapping than other PGRs (salicylic acid - SA and gibberellic acids - GA) and control samples. Endogenous SA and JA were significantly activated by exo-JA and SA compared to controls. This was in concordance with gene expression patterns of terpenoid, cell-wall and wounding-stress signaling cascades, which showed significantly higher transcript levels after 30min compared with 3days of tapping. The findings elucidates for the first time a detailed transcriptome of site-specific tapping in one of the ecologically, economically and medicinally important frankincense producing tree species. In addition, this has not been done for any of the species at genus and Family Burseraceae levels. Thus, this transcript dataset would also work as references for more studies on this and related species. In conclusion, our study showed that tapping immediately activated several cell development and regeneration processes along with defense-induced terpenoid and phytohormonal metabolism to heal damaged tissues in the epidermis.

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