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3rd Annual Conference of the American Council for Medicinally Active Plants (ACMAP)

3rd ANNUAL CONFERENCE

OF THE AMERICAN COUNCIL FOR MEDICINALLY ACTIVE PLANTS

May 22-25, 2012

Arkansas State University, Jonesboro, Arkansas, USA

Program Chair and Host: Fabricio Medina-Bolivar

Co-Organizer: Agnes Rimando

PLENARY SPEAKER ABSTRACT

PL1. Bioexploration for natural health products: From the cloud forests of Ecuador to the plants in your own backyard

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Abstract. Particularly in developing countries, millions of people suffer ill health or die each year due to chronic and infectious diseases, but the large Western pharmaceutical industries have not focused on development of effective preventions or therapies for this underserved sector in the global population. Especially vulnerable are populations afflicted with geographically-localized diseases, which have not reaped the benefit of any major research initiatives. To seek new drug treatments, both science and traditional ecological knowledge often turn to natural products from organisms (plants, microbes) that have evolved and adapted to biotic stressors and pathogens over a millennium. Indigenous cultures have established traditional practices for using these natural products to treat injuries and ailments, and while ethnobotanical recommendations can pave the most efficient, targeted route to phytopharmaceutical drug discovery, local healers frequently (and, understandably) resist the intrusion of modern science into their realm of expertise. In a multi-investigator, multi-university initiative, a new bioexploration paradigm has overcome these obstacles and fostered strong partnerships and valuable discoveries that were previously inaccessible. This novel approach, funded through a mélange of federal agencies and NGOs, has catalyzed not only unique scientific discoveries, but also enabled training and biological education initiatives, throughout the developing world, and even in tribal communities in the USA. This presentation will focus on discovery of unique health-protective polyphenolic chemistries that have evolved within some of the wild plants which exist in the most extreme, remote environments, and on efforts to translate the biodiscoveries into practical, cost-effective deliverables that can benefit consumers all over the world.

CONCURRENT SESSIONS ABSTRACTS

SESSION 1A.ETHNOBOTANICAL INFORMATION / METABOLISM OF PHYTOCHEMICALS

Chair: Travis Marsico (Arkansas State University, AR, USA)

KEYNOTE PRESENTATION:

K1. The value of dereplications on understanding the worth of traditional pharmacopeias

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Abstract. As aculturization and globalization continues, there is an urgent need to carefully record and delineate traditional pharmacopeias so that their true worth is understood and protected and any possible benefits related to their commercial development are equitably distributed. In the past most of these endeavors resulted in a list of plants with their associated uses without providing further explanations as to the extent of this knowledge within the traditional group, or if this knowledge was known elsewhere. This practice tended to generate the notion of finite exclusivity without providing proof that this was actually the case. Moreover, since the talents and methods of those conducting these initial studies varied widely, little effort was made to provide adequate information on how selective processes and preferences as well as modes of collection, preparation and use were achieved. Without these data, the potential of their clinical worth, bioreactive capacities or chemical compositions were often compromised. This frequently led to expending much time, effort and treasure on a pharmacopeia's evaluation without guidance on how these efforts could be optimized to achieve its best possible medicinal potential. This presentation will review how types of dereplications and other techniques are helpful in amplifying this process.

ORAL PRESENTATIONS:

Oral 1.Under-developed potential of Ozark medicinal plants

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Abstract. The Ozark Plateau, Ozark Highlands or Ozark Dome as it is variously known, is characterized by exposed marine sediments, averaging 500 m in depth, primarily made up of sedimentary deposits including calcitic limestone and dolomite, sandstone, shale, clay, and conglomerates, creating a calcareous substrate which forms a karst topography not subject to major structural geological changes, saline water flooding or glaciation since flowering plants first evolved 200 million years ago. An initial survey of floristic sources compared with ethnobotanical data and historical works on American medicinal plants identified 477 species of known medicinal plants in the Ozarks. At least 195 species, including both introduced and indigenous elements of the flora are represented in international trade in medicinal and aromatic

plants. At least 35 species are traded in significant tonnage. A brief survey of the Ozark medicinal flora and potential under-developed germplasm resources will be presented, with emphasis on *Echinacea* (Asteraceae), *Solidago* (Asteraceae), *Hamamelis* (Hamamelidaceae), among others.

Oral 2.Phytochemical modulators of human drug metabolism: An introduction to herb-drug interactions

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Abstract. Over the past decade, an upsurge in botanical supplement usage, particularly within the United States, has engendered concerns among health care professionals regarding herb-drug interactions. Such concerns may be well founded given that recent surveys indicate 21-31% of prescription drug users take medications concomitantly with herbal dietary supplements, oftentimes without notifying their physician. Two general categories of herb-drug interactions have been recognized: pharmacodynamic and pharmacokinetic. Pharmacodynamic herb-drug interactions are those in which a botanical supplement can either enhance or diminish a drug's pharmacological activity without affecting its metabolism or transport. On the other hand, pharmacokinetic herb-drug interactions involve phytochemical-mediated alterations in a drug's metabolism and/or transport. One mechanism that appears to underlie many herb-drug interactions is the modulation of human cytochrome P450 drug metabolizing enzyme (CYP450) activity. CYP450s are heme-containing monooxygenases located in the small intestine, liver, and other tissues that play pivotal roles in the detoxification and bioactivation of diverse xenobiotic substances. Phytochemical-mediated changes in the activity of specific CYP450 isoforms (e.g. CYP1A2, CYP2D6, CYP3A4, etc.) may significantly alter the efficacy or toxicity of conventional medications whose biotransformation pathways are dependent on these same isoforms. Modulation of drug transport protein activity (e.g. P-glycoprotein, organic anion transporting polypeptide, breast cancer resistance protein, etc.) is another mechanism by which phytochemicals can affect the pharmacokinetic profile of conventional medications. The purpose of this presentation is to provide an introduction to herb-drug interactions using clinically relevant examples of each category.

Oral 3.Pharmacokinetics & bioavailability of *Nepeta cataria* (Catnip) used by rural African-Americans

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Abstract. The literature related to the use of plant therapies in the African-American community is limited and there is little documentation that describes the historical and present day use of plant remedies, specifically for health promotion and illness in African-Americans. The specific aims of this pilot study were to: document & describe the historical/present day use of *Nepeta cataria* (Catnip) for health & illness; identify, collect, and authenticate the Catnip used by rural African-Americans in the Alabama Black Belt; examine the pharmacologic properties and

bioavailability of *Nepeta cataria* (Catnip) plants through laboratory analysis and a review of the literature. Findings suggest that *Nepeta cataria* (Catnip) is used for a variety of aliments related to the gastrointestinal tract. Nepetalactones were found to be the main component of essential oil in catnip. Further, liquid chromatography-mass spectrometry analysis revealed luteolin, an antioxidant, and apigenin, a potent inhibitor of CYP2C9 enzyme. Both luteolin and apigenin are believed to play an important part in the prevention of cancer. Further study is needed to investigate the efficacy of the plant remedies used by African-American populations for illness and health promotion.

SESSION 1B.ISOLATION, STRUCTURE CHARACTERIZATION AND MODIFICATION OF MEDICINALLY ACTIVE COMPOUNDS

Chair: Agnes Rimando(USDA ARS, Natural Products Utilization Research University, MS, USA)

KEYNOTE PRESENTATION:

K2. The discovery of bioactive compounds: From ancestral medicine to molecular modeling

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Abstract. Humans as we know have been around for at least 200,000 years. Fleming discovered penicillin in 1928, and opened the era of “modern” therapeutic treatments. However, does it make sense that only in the last 84 years we have discovered medicines worth to learn about? How did mankind survive the other 199,916 years? It is clear that nature has played a key role as source of medicines for mankind. Natural occurring compounds represent a unique source of bioactive compounds that cannot be substituted by synthetic compounds. Either by themselves or as templates for further development, natural products continue to surprise us by their chemical diversity and intriguing biological activities. However, due to enormous losses in biodiversity that produce massive rates of extinction there is an urgent need to investigate new sources of natural products. In this presentation we will show how we have used historical sources, and ethnobotanical fieldwork coupled with computational and molecular modeling techniques to uncover new lead bioactive compounds.

ORAL PRESENTATIONS:

Oral 4.Plant-derived human glycogen synthase kinase 3 beta inhibitors

Dion A. Kevin,[1] Sivaprakasam Prasanna,[1] Olivia R. Dale,[1] Susan P. Manly,[2,3] Stephen J. Cutler,[1,3]Robert J. Doerksen[1,3]. [1]Department of Medicinal Chemistry, [2]National Center for Natural Products Research and [3]Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS, 38677, USA. E-mail: rjd@olemiss.edu

Abstract. *Aristolochia* species are widely used in traditional medicine in Chinese and Indian society. However, the main constituent, aristolochic acid, has been studied and found to be carcinogenic and nephrotoxic. Nevertheless, there is hope that other compounds isolated from the plants may have useful activity without toxicity, such as has been found for aristolactam, and/or that these compounds may serve as insightful scaffolds for drug design. Virtual screening featuring docking of molecules isolated from traditional Chinese medicine into the ATP-binding pocket of a 3D X-ray crystal structure of human glycogen synthase kinase-3β (GSK-3β) identified hit compounds which had been isolated from *Aristolochia* species. Several *Aristolochia* plant samples available in the University of Mississippi's National Center for Natural Products Research repository were subjected to extraction in an 80/20% mixture of methanol/formic acid solution and the resulting dried crude extracts showed significant GSK-3β binding at 10 μg/ml. Larger scale extraction was commenced starting from two *Aristolochia* samples. 1 kg of each macerated, dried plant source was subjected to methanol extraction at low temperature, yielding 1-10 g of material. Preparative scale flash column chromatography (normal phase extraction) was used on this material to obtain 12 fractions from each source. Significant GSK-3β activity was obtained for the crude samples as well as for some of the fractions. Focusing on the plant sample with greater activity, several of the more polar fractions with the highest activity were combined and sub-fractionated using reverse-phase column chromatography. 12 subfractions were obtained and bioassayed. HPLC was used for the 3 combined more active subfractions. Sixteensubsubfractions were obtained. Tests of purity and characterization of the purified compounds are currently underway and will be reported in the presentation.

Oral 5.Cannabis: Old plant, new constituents, potential therapeutic uses

Samir A. Ross[1,2], Mohamed M. Radwan[1], Safwat Ahmed[1], Desmond Slade[1], Susan Manly[1], Stephen Cutler[3], Mahmoud A. Elsohly[1,4]. [1]National Center for Natural Products Research, [2] Dept. of Pharmacognosy, [3] Dept. of Medicinal Chemistry, [4]Dept. of Pharmaceutics; School of Pharmacy, University of Mississippi, University, MS 38677, USA. E-mail:sross@olemiss.edu

Abstract. The chemistry of cannabis is very diverse with compounds from a wide range of chemical classes. The best-known and most-specific class is the Cannabinoids. Δ^9-tetrahydrocannabinol (Δ^9-THC) is the main psychologically active constituent and has been approved by FDA as antiemetic for cancer patients receiving chemotherapy and as an appetite stimulant for AIDS patients with wasting syndrome. The availability of high potency marijuana on the illicit market with unprecedented Δ^9-THC concentrations (above 20% by dry weight) prompted us to investigate these varieties. From high potency *Cannabis sativa* plant material, ninety nine compounds were isolated in a pure form (68 cannabinoids and 31 non-cannabinoid compounds), and their structures were established by UV, IR, GC, GC/MS, HR-ESI-MS, 1D and 2D NMR experiments, optical rotation, CD, chemical reactions, and chemical correlations. Of the above isolated compounds, sixty one compounds are new and isolated from cannabis for the first time. The new compounds include 42 new cannabinoids, 17 new non-cannabinoid phenolics, 1 new steroid, and 1 new aldehyde derivative. The *in-vitro* antibacterial, antifungal, antiprotozoal, and CB1 and CB2 receptor binding activities of the isolates have been evaluated.

Oral 6.Biotransformation of taxadiene in transgenic moss

Aldwin Anterola[1], Laxmi Sagwan[1], Daniel Medina[1], Kris Kirmess[2], Gary Kinsel[2], Pierre-Francois Perroud[3] and Ralph Quatrano[3]. [1]Dept. of Plant Biology and [2]Dept. of Chemistry and Biochemistry, Southern Illinois University Carbondale, Carbondale, IL 62901; [3]Dept. of Biology, Washington University, St. Louis, MO 63130, USA. E-mail: anterola@siu.edu

Abstract. Taxadiene is a metabolic precursor to the anticancer drug Paclitaxel, a highly substituted diterpenoid natural product found in *Taxus* species. This compound is expensive to manufacture (or isolate) so our group has been developing biotechnological alternatives for its production. In one approach, the moss *Physcomitrella patens* was transformed with taxadiene synthase (TS) gene from *Taxus brevifolia* under the control of a constitutive ubiquitin promoter. Gas chromatography-mass spectrometry (GC-MS) analysis revealed the formation of not only the expected product (taxadiene), but also other metabolites that are probably derived from, or structurally related to, taxadiene based on their mass spectra. Taxadiene 5-hydroxylase (T5H) gene was coexpressed (using a 35S promoter) with TS (still controlled by the ubiquitin promoter) in a stable transgenic moss, which resulted in the biotransformation of taxadiene into two main products. One of the products was identified by GC-MS as 5(12)-oxa-(3(11)-cyclotaxane (a.k.a. OCT), which had been observed previously in transgenic tobacco coexpressing TS and T5H. The other product was thought to be taxadiene 5-ol (as was expected) by another group coexpressing TS and T5H in bacteria. However, our preliminary characterization of this compound by mass and infrared spectroscopy suggested that this was not taxadiene 5-ol. On the other hand, we had detected what appeared to be taxadiene 5-ol (based on GC-MS) in our transgenic moss, although only as a minor product. Nevertheless, compared to other potential biotechnological hosts, moss is still arguably the most promising in the production of Paclitaxel precursors.

SESSION 2A. NATURAL COMPOUNDS AS THERAPEUTIC AGENTS

Chair: Prahlad Parajuli (Wayne State University & Karmanos Cancer Institute, MI, USA)

KEYNOTE PRESENTATION:

K3. IPI-926: A novel, oral, semisynthetic analog of the *Veratrum californicum* derived natural product cyclopamine currently under investigation for the treatment of Hedgehog ligand-dependent cancers

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Abstract. The Hedgehog (Hh) signal transduction pathway plays a critical role in cell differentiation and patterning during development, but is inactive in most adult cells. Malignant activation of the Hh pathway through a key signaling protein called Smoothened (Smo) has been demonstrated in a broad range of cancers.Although ligand-independent signaling occurs in some tumors due to genetic mutations, the greatest potential for Hh inhibition in cancer therapy is in ligand-dependent settings, in which Hh signaling occurs either directly to the tumor

cells or to the tumor microenvironment. IPI-926 is a potent, orally delivered small molecule that targets the Hh pathway by inhibiting Smo. IPI-926 (Saridegib), a semisynthetic analog of the *Veratrum californicum* derived natural product cyclopamine, was designed to have improved potency, solubility, and chemical and metabolic stability. In preclinical studies, IPI-926 has a demonstrated anti-tumor activity in a number of tumor models. IPI-926 is currently being evaluated in Phase 2 clinical trials that are designed to exploit multiple approaches to target ligand-dependent activation of the Hh pathway, namely: metastatic or locally advanced chondrosarcoma, and myelofibrosis. An overview of the IPI-926 program will be presented with a focus on the sourcing of the natural product cyclopamine.

ORAL PRESENTATIONS:

Oral 7.Natural prenylated and synthetic resveratrol analogs as novel ligands for cannabinoid receptors

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Abstract.*Trans*-resveratrol (tRes) has demonstrated significant therapeutic promise but its use is limited by poor bioavailability following rapid metabolism. Natural isoprenylated analogs of tRes derived from peanut hairy root cultures, *trans*-arachidin-1 (tA1) and *trans*-arachidin-3 (tA3), exhibit similar protective properties with significantly improved metabolic profiles. Although the molecular targets responsible for mediating the actions of tRes and its analogs are unknown, tRes and several other polyphenols bind to a distinct, yet unidentified, plasma membrane bound receptor that occurs in high density throughout the brain. Interestingly, cannabinoid (CB) receptors appear to share many characteristics with this newly discovered, uncharacterized tRes receptor. As such, initial studies in our laboratories were conducted to determine whether tRes and its analogs might act as novel ligands at CB receptors. The affinity and activity of the naturally synthesized isoprenylated analogs tA1 and tA3 at CB1 and CB2 receptors were compared to that exhibited by their non-prenylated parent compounds *trans*-piceatannol (tPice) and tRes, respectively. CB receptor binding studies demonstrated that all analogs bound to CB1 receptors with similar affinities ranging from 5-18 μM. However, only tA1 and tA3 bound appreciably to CB2 receptors with affinities similar to those observed for CB1 receptors. Molecular modeling studies confirmed that the isoprenyl moiety of tA1 and tA3 improved, while increasing the hydrophilicity of the primed stilbene ring.reduced binding affinity to CB2 receptors. Finally, although tA3 acted as a competitive CB1 receptor antagonist, tA1 antagonized CB1 receptor agonists by both competitive and non-competitive mechanisms. Prenylated stilbenoids may therefore be preferable alternatives to tRes due to increased CB1 receptor affinity and similar structural analogs might be developed as novel CB therapeutics for obesity and/or drug dependency.

Oral 8.Pterostilbene exhibits anti-anxiety effect in a model for anxiolytic assessment

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Abstract. Earlier studies that demonstrated pterostilbene has neurological activities (e.g., reversal of age-related cognitive deficits and improvement of memory function) triggered investigation of its anti-anxiety effect. Eight-week old male Swiss Webster mice were administered with pterostilbene (1, 2, 5, and 10 mg/kg BW) by oral gavage, and were subjected to elevated-plus maze (EPM) test, a behavioral model for anxiolytic assessment. Pterostilbene manifested anxiolytic activity at 1 and 2 mg/kg doses; mice showed increases in % permanence time and number of entries in open arms (established determinants of anxiolytic action), and increase in the % traveled distance in open arms. Furthermore, the % traveled distance and the % permanence time in the enclosed arms were decreased. The anxiolytic activity of pterostilbene was comparable to that of diazepam, one of the most frequently used anxiolytics, at effective doses (1 and 2 mg/kg BW; i.p.) in the EPM. Anxiolytic activity was not observed at the higher doses (5 and 10 mg/kg). Locomotor activity was not impaired, neither was sedating tendency observed, at all the doses suggesting this compound's favorable effects. Western blot analysis of hippocampal homogenates were in agreement with the observed behavior in the EPM, showing a decrease in both ERK1 and ERK2 phosphorylation in hippocampal homogenates from mice treated with 1 and 2 mg/kg pterostilbene, while no significant effect on the phosphorylation of ERKs was observed at the 5 and 10 mg/kg doses. Pterostilbene was detected in the plasma and brains of mice following single oral administration. These results suggest that pterostilbene has the potential to alleviate or treat anxiety disorders.

Oral 9.A novel antimalarial strategy based on whole plant delivery of *Artemisia annua* L.

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Abstract.*Artemisia annua,* which produces artemisinin (AN), has been used as a therapeutic tea to treat "fever" and other ailments for millennia; this GRAS herb is thus, safe to eat. The current best use of AN therapy for malaria, is artemisinin + an older drug: artemisinin combination therapy (ACT). We propose a new form of ACT: inexpensive, dose controlled, rapid delivery of the drug as dried, encapsulated *A. annua* leaves. Delivery of the drug is edible, but

dose controlled because the dried whole plant AN (WP-AN) can be homogenized and easily assayed for AN content prior to encapsulation with the number and size of capsules adjusted for AN content and patient weight. About 40 times more AN entered the bloodstream of a mouse fed WP-AN than with equal amount of pure AN. Ongoing experiments with *Plasmodium chabaudi*-infected mice treated with AN or WP-AN demonstrated that WP-AN effectively reduces parasitemia as well as or better than AN. Groups of five mice were infected with *P. chabaudi* and after allowing six days for parasitemia to reach log growth, mice were fed either pure AN or an equivalent amount of dried WP-AN (SAM cultivar). Treatments were delivered in LO and HI dosages, at 24- and 120-mg of AN per kg of mouse body weight, respectively. While parasite killing of AN and WP-AN was roughly equivalent between the two HI groups, among the LO concentration groups WP-AN actually was much more effective at reducing parasitemia than the LO AN group. We, thus, have unequivocal proof-of-concept that whole plant *Artemisia annua* (WP-AN) kills rodent malaria parasitemia *in vivo*. Together with our studies on resilience to AN resistance and also bioavailability, our results suggest a new paradigm in malaria artemisinin therapy is emerging.

SESSION 3A. NATURAL COMPOUNDS AS THERAPEUTIC AGENTS

Chair: Prahlad Parajuli (Wayne State University & Karmanos Cancer Institute, MI, USA)

ORAL PRESENTATIONS:

Oral 10.Flavonoids affect a direct loss of rotavirus infectivity in the cell-free experimental system

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Abstract. Secondary plant metabolites (*e.g.,* flavonoids) display antiviral activity upon infectivity titration testing in susceptible host cells. However, no studies have reported the direct loss of virus infectivity/virus structural integrity by flavonoids in the cell-free assay system. Accordingly, experiments were performed to determine the extent of antiviral activity by structurally diverse flavonoids present in cranberry and grape juices, citris fruits, and green tea [*viz*., proanthocyanidins (PACs), epigallocatechin galate (EGCG), catechin, epicatechin (EC), hesperidin, naringin, and diosmin] using a cell-free antigen capture enzyme immunoassay (EIA) system. A cranberry PAC concentrate was evaluated as well. A quantitative viral antigen capture EIA was used to measure loss of viral infectivity. The simian rotavirus stain SA-11 (RTV), was used as a representative enteric virus. RTV stock titers were determined in cell cultures. Two hundred and 100 ug/ml PACs affected a direct loss of virus infectivity to 92 and 56% of control, respectively. One-hundred sixty and 80 ug/ml EGCG affected a loss of RTV infectivity >99 and 45%, respectively. PAC component flavan-3-ol monomers consisting of catechin and EC, mandated increased concentrations (*ca.*, 5,000 to 10,000 ug/ml) to affect a RTV loss of infectivity approaching 50% of control. Synergistic studies did not enhance these

monomers' antiviral effect. Neither hesperidin, naringin, diosmin, displayed antiviral activity. At a concentration of 1,000 ug/ml, catechen but not EC, displayed an inhibitory effect on RTV-induced hemagglutination of guinea pig red blood cells. These data suggest an inhibitory effect on the binding of RTV antigenic determinants to RBC sialic acid or integrin receptor sites. Utilizing gold-labeled immunoelectron microscopy, RTV particles were found to be entrapped within PAC aggregates. The findings from this study suggest a significance of flavonoid groups as potential antiviral agents. Furthermore, our work points out a more directed or canalized approach in the use of plant metabolites as antiviral moieties.

Oral 11.Molecular insight and chemosensitisation potential of thymoquinone in colon cancer cells

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Chemotherapy remains an essential module in clinical management of patients diagnosed with colon cancer with 5-Flurouracil (5-FU) or 5-Flurouracil plus Oxaliplatin- (FOLFOX) as standard of care and treatment. Unfortunately, partly due to the high degree of inherent and acquired chemo-resistance,chemotherapy fails after multiple cycles of therapy underscoring the need for improved therapies. Sensitivity to low concentrations of chemotherapy severely compromises therapeutic advantages. As an alternative to overcome resistance to chemotherapy, use of chemosensitizers in restituting sensitivity to lower concentrations of chemotherapy have been proposed. Corollary to this conception, non nutritive dietary agents with pleiotropic effects (including chemosensitisation) have been reported for various site specific cancers. This led our attention to evaluate beneficial effect of Thymoquinone (TQ)- the bioactive compound derived from black seed Nigella sativa oil. As proof of principle complimenting our hypothesis, we used HCT-116 and HT-29 colon cancer cells *in vitro* and *in vivo* in xenograft model to evaluate chemosensitisation potential of TQ towards 5-FU and Oxaliplatin. TQ pretreatment significantly enhanced the anti-proliferative and apoptotic effect of 5-FU and Oxaliplatin in colon cancer cells relative to control and single agent regimen. Several regulatory target molecules such as Bcl-xL, Bcl-2, cyclin D1, survivin and ABCG2 were significantly downregulated by TQ pretreatment. *In vivo* treatment by TQ and oxaliplatin also caused a significant reduction in tumor volume relative to monotherapy ($p<0.01$).Our results strongly suggest chemosensitization by TQ can be utilized as basis for colon cancer therapy.

Oral 12.Antifungal drug discovery using natural products

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Abstract. The need for new antifungal agents continues, fueled by opportunistic infections in immune compromised patients and by the development of resistance to existing agents. Natural products offer a virtually unlimited source of unique molecules and not only serve as a reservoir for new potential drugs and drug prototypes, but also for probes of fungal biology. A summary of the work at the National Center for Natural Products Research, University of Mississippi towards antifungal drug discovery, including whole-cell screening methods targeted for natural products and mode of action studies, will be presented.

SESSION 2B. MEDICINAL PLANT RESEARCH IN MEXICO AND SOUTH AMERICA

Chair: Argelia Lorence(Arkansas State University, AR, USA)

ORAL PRESENTATIONS:

Oral 14.Reversal of multidrug resistance by morning glory resin glycosides in bacterial and human cancer cells

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Abstract. Resin glycosides are complex amphipatic glycolipids of high molecular weight derived from species of the morning glory family (Convolvulaceae). The modulatory effect of microbiologically inactive resin glycosides evaluated on nosocomial multidrug-resistant Gram-positive (*Staphylococcus aureus*) and -negative bacteria (*Salmonella typhi* and *Shigella flexneri*) led to the characterization of these compounds as substrates for efflux pumps. One of the most frequent mechanisms in bacterial resistance development is the expression of transmembrane proteins, which produce the MDR phenotype (e.g., NorA and TetK in *Staphylococcus aureus*) and, in addition, play a significant role in the acquired clinical resistance of both gram-positive and negative bacteria. These proteins are activated to expel xenobiotics and, in this manner, prevent drugs from reaching toxic concentration within the cell. These compounds exerted a potentiation effect of the clinically useful antibiotics tetracycline, kanamycin, and chloramphenicol against the tested bacteria by increasing antibiotic susceptibility up to 64-fold at concentrations of 25 ug/mL. Reversal of multidrug resistance (MDR) by this class of plant metabolites was also evaluated in vinblastine-resistant human breast carcinoma cells (MCF-7/Vin). Active non-cytotoxic compounds exerted a potentiation effect of vinblastine susceptibility to over 1906-fold at tested concentrations of 5 and 25 ug/mL and also, based on flow cytometry, significantly increased the intracellular accumulation of rhodamine 123 (a substrate for an effluxing P-gp pump) with the use of reserpine as a positive control for a MDR reversal agent. Decreased expression of P-glycoprotein by resin glycosides was detected by immunofluorescence flow cytometry after incubation with an anti-P-gp monoclonal antibody. These results suggest that morning glory oligosaccharides represent potential efflux pump inhibitors for overcoming MDR by lowering current effective therapeutic doses, thereby decreasing toxic side-effects in refractory malignancies. These results also indicate that combining an anticancer or antimicrobial agent with a MDR inhibitor is an approach that promises positive possibilities for future applications.

Oral 15.Cardenolides and cucurbitacins as antiviral and antitumor agents

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Abstract. Cardenolides belong to a group of naturally derived compounds that bind to and inhibit Na^+K^+ATPase. They have been used for the treatment of heart failure and atrial arrhythmia. Recently, important applications have been suggested for these compounds related to their potential antiviral and anticancer properties. We screened 65 cardenolides derivatives obtained from plants, by synthesis or by fungi biotransformation for anti-HSV-1 and HSV-2 activity (anti-herpes), as well as for their cytotoxic effects against a panel of human tumor cell lines. Among them, glucoevatromonoside, isolated from a Brazilian cultivar of *Digitalis lanata*, presented an important anti-HSV action and strong cytotoxic effects, and their mechanisms of action were elucidated.
Cucurbitacins are natural tetracyclic triterpenoids presenting a cucurbitane skeleton. Some of them have been investigated for their cytotoxic, hepatoprotective, anti-inflammatory, and anti-diabetic effects. We also screened 60 cucurbitacins derivatives for their cytotoxic effects against a panel of human tumor cell lines. Among them, one novel cucurbitacin isolated from a Brazilian medicinal plant [*Wilbrandia ebracteata* (taiuiá)] was the most active and its mechanism of action on human non-small-cell lung cancer (A549) was elucidated. The set of the obtained results will be presented, and they suggest that both groups of natural compounds could be considered potential candidates for anti-HSV and antitumor drugs.**Financial support:** CNPq (MCT) and CAPES (MEC), Brazil.

Oral 16.Biosynthesis of benzylisoquinoline alkaloids in *Argemone mexicana*,the Mexican prickly poppy

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Abstract. *Argemone mexicana*, commonly called Mexican prickly poppy, belongs to the Papaveraceae family and it has been used as a medicinal plant by traditional healers in Mexico and southern USA. The presence of different benzylisoquinoline alkaloids (BIA's) may explain the alleged medical properties of this plant. Although all BIA's are derived from two tyrosine units, they can be grouped in different families depending on their core structure, including the benzophenanthridines, protoberberines and morphinans. *Argemone* is one of the few plants producing both benzophenanthridine- and protoberberine-type alkaloids, since sanguinarine (a benzophenathridine) and berberine (a protoberberine) are accumulated in its tissues. This feature makes this plant an interesting model for studying the coordination between the biosynthetic branches leading to these products. In developing seedlings, berberine and sanguinarine occur both in radicles and aerial parts. However, as development continues, sanguinarine becomes restricted to roots, whereas berberine remains uniformly distributed throughout the plant. Specific cell types containing these alkaloids have been identified in roots and stem by *in situ* fluorescence, showing an association to vascular tissues. Such cell types coincided with areas where transcripts coding for two biosynthetic enzymes [the berberine bridge enzyme (BBE) and norcoclaurine synthase (NCS)] were detected. On the other hand, *Argemone* cell cultures accumulated mainly sanguinarine and its dihydro form. These forms were convertible, one in each other, along a culture cycle. Exposure of these cultures to yeast extract promoted sanguinarine accumulation, and this response could be increased by a previous exposure to salicylic acid and jasmonate. Nevertheless, no clear relationship could be

drawn between such an increase and BBE and NCS transcript accumulation. Results obtained with both plants and cell cultures will be discussed in terms of the suggested role of these alkaloids in chemical defense. (Supported by CONACYT).

Oral 17. Multidisciplinary research on Peruvian medicinal plants

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Abstract. It is estimated that over 1,000 plants are used in Peruvian traditional medicine for the treatment of several diseases. The Natural Products Research Unit of the Cayetano Heredia University has been performing basic and applied research on hundreds of Peruvian medicinal plants, with the aim of adding value to these natural resources. In this research unit we have used a multidisciplinary approach in order to isolate and characterize natural compounds with larvicidal, antioxidant, antitrypanosomal, anti-*Mycobacterium tuberculosis*, antifungal, cytotoxic, antileishmania and immunomodulatory activities. During our presentation we will present various examples of isolation and identification of active principles from Peruvian plants, as well as a summary of the ongoing projects that our research unit is carrying out in collaboration with several Peruvian companies for the development of phytomedicines, veterinary products, nutraceutics and phytocosmetics.

SESSION 3B. POSTDOCTORAL FELLOW PRESENTATIONS

Chair: Gary Stutte (John F. Kennedy Space Center, FL, USA)

ORAL PRESENTATIONS:

Oral 18.*Thymus vulgaris*extract inhibited mold growth, extended shelf life, and improved the flavor of enzymatic protein hydrolyzate beverage for athletes

Wudeneh Letchamo[1], Thomas Hartman[1],Andre Gosselin[2], Serete Hordofo[3].[1]Center for Advanced Food Technology, SEBS Rutgers University, 63 Dudley Rd, New Brunswick, N.J 08901,USA. [2]Hort. Res. Center, Université Laval, Québec Canada, G1K 7P4,[3]Bogiido Farm, Hadiya, Ethiopia·E-mail:Wletchamo@hotmail.com

Abstract. Among the major challenges in the formulation of beverages based on enzymatic protein hydrolyzate (EPH) is the relative high content of amino acids, minerals, and peptides that favors microbial/mold growth. Furthermore, the EPH beverages in general tend to accumulate odoriferous headspace due to semi volatile/ volatile compounds, and ammoniac. Our objective was to develop a natural ingredient that could be used commercially to inhibit microbial/mold growth, extend shelf life, and product safety while improving the flavor, and headspace composition of the EPH sports beverage. A 1:1 thyme leaf hydro-alcoholic extract obtained from our previous eco-genetic studies program was used in our experiment. Pure EPH was obtained from Zymtech. Gas chromatographic analysis of leaf oil has been shown to contain 46.3 % thymol, 8.1 % carvacrol and other components. We used 0 (control), 0.5 %, 1.0

% and 1.5 % extract by directly incorporating and mixing in the EPH juice formulated with mango (*Mangifera indica*), sea berry (*Hippophae rhamnoides*), and aronia (*Aronia melanocarpa*) berry juices. The treatments were replicated three times, and stored at 18 oC - 20 oC in the darkness for 40 days. The EPH samples were taken at regular intervals of 2, 6, 10, 14, 20, 30, and 40 days for visible analysis of mold growth, color change, etc, while examining microbial load. We found significant difference between the control and the treatments. Untreated control samples showed visible signs of mold growth, gas formation, and color changes after 6 and 10 days of storage. However variants containing 1.5 % thyme extract showed mold and gas free shelf-life of 40 days. The results suggested that *T. vulgaris* leaf extract inhibited mold growth, prolonged the shelf life, delayed color changes, improved the flavor, taste, the head space, and palatability of EPH beverage during storage, thus exhibiting high potential for commercial shelf-life extension of EPH based sports beverage.

Oral 19. QTL associated with waterlogging tolerance and related physiological traits in wheat

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Abstract. Waterlogging is a major constraint to crop yield worldwide. In the soft winter wheat growing region of the eastern United States, yield losses as high as 30% have been observed in years with high rainfall during early vegetative growth. It is also predicted that global climate change will result in increased winter precipitation and waterlogging of cereals. Waterlogging causes an 'energy crises' due to the depletion of oxygen available to the plant for respiration, resulting in low ATP production. Wheat has developed different mechanisms to adapt to waterlogging including both anatomical and biochemical changes. Anatomical changes include the development of adventitious roots, aerenchyma and stem elongation. Differences in physiological traits such as photosynthesis, chlorophyll content and stomatal conductance have also been associated with variation for waterlogging tolerance. However, despite its impact on wheat yields, little is known about the genetic control of waterlogging tolerance. In this study we report data from a set of 130 Jaypee/USG3209 RILs segregating in their agronomic and physiological response to waterlogging. Jaypee is a waterlogging tolerant cultivar which maintains 15-20% higher vegetative and root biomass under waterlogging compared to USG3209. A strong phenotypic correlation and co-localization of QTL for flooding tolerance index (FTI), quantum yield of PSII and chlorophyll content was observed in the RIL population. Other genome regions show co-localization of QTL for FTI and root characteristics including root biomass and the formation of root aerenchyma. Current work is focusing on developing spectral reflectance indices for waterlogging tolerance and identifying associated QTL regions.

Oral 20.Stable endosperm-production of CBHI exocellulase in maize

Deborah Vicuna Requesens[1], Yeun-Kyung Chang[1], Shivakumar P. Devaiah[1] and Elizabeth E. Hood[1,2].Arkansas Biosciences Institute[1] and College of Agriculture[2], Arkansas State University, Jonesboro, AR, USA 72467. E-mail: dvicuna@astate.edu

Abstract. Breaking down cellulose into glucose molecules is one of the most important steps in the biomass to fuels industry. Increasing the amount of cellulases to efficiently digest cellulose will have an enormous impact in the production of biofuels. One way to achieve this is to minimize the cost of cellulases by maximizing protein accumulation in the production material. We have previously successfully utilized the plant production system for industrial enzymes by expressing CBH1, an exocellulase from *Trichoderma reesei* in transgenic corn kernels. We expressed this cellulase using the embryo-preferred globulin-1 promoter from maize, with expression of up to 17.9% total soluble protein (%TSP) in first generation seed (Hood *et al*., 2007). To further increase seed-based protein accumulation, two endosperm-specific promoters from rice (a globulin and a glutelin) as well as four maize Zein promoters were employed. They have been tested using the GUS gene as a reporter and also in transient expression experiments on maize endosperm (Vicuna Requesens *et al*., 2011). Constructs containing CBHI under the control of these endosperm-specific promoters have been transformed into corn using co-cultivation with immature zygotic embryos. Transgenic maize plants resulting from these experiments were obtained and T1 seeds tested for cellulase activity. Expression driven by the endosperm-preferred glutelin promoter from rice was up to 1.2% TSP and those from the combination of this glutelin promoter and the endosperm-preferred globulin promoter from rice were up to 7% TSP of CBHI. Other constructs are being tested at this time. In the future, we foresee crossing these endosperm-expressing CBHI plants to those already over-expressing the embryo-specific promoter from maize in order to achieve a synergy in expression of this exocellulase, providing larger volumes of this enzyme at lower costs.

SESSION 4A. SELECTED GRADUATE STUDENT PRESENTATIONS

Chair: Wudeneh Letchamo(Rutgers University, NJ, USA)

ORAL PRESENTATIONS:

S1. Engineering rice for elevated vitamin C content

Katherine A. Lisko[1], Gwendolyn Wilson[1], Jamie Underwood[2], Vibha Srivastava[2], John Hubstenberger[1],Gregory Phillips[1,3], and Argelia Lorence[1,4]. [1]Arkansas Biosciences Institute, [2]Dept. of Crop, Soil, and Environmental Science, University of Arkansas,[3]College of Agriculture and Technology, Arkansas State University, [4]Department of Chemistry and Physics, Arkansas State University, Jonesboro, AR, USA. E-mail: alorence@astate.edu

Abstract. Vitamin C (L-ascorbic acid, AsA) is the most abundant water-soluble antioxidant in plants. In addition to serving as a key enzyme cofactor, AsA is a modulator of key processes in plant physiology including photosynthesis, cell division, growth, flowering, and senescence. During exposure to abiotic stresses, AsA counteracts excessive reactive oxygen species within the cell and protects key macromolecules from irreversible damage. In this study we focus on understanding how AsA levels are controlled in rice (*Oryza sativa*) and using this knowledge to engineer elevated AsA levels in this important crop. Our results indicate that AsA metabolism in

rice follows a unique pattern compared to other species such as Arabidopsis and tomato. The steady state foliar AsA in several rice varieties increases during development and peaks at the vegetative 2 and reproductive 4 stages, whereas in Arabidopsis, tomato, and tobacco foliar AsA content declines with age. Preliminary results from substrate feeding show that *myo*-inositol, L-gulose, and L-galactose are converted to AsA by detached leaves, indicating the operation of the D-mannose/L-galactose, L-gulose, and *myo*-inositol pathways in rice. We have transformed *O. sativa* var. Taipei and Nipponbare with genes encoding a *myo*-inositol oxygenase (AtMIOX), and an L-gulono-1,4-lactone oxidase (rGLOase), enzymes involved in the inositol pathway to AsA. We have confirmed the presence and expression of the transgenes via PCR and RT-PCR, respectively. Most importantly, we have found that expression of AtMIOX and rGLOase in rice leads to plants with elevated AsA content by 2 to 5-fold. Our future plans include completing the feeding studies and analyzing the expression of key genes that participate in the various routes involved in AsA synthesis using quantitative RT-PCR. We also plan to determine whether elevated AsA content in rice leads to enhanced biomass, growth, and tolerance to abiotic stresses, similarly as in Arabidopsis.

S2. Utilization of endangered American plant resources: **Volatilecomponents of *Lindera melissifolia* (Lauraceae) drupes repels ticks**

Joonseok Oh[1], John J. Bowling[1], John F. Carroll[2], Betul Demirci[3], K.Hüsnü Can Başer[4], Theodor D. Leininger[5]and Mark T. Hamann[1].[1]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, [2]USDA, ARS, IIBBL, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, [3]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, Turkey, [4]Botany and Microbiology Department, King Saud University, College of Science, 1145, Riyadh, Saudi Arabia, [5]USDA Forest Service, Center for Bottomland Hardwoods Research, 432 Stoneville Road, Stoneville, Mississippi 38776. E-mail: joh3@olemiss.edu

Abstract. Ticks are vectors for Lyme disease, a significant human disease with over twenty thousand new cases reported in 2010 according to surveys conducted by the United States Centers for Disease Control. Chemical repellents are considered a means of personal protection against the bites of these hematophagus arthropods. DEET (*N*, *N*-diethyl-3-methylbenzamide) has been used as a broad-spectrum repellent effectively for over five decades, but the synthetic agent has been reported to exert toxic reactions in humans. *Lindera melissifolia* (Walt.) Blume (Lauraceae), commonly known as pondberry, is an aromatic, rhizomatous, dioecious shrub that is found in limited numbers within wetlands and on the edges of sinks and ponds in the Southeastern US. The essential oil acquired from the drupes of this endangered plant showed a significant dose dependent repellency and with potency comparable to DEET while a hexanes extract was inactive. Fractional freezing enriched the active tick repellent components of the essential oil. Several tick repellent components were detected by GC-MS analyses of the fractions and based on the repellent activity of those fractions β-caryophyllene, α-humulene, germacrene D and β-elemene were identified as new repellent leads. The number of endangered plant species in the United States is significant but little attention has been directed towards endangered or rare U.S. plant species that could play

a vital role in pharmaceutical, food or fiber development. Identifying pondberry as a potential renewable resource for a broad spectrum repellent supports efforts to conserve similar American endangered or threatened plant species.

S3. Antioxidant and anti-hyperglycemic properties of *Tridax procumbens* asava

Gauri S. Desai[1,2], Shirish V. Desai[2], Rajendra S. Gavaskar[3] and Suresh T. Mathews[1]. [1]Department of Nutrition and Dietetics, Auburn University, AL 36830.[2]Bhagawati Industrial and Organic Products, Kolhapur, Maharashtra, India.[3]GTL Foundation, Sindhudurg, Maharashtra, India. E-mail: mathest@auburn.edu

Abstract. *Tridax procumbens*Linn., has been traditionally used in India to treat bleeding wounds, inflammation and hyperglycemia. The goal of our study was to evaluate antioxidant and anti-hyperglycemic potential of *T. procumbens* asava, a hydro-alcoholic preparation which retains heat-sensitive bioactive compounds, and formulated according to Ayurveda guidelines. Microbial and chemical analyses of the asava showed absence of microbial contamination, aflatoxins, and heavy metals including lead, cadmium, arsenic and mercury. *T.procumbens* asava demonstrated significant inhibition of lipid peroxidation and strong antioxidant activity, as indicated by higher trolox equivalent antioxidant capacity, ferric reducing antioxidant potential, DPPH free radical scavenging ability, hydrogen peroxide scavenging activity and metal ion-chelating effect compared to buylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol and curcumin. Blood glucose lowering potential of the asava was evaluated in 20 established, type 2 diabetic patients (10 men, 56 ± 7.8 years; 10 women 61 ± 15.0 years) who received conventional treatment for 16 ± 8 years. This study was approved by the Kolhapur Independent Ethics Committee, Kolhapur, India. Patients were supplemented with *T. procumbens* asava, twice daily, for 4 weeks. During this period, patients continued to receive their anti-diabetic medication. Fasting blood glucose levels were significantly reduced (Pre-treatment: 152.4 ± 34.09 mg/dl vs. Post-treatment: 128.5 ± 16.98) after 4 weeks of asava supplementation. A highly significant reduction was also observed in the 2-hour post-prandial blood glucose levels (Pre-treatment: 262.0 ± 75.16 vs. Post-treatment: 189.6 ± 41.98) following the 4-week supplementation period. No side-effects or adverse effects were reported. To our knowledge, this is the first clinical report of the anti-diabetic effect of *T. procumbens,* validating its traditionally claimed blood glucose lowering properties. Further, the strong antioxidant activity and significant blood glucose lowering effect of the prepared *T.procumbens* asava, suggest its potential as a complementary option in the management of type 2 diabetes.

SESSION 4B. SELECTED GRADUATE STUDENT PRESENTATIONS

Chair: Rao Mentreddy (Alabama A & M University, AL, USA)

ORAL PRESENTATIONS:

S5. Plant-made pharmaceutical proteins: Targeting enzyme bioproduction and delivery

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Abstract. Many recombinant human therapeutic proteins are currently produced using mammalian expression systems. Molecular technologies make possible the plant-based bioproduction of novel and high value recombinant proteins for pharmaceutical applications. The delivery of recombinant proteins into disease-appropriate tissues, cells, and subcellular compartments within cells, remains a challenge for many therapeutic applications including enzyme replacement therapeutics (ERTs). The RTB plant lectin mediates endocytotic uptake into mammalian cells and trafficking to lysosomes or the endoplasmic reticulum (ER) of associated proteins or "payloads". In order to test the potential of RTB to deliver lysosomal and ER ERTs, RTB was genetically fused to three different human enzymes: L-alpha-iduronidase (IDUA; the lysosomal enzyme deficient in Hurler disease patients), beta-glucocerebrosidase (hGC; the lysosomal enzyme deficient in Gaucher Disease), and UDP-glucuronosyltransferase 1A9 (UGT-1A9; ER-localized enzyme involved in detoxification of small molecules). Gene constructs encoding RTB:IDUA, RTB:hGC and UGT-1A9:RTB were developed and expressed in leaves of Nicotiana benthamiana plants using an Agrobacterium-mediated transient expression system. Recombinant fusion products were purified and characterized for RTB lectin activity, human enzyme activity, and the ability to direct uptake into human cells. In order to determine whether RTB effectively delivers bioactive IDUA or hGC enzyme to the site of disease substrate accumulation, fibroblast cell lines from Hurler and Gaucher patients were treated with the recombinant protein and analyzed for glycosaminoglycan content (Hurler disease substrate) or hGC enzyme activity of lysed cells (Gaucher) compared with non-treated cells. Results of these studies support strategies to use plant-based bioproduction of these RTB:human enzyme therapeutics to provide replacement enzymes that address key delivery and targeting issues as well as providing a safe and scalable commercial production platform.

S6. Characterization of an *Arabidopsis* L-gulono-1,4-lactone oxidase (GLOase) in *Nicotiana benthamiana*

Siddique Imran Aboobucker[1], Walter P. Suza[1,2] , and Argelia Lorence[1,3], [1]Arkansas Biosciences Institute, [2]Department of Agronomy, Iowa State University, and [3]Department of Chemistry and Physics, Arkansas State University, P.O. Box 639, State University, AR, USA 72467 Email: alorence@astate.edu

Abstract. Vitamin C (L-ascorbate, AsA), the most abundant water-soluble antioxidant, is essential for plant and animal health. There are four known AsA biosynthetic pathways in plants: the D-mannose/L-galactose, L-gulose, D-galacturonate, and *myo*-inositol routes. Our group has made significant progress in the study of the first three enzymes of the inositol pathway to AsA. This work focuses on the study of L-gulono, 1,4-lactone oxidase(GLOase), the enzyme that works at the intersect of the L-gulose and the inositol routes. Feeding L-gulono lactone, the known GLOase substrate, to multiple plant species, together with enzyme activity measurements in *Arabidopsis* support the presence of a functional GLOase in plants. Using the rat GLOase as bait we have been able to identify seven putative *GLOases* in the *Arabidopsis*

genome. Gene transcript profiling data we have analyzed through Genevestigator, a public microarray repository, show an increased expression of one of these genes, *At2g46740,* in the roots of *Arabidopsis*, and this is the protein we are focusing on first. A *Nicotiana benthamiana*-based transient expression system was chosen for the functional characterization of *At2g46740*. The identity of the transiently expressed protein was confirmed by MALDI-TOF. Feeding L-gulono lactone through the petiole of *N. benthamiana* leaves expressing a *35S:At2g46740:pBIB-Kan* construct led to an increase in foliar AsA, indicating that *At2g46740* encodes a functional GLOase. Surprisingly, over expression of *At2g46740* in *Arabidopsis* did not affect the AsA content. Nonetheless, Western blot analysis of the foliar tissue from plants over expressing *At2g46740* confirmed the presence of the protein in that tissue. The Western blot and substrate feeding results led us to hypothesize that in the *ArabidopsisAt2g46740* over-expressers the substrate pool is the limiting factor for the reaction to form AsA. Our ongoing experiments are focused on understanding the substrate specificity of the At2g46740 enzyme in *N. benthamiana* and *Arabidopsis*.

S7. Bioproduction of a thermostable enzyme for processing Arkansas energy beets

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Abstract. Production of energy beets (*Beta vulgaris* L.) in Arkansas is targeted for establishing an industrial-scale sugar (sucrose) feedstock platform as part of a regional strategy to produce advanced biofuels in the Mid-South Delta. Energy-efficient processing technologies and development of co-product streams are critical for the overall economic viability of bioenergy crops. Expanded beet production into the Mid-South will generate millions of tons of wet pulp as a processing residue that will have to be dealt with. The overall goal of this research is to establish the utility of a naturally thermally-tolerant pectinesterase (TT-PME) for innovative applications in processing pectin-rich beet pulp. We hypothesize TT-PME can impart specific structural modifications to cell walls in beet pulp under process conditions (which are optimal for enzyme activity) that will reduce water-holding capacity. Reduced water-holding capacity will directly lower energy inputs to dry pulp for storage and shipping. We present current work to develop a bioproduction platform for this thermostable enzyme using the yeast *Pichia pastoris*. Results include: 1) design of expression constructs using synthetic gene technology for producing enzyme, 2) selection and initial characterization of *Pichia* transformants, 3) design of a TT-PME-based immunogen using a selected peptide epitope, and 4) characterization of a monospecific TT-PME antiserum produced using this immunogen. These results will provide TT-PME in technical quantities sufficient for biomass treatment trials and an analytical tool useful for selective detection and quantification of TT-PME in the presence of other closely related isoforms.

S8. Kinetic Modeling of xylose oligomers degradation during dilute acid hydrolysis

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Abstract. Dilute acid hydrolysis is a common pretreatment method for cellulosic ethanol production by hydrolyzing hemicellulose into simple monomeric sugars, such as xylose, which can be fermented to produce ethanol. However, the severity of pretreatment generates degradation by-products such as furfural and formic acid, which reduce yields of monomeric sugars and inhibit the fermentation process. Conversely, if pretreatment conditions are less severe, a high accumulation of oligomers, the intermediate products of hemicellulose depolymerization into monomers, will inhibit the enzymatic hydrolysis of cellulose. Therefore, studying the kinetics of xylose oligomers degradation is critical to describe hemicellulose depolymerization because these results will help optimize the monomeric sugar yields from biomass.

Birchwood xylan was partially hydrolyzed to produce xylose oligomers, which were then fractionated using centrifugal partition chromatography (CPC). A solvent system of butanol: methanol: water (5:1:4, V/V/V) was used in the CPC instrument to produce high purity fractionated xylose oligomers, which were subsequently hydrolyzed using sulfuric acid concentrations ranging from 0 to 0.5% at 120 to 200 ˚C for 0 to 60 min. The hydrolysates were analyzed for xylose monomer, oligomers, and by-products to determine rates of formation and degradation.

The depolymerization of xylose monomer and oligomers was found to follow first order kinetics. Using water at 160 ˚C, the degradation rates of xylose (DP1), xylobiose (DP2), and xylotriose (DP3) were 0.0040, 0.0391, and 0.0155 min^{-1}, respectively. The overall degradation rates of DP1, DP2, and DP3 were comparable to the values reported in literature, even though our starting material was produced and purified in-house as compared to commercially available xylose oligomers used in the literature. Our future work will focus on expanding the kinetic model of xylose monomer and oligomers decomposition to different pH and temperature conditions.

THURSDAY, MAY 24, 2012

PLENARY SPEAKER ABSTRACT

PL2. Engineering phenylpropanoid production for healthy foods

Cathie Martin[1], Concetta Licciardello[2], Katharina Bulling[1],Yang Zhang[1], Simon Carding[3],Matt Tomlinson[3], Paul Bailey[1], Giuseppe Reforgiato-Recupero[2] and Eugenio Butelli[1].
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Abstract. The past 20 years has seen an enormous rise in publicity about super foods that promote health and reduce the risk of cardiovascular disease, cancer and age-related

degenerative diseases, related specifically to the metabolic syndrome. These claims are supported by evidence from cell studies, animal feeding trials, human intervention studies and epidemiological studies. However, despite all the positive messages about the value of eating fruit and vegetables (the 5-aday program has been running for 25 years) the numbers of people meeting these dietary recommendations in the US remains below 25% of the population, numbers are falling, and chronic diseases, especially those associated with obesity and the metabolic syndrome, are reaching epidemic proportions in Western societies.

There is a need to engineer high levels of protective bioactives in the foods that people actually do consume, to help combat this rise in chronic diseases. Most attempts at engineering the levels of bioactives have focused on increasing the activity of key, rate-limiting steps, but such strategies usually result in only modest improvements in flux to bioactive end-products. Use of transcription factors to up-regulate entire pathways of plant secondary metabolism is a far more effective strategy and results in food material with very significantly elevated levels of health-promoting bioactives. While such improvements may, in part, be achievable for some crops through selective breeding, genetic modification offers bigger improvements because it can overcome limits in the natural variation available in transcription factor specificity and activity. Use of genetically-improved foods in animal feeding studies with models of tumorigenesis has revealed that protection is afforded by diets enriched in high bioactive foods. Such health-promoting foods will offer consumers tangible improvements in the products available to them, and have the potential for public approval of genetically improved plant varieties and foods derived from them, in Europe.

CONCURRENT SESSIONS ABSTRACTS

SESSION 5A. PLANT BIOACTIVES IN CANCER CHEMOPREVENTION

Chair: Anait S. Levenson (University of Mississippi Medical Center, MS, USA)

KEYNOTE PRESENTATION:

K4. Using soy to inform anti-metastic therapeutic strategies

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Abstract. Natural products contain highly informative bioactive compounds. Their properties can facilitate the identification of heretofore unknown regulatory pathways of therapeutic relevance, and can then be used to target those pathways. Starting from the anti-motility properties of the soy constituent, genistein, we have gone on to elucidate novel and key pathways responsible for regulating transformation to a metastatic phenotype in human prostate cancer. We then used genistein to target those pathways, and have done so in pre-clinical models and in humans. From this platform, we used genistein as chemical scaffold to support the synthesis and

discovery of a more potent and specific new drug that inhibits human prostate cancer metastasis.

ORAL PRESENTATIONS:

Oral 22.Chemoprevention of prostate cancer by resveratrol: Novel epigenetic mechanisms of action

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Abstract. One of the most important features of epigenetics in cancer is that histone and DNA modifications can be influenced by dietary and pharmacological agents. “Epigenetic” drugs are currently represented by HDAC inhibitors and inhibitors of DNA methyltransferases; however, these drugs have limitations such as lack of specificity and high toxicity. Dietary polyphenols and their analogues with anticancer properties hold great promise as potentially safer epigenetic chemopreventive and therapeutic agents. We found that Resveratrol (Res) acts as a natural HDAC inhibitor by reversing the activity of co-repressor NuRD (nucleosome remodeling and deacetylation) complex. The multi-protein NuRD complex consist of metastasis associated protein 1 (MTA1) and connected histone deacetylases (HDAC1 and 2). Resveratrol downregulated MTA1 by promoting its degradation and destabilized NuRD complex, which affected the deacetylation/acetylation balance of p53, PTEN, and HIF-1α in prostate cancer (PCa) cells. Resveratrol treatment of PCa cells, in which MTA1 was silenced by shRNA-mediated knockdown, significantly enhanced p53 acetylation and apoptosis. Combination treatments of Res and HDAC inhibitor SAHA synergistically increased p53 acetylation and apoptosis. Further, MTA1 knockdown promoted acetylation of PTEN in Du145 cells, and combination treatment with HDAC inhibitor trichostatin A (TSA) further increased PTEN acetylation. As a part of a gene-controlling network that can be regulated by Res, we also identified Res-induced MTA1-associated microRNA changes in PCa cells, which may ultimately lead to novel chemopreventive and prognostic biomarkers. Finally, preliminary preclinical studies demonstrated that Res in diet (205 mg/kg) inhibited the growth of orthotopically implanted tumor cells in xenografts. Res was found in serum at concentrations from 0.29 to 3.82 ng/μl, and, importantly, in the prostate tissues of mice fed with Res.

Oral 23. Selective reactivation of mutant p53 by methylseleninic acid through thiol modification

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Abstract. Mutations of the p53 gene constitute the most frequent genetic alteration known in human cancers. The vast majority (~80%) of the mutations is missense mutations. p53 mutation leads to checkpoint defects, genomic instability, and propagation of cells with genetic damage. Developing therapeutic agents that could directly bind to the mutant p53 and reconstitute p53 function has been intensively pursued and proven to be feasible. However, the challenge remains to develop a drug that specifically reactivates mutant p53 without activating the pro-apoptotic function of wild-type p53 in normal tissues. In the present study, we show that methylseleninic acid (CH_3SeO_2H, MSA), a stable active metabolite of organoselenium compounds, can selectively reactivate mutant p53 to induce apoptosis in cancer cells. Organoselenium compounds are bioactive food components. Brazil nuts are the richest known food source of organoselenium compounds. We show that MSA can be used at a concentration at least one order of magnitude lower than that of other mutant-p53-rescue agents to achieve the same p53-reactivating and growth-inhibitory effects. MSA reduces redox-active free thiols in mutant p53 without the requirement of other proteins, potentially through forming adducts with thiols in mutant p53. The modification restores wild-type conformation to mutant p53, leading to increased DNA binding and transactivation as well as expression of growth-suppressive and pro-apoptotic target genes in both cultured cancer cells and an orthotopic breast tumor model. Although MSA also redox modifies recombinant wild-type p53, the modification does not lead to the induction of growth-suppressive or pro-apoptotic target genes in normal fibroblasts. Instead, the expression of p53-regulated DNA-repair genes is significantly induced in normal fibroblasts, suggesting that MSA may selectively modify p53 for DNA repair or apoptosis depending on the level of endogenous or exogenous DNA damage. Our findings, therefore, demonstrate the effectiveness and selectivity of MSA as a novel potent mutant-p53-rescue drug.

SESSION 5B. BIOPRODUCTION AND PURIFICATION OF BIOACTIVE PLANT PRODUCTS

Chair: Rebecca Parr (Arkansas State University, AR, USA)

KEYNOTE PRESENTATION:

K5. Root cultures as bioproduction systems of health-beneficial compounds

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Our group has used *Agrobacterium rhizogenes*- and *A. tumefaciens*-mediated transformation to develop hairy root cultures of peanut and American skullcap (*Scutellaria lateriflora*) as bioproduction systems for stilbenoids and flavonoids, respectively. These polyphenols have been associated with health benefits impacting cancer and neurodegenerative diseases. In order to increase their levels in plant systems, elicitation and metabolic engineering strategies were investigated. The combination of methyl jasmonate with cyclodextrin was the most effective elicitor treatment for both species. High levels of the bioactive prenylated stilbenoids arachidin-1 and arachidin-3 were induced in peanut. Furthermore, we utilized high performance countercurrent chromatography to purify these stilbenoids from the culture medium and studied

their biological activity. In skullcap, the non-elicited cultures produced the anticancer flavonoid wogonin. Interestingly, several putative phenolics were induced upon treatment with these elicitors. In addition, hairy roots of skullcap harboring a flavonoid-specific transcription factor (AtMYB12) were produced under the control of the superP:TEV expression system in efforts to increase the levels of wogonin. Hairy root cultures and these elicitation/metabolic engineering strategies provide a valuable tool for increasing the levels of valuable compounds in these tissue culture systems.

ORAL PRESENTATIONS:

Oral 25.The extraction of high value phytochemicals in the context of a biorefinery: Sweetgum as a possibility

Dinesh Babu[1], Philip Crandall[1], Shiloh Hurd[2], LaRae Brown[3], Elizabeth Martin[2], Matt Pelkki[4] and Danielle Julie Carrier[2]. [1]Food Science, [2]Biological and Agricultural Engineering, [3]Biological Sciences, University of Arkansas, Fayetteville, AR 72704, USA; [4]Arkansas Forest Resources Center, University of Arkansas-Monticello, AR 71656, USA. E-mail: carrier@uark.edu

Abstract. Biorefinery revenues can be increased by the extraction of valuable phytochemicals prior to or after the biochemical conversion of biomass to biofuels or other biobased products. These phytochemicals could find use in human and animal health care products, cosmetic applications and as essential ingredients in green cleaning products. A phytochemical extraction scheme can be nestled within the biorefinery or as a part of different operation located nearby. To be realistic, the extraction step must not hinder the conversion process by decreasing carbohydrate recovery.

Sweetgum (*Liquidambar styraciflua*), a fast-growing southeastern US tree, is a biorefinery feedstock that also contains valuable phytochemicals. In this study, a solvent-free bark extract was tested for antioxidant and antimicrobial properties. The crude sweetgum bark extract were further analyzed for their antioxidant effect using the *in-vitro* Cu^{2+} induced low density lipoprotein oxidation (LDL) thiobarbituric reactive substance (TBARS) assay. The crude sweetgum bark extract inhibited LDL oxidation by at least 90%. The solvent-free bark extract was also tested for its inhibition activity against major food pathogenic bacteria, such as *Listeria monocytogenes* strains. The minimum inhibitory concentrations (MICs) of extract showed complete inhibition of the growth of seven *L. monocytogenes* strains at 0.38%.

These results demonstrate that it could be possible to devise a biorefinery operation that, in addition to producing biobased fuels or chemicals, also recovers high value phytochemicals from feedstock.

Oral 26.Beyond extraction: biosynthesis of active ingredients of medicinal plants by metabolic engineering

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Abstract. Traditionally, majority of the bioactive ingredients of medicinal plants are extracted from plant sources. The plant origin of these ingredients limited their availability and increased their prices. Most importantly, cultivating these medicinal plants uses a significant amount of arable land, and collection of some medicinal plants from their natural habitat can damage their fragile natural environment. With the advent of modern metabolic engineering, more and more of these active ingredients can be produced through synthetic biology. Our group has successfully engineered more than 50 different types of phenylpropanoid and flavonoid compounds. Some of these compounds are important food additives or flavor and fragrance compounds. To produce them, we have developed various engineering strains with unique metabolic channels to increase the pathway efficiency. And we have also developed specific transporters that pump these compounds out of the cells into the culture media. Protein engineering allowed us to produce compounds that have not been reported previously. By combining these engineer approaches, we have developed E coli and yeast production platform for large-scale fermentation of these compounds. The yields of many engineered phenylpropanoids and flavonoids are at more than 1 g/L in small scale fermentation.

Oral 27.Bioproduction of stilbenes and localization of resveratrol in grapevine cells using confocal microscopy

Thomas Chastang[1,3], David Donnez[1], Christine Terryn[2], Aziz Aziz[1], Behnam Taïdi[3], Philippe Jeandet[1], Marc-André Théoleyre[3], Dominique Pareau[3], Christophe Clément[1] and Eric Courot[1]. [1]Research Unit N°2069 Vine and Wines of Champagne, Faculty of Sciences, University of Reims Champagne-Ardenne, PO Box 1039, 51687 Reims cedex 02, France. [2]IFR 53, 51 Cognacg-Jay street, University of Reims Champagne-Ardenne, 51095 Reims cedex, France, [3]Ecole Centrale Paris, LGPM, Centre d'Excellence en Biotechnologies Blanches, Route de Bazancourt, 51110 Pomacle, France. E-mail: eric.courot@univ-reims.fr

Abstract. Considering the interests in resveratrol (3,5,4'-*trans*-trihydroxystilbene) on human health and its cosmetic and nutraceutical uses, we have developed a process to produce both resveratrol (up to 250 mg/L of culture) and viniferins in a 2 L stirred bioreactor (Donnez *et al.* 2011). Cell suspensions of 41B rootstock used produced stilbenes only when elicited and this cell line represents a very good system to study the early synthesis of resveratrol within the cells. Based on the spectral properties of resveratrol under UV light, we have characterized the level of fluorescence emitted by a single elicited-cell and compared it with a non-elicited cell using a UV-videomicroscope. This led us to follow the appearance of resveratrol within the cells in real-time. In complement, Z-stacks images from resveratrol autofluorescence were acquired using confocal laser scanning microscope with dedicated fluorescence emission filter. The results clearly showed that the synthesis of resveratrol in 41B cells is cytosolic and close to the plasma membrane, in agreement with the stilbene synthase localization in cell grapes (Fornara *et al*. 2008). Following synthesis, resveratrol is rapidly observed in the cell wall, confirming its secretion and release into the culture medium has been confirmed by UPLC. In our system, 90% of the resveratrol produced (in the free form: *trans*-resveratrol) was excreted into the medium. Our results show that the use of specific microscopic techniques could be of interest for following the dynamics of resveratrol biosynthesis in a single cell at the subcellular level.

This led us to better understand how and where this molecule of interest is produced in grape cells with the aim of optimizing its bioproduction.

SESSION 7A.PROPAGATION / CROP IMPROVEMENT / MICROPROPAGATION AND GERMPLASM CONSERVATION

Chair: Nirmal Joshee (Fort Valley State University, GA, USA)
Co-chair: Hazel Wetzstein (University of Georgia, Athens, GA)

KEYNOTE PRESENTATION:

K6. Ex situ conservation of medicinal plants – diversity for the future

David D. Ellis. USDA-ARS National Center for Genetic Resources Preservation, Fort Collins, CO 80526 E-mail: david.ellis@ars.usda.gov

Abstract. The Food and Agriculture Organization of United Nations (FAO) estimates that there are over 1,750 individual plant genebanks worldwide conserving some 7.4 million ex situ accessions (unique collections) of plant genetic resources. This exemplifies the incredible job Mother Nature did in the evolution of the seed, a compartment to hold, protect and preserve the plant embryo for 100's of years if well prepared. For the vast majority of plant seeds, seed quality, moisture content and storage temperatures are the main determinants effecting long-term storage viability in seed banks. Long-term storage of global collections is maintained at 20^{o}C where they will remain alive for 50+ years. For seedsthat are not desiccation-tolerant, and hence cannot be stored frozen, cryopreservation of seed or emrbryo tissues offers an alternative for long-term storage. Cryopreservation technologies are also rapidly being developed for vegetatively-propagated genotypes expressing elite fruit or phytochemical properties where clonal properties can be preserved for centuries. The U.S. National Plant Germplasm System (NPGS) contains almost 550,000 accessions from over 14,000 species and 2,355 genera. While it is difficult to validate which taxa actually have active compound(s) versus folkloric use, it is intiguiing that over 300,000 accessions in the NPGS have some record of having a mention of medicinal use. These accessions can be easily searched on the NPGS Germplasm Resources Information Network (GRIN), a public web database, at http://www.ars-grin.gov/~sbmljw/cgi-bin/medicinal.pl. This link allows searching of plants in the NPGS with medicinal history by taxa, repository, literature reference, country of origin or taxa with known pharmaceutical agents. The accessions in the NPGS are a public asset and as such most are available to researchers worldwide with GRIN.

ORAL PRESENTATIONS:

Oral 28.Health benefits, production and consumption of pomegranates

Hazel Y. Wetzstein and Weiguang Yi. Department of Horticulture, University of Georgia, Athens, GA 30602. E-mail: hywetz@uga.edu

Abstract. Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and has been used for medicinal purposes since ancient times. Research studies employing in vitro, animal and human models have identified extensive health benefits associated with pomegranates. Reported health benefits include efficacy against a wide range of conditions, including coronary heart disease, atherosclerosis, cancer, hypertension, hypercholesterolemia, aging, and infectious diseases. Pomegranate is consumed and marketed as whole fresh fruit, extracted arils, juice, syrup (grenadine), wine, teas, and seed oil. A review of the health benefits and recent increases in the consumption of pomegranate products will be discussed. In addition, physiological studies on crop production and how they influence fruit quality such as fruitset, size and anthocyanin will be presented. In general, pomegranates have significant potential human health benefits. Modification of crop culture can be used to increase yield and fruit quality, and in conjunction with new product development, may help increase consumption by the public of this healthy fruit.

Oral 29.Controlled environments: A tool for enhancing productivity and bioactivity of medicinal plants

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Abstract. Controlled Environments [(CE) growth chambers, growth rooms, and greenhouses] are powerful tools for providing consistent growth parameters for propagation, research and production of medicinal plants. CE technology is widely used for micropropagation of plant material, conservation of germplasm, and, to a lesser degree, commercial production of medicinal plants. Advances in lighting and control system technology, coupled with increased knowledge on physiology and biochemistry of secondary metabolism of medicinal plants, and has provided new opportunities to use CE for medicinal plants. The advances in CE technology, also present new challenges that need to be recognized. Light Emitting Diode (LED) technology is being increasingly used in CE due to its energy efficiency, long life, and spectral specificity. Effective application of LED in CE requires an understanding spectral quality effects on plant morphology, physiology and development to avoid unanticipated and undesirable effects on plant appear, reproductive biology, and bioactivity. Similarly, modifications of atmospheric composition, such as elevated CO_2, can greatly increase growth rate, but may alter the composition and distribution of secondary metabolites.

Oral 30.Effects of pre- and post-harvest conditions on the health bioactivity of medicinal herbs

Weiguang Yi and Hazel Y. Wetzstein. Department of Horticulture, University of Georgia, Athens, GA 30602-7273. E-mail: hywetz@uga.edu

Abstract. A number of herb species has been used for medicinal purposes for thousands of years throughout the world. Growing numbers of studies have supported the potential health benefits of herbs with plant extracts displaying antioxidant, anti-inflammatory, antibacterial, analgesic, and antitumor activities. We here review how pre- and post-harvest conditions may impact the biochemical and biological activity of herb species. Phenolic content, antioxidant capacity, antitumor, and anti-inflammatory activity were evaluated in five culinary and medicinal herbs: thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), peppermint (*Mentha piperita*), and spearmint (*Mentha spicata*). Leaves from thyme, sage, spearmint, and peppermint grown in the greenhouse, showed significantly higher total phenolic content and TEAC antioxidant capacity than those grown under field conditions, with a 3-fold difference observed in peppermint. Rosemary, spearmint and peppermint extracts showed stronger inhibition to cycloxygenase COX-2 than to COX-1. In addition to pre-harvest growth condition, post-harvest conditions such as thermal drying can impact biochemical and biological activity. In peppermint, sun drying and 40 ^{o}C oven drying resulted in significantly higher antioxidant capacity and antitumor activity than 70 ^{o}C oven dried samples and fresh leaf samples. In conclusion, producing herbs under greenhouse conditions can improve their biological activities by increasing total phenolic contents and antioxidant capacities. Low temperature drying of certain herbs species may produce products with enhanced biochemical and biological activities than fresh samples.

SESSION 7B. NOVEL APPLICATIONS IN PLANT BIOTECHNOLOGY

Chair: Maureen Dolan (Arkansas State University, AR, USA)

KEYNOTE PRESENTATION:

K7. Plant-based bioproduction of pharmaceutical proteins – pushing the 'medicinally active' envelope

Carole L. Cramer, PhD. Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401 and BioStrategies LC, State University, AR 72467; E-mails: ccramer@astate.edu; cramer@biostrategies-lc.com

Abstract. Plants have emerged as effective systems for the bioproduction of complex high-value proteins for medical applications. Production platforms range from stable transgenic plants to transient leaf-based expression systems to plant cells grown in bioreactors. When compared to human proteins produced in cultures animal cells, these plant-based systems provide advantages in safety, scalability and cost, especially up-front capitalization to establish production capacity. The first generation plant-made pharmaceutical proteins are in patients and are showing increasing acceptance by the pharmaceutical industry. The US government has made a significant investment in infrastructure and capacity-building for plant-based production of vaccine antigens. A new generation of plant-made pharmaceutical proteins is integrating innovative strategies to facilitate protein delivery; to selectively target key tissues, cells, or organelles; and to impact protein stability both during bioproduction and when administered as a therapeutic or vaccine.

ORAL PRESENTATIONS:

Oral 31.Advancing drug development from plants – The Medicinal Plant Consortium experience

Yun-Soo Yeo[1], S. Eric Nybo[1], Amar Chittiboyina[2], Aruna D. Weerasooriya[2], Yan-Hong Wang[2], Troy Smille[2], Ikhlas A. Khan[2], Natalia Dudareva[3] and Joe Chappell[1].[1]University of Kentucky, Lexington, KY; [2]University of Mississippi, Oxford, MS; [3]Purdue University, West Lafayette, IN. E-mail: chappell@uky.edu

Abstract. Medicinal plants produce a wealth of pharmaceutical compounds. Yet, the specialized metabolic pathways leading to such compounds remain poorly understood and progress in elucidating and manipulating these taxonomically-restricted metabolic pathways has been correspondingly slow. Development of "omics"-level resources for medicinal plants has been limited meaning that research in medicinal species has not benefited to the same extent from the genomics revolution as has research in model plants and agronomic crop species. With the combined use of state-of-the-art sequencing technologies, metabolomics capabilities, and bioinformatics, the Medicinal Plant Consortium has developed an unrestricted, public resource to address this growing gap in our knowledge. This resource includes a publicly accessible metabolomics and transcriptomics database for 14 widely used medicinal plant species and is designed to accelerate the identification and functional analysis of genes involved in natural product biosynthesis. Progress will be illustrated by describing the discovery of a very unusual sesquiterpene pathway in *Valeriana officinalis*.

Oral 33.Image-based high-throughput screening and plant phenotyping – From active ingredient screening to plant breeding

Ben Niehaus[1], Joerg Vandenhirtz[1], Ralph Schunk[1], Hans-Georg Luigs[1], Matthias Eberius[1]
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Abstract. The task of measuring the impact of medicinally active substances on model organisms requires the reproducible screening of statistically relevant, large numbers of probes. Using complex organisms or cell cultures rather than enzymatic tests for the testing of medicinally active ingredients will lead to more relevant results. As the effect of the active substance might be unknown, any deviation in e.g. movement, colour, size or other, even more specific variables between the test and control group can be of importance, either to identify or to improve the test compound. The LemnaTec scanalyzerHTS has been found to be the ideal image-based approach for a wide range of test structures such as those with mosquito larvae, water fleas, fish eggs, nematodes, cell cultures and in situ tests. The LemnaTec scanalyzerHTS provides a flexible solution to screen anything from cell cultures to small plants like Arabidopsis thaliana, providing images in visible light as well as near infrared, infrared and fluorescence.
Keeping in mind that many medicinally active substances can best be produced by plants, efficient plant breeding using image-based plant phenotyping becomes more and more important. Such non-destructive assessment of plant growth, morphology and architecture

under normal as well as under stressful environmental conditions represents the optimal way to characterize and select high-yielding plants. Growing plants under controlled conditions on the LemnaTec scanalyzer3D conveyor system or assessing the development of small plants in the scanalyzerHTS system provides a comprehensive set of phenotyping data to complement the chemical and biological analysis of the active ingredients. Yields that originate from crossings, wild collections or germplasm banks can thus be optimized for specific environmental conditions, longer harvesting periods and higher concentrations of the desired active compound.

SPECIAL SESSION 8.LEGALESE OF RESEARCH: CONFIDENTIALITY, CONTRACTS, PATENTS, STARTUPS

Brian Rogers (Arkansas Biosciences Institute Commercial Innovation Center, AR, USA). E-mail: brogers@astate.edu

Abstract. There is a need for a better understanding of the various intersections between law and innovation. There is also a need to debunk many misapprehensions about the lack of common purpose among grant sponsored academic endeavors, the patent system, and commercialization. This is also a need to inspire a culture of innovative entrepreneurship. This thesis will seek to address as many of the foregoing matters as is possible within thirty minutes, as well as provide some tips and tools that those in attendance can employ in their daily endeavors. Following are some specific topics to be addressed as time permits: (1) defining and thinking about intellectual property; (2) reviewing the life of a research project and some of the common legal matters not to be neglected; (3) reviewing policies behind patenting and commercializing sponsored projects; (4) reviewing the patenting process; (5) using the patent databases as a primary source of scholarly works; and (6) reviewing university technology transfer.

SESSION 9A. VOLATILE OILS: CHARACTERIZATION, MEDICINAL USES AND PRODUCTION

Chair: Valtcho Jeliazkov (University of Wyoming, WY, USA)

KEYNOTE PRESENTATION:

K8. Trends, challenges and new discoveries with volatile oils

James E. Simon[1], H.R. Juliani[1], Q. Wu[1], Kelsey Gustafon[2] and Derek Hawkins[2]. New Use Agriculture & Natural Plant Products (NUANPP), Department Plant Biology and Plant Pathology, and [2]Medicinal Chemistry, School of Pharmacy, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901. E-mail: jimsimon123@rci.rutgers.edu

Abstract. As the search for new aromas and fragrances continues around the world, traditional focus by those in plant biology and agricultural sciences have centered on plant genetics, genetic diversity, sourcing, optimizing production and processing with fruitful results. The

paradigm shift into cross disciplinary areas has opened up new doors of scientific discoveries that can connect human perceptions of aroma and scent to emotion, behavior, health, well-being and nutrition. From custom designing plants to custom designing aromas and fragrances to induce human responses can further connect plant biologist and chemist to those in psychology, pharmacy and neuroscience as new applications of aromatic volatile oils are explored.

Increasing interest in biologically active volatile oils and carriers also opens new opportunities. Case studies of plant-based volatile oils as well as plant-based butters will illustrate a number of trends, challenges and applications. Shea butter (*Vitellaria paradoxa* C.F. Gaertn.), from sub-Sahara Africa, popular in cosmetics, personal care products and foods popular because of its unsaturated fatty acids composition as well as the potential utility of its unsaponifiable fractions now being used in cosmeceutical, pharmaceutical and nutraceutical applications. *Pycnanthus angolensis* (Welw.)Warb.commonly known as African nutmeg, is a tree species found in West and Central Africa. The seed fat, from which kombo butter is produced, contains unusually high levels of myristoleic acid, a precursor of cetyl myristoleate of interest for its potential use in the treatment of arthritis. Kombo butter is also a source of phytochemicals that are structurally related to the tocotrienols and have shown significant anti-inflammatory antioxidant activity and neuroprotection benefits.

ORAL PRESENTATIONS:

Oral 35.Unique essential oils from Africa for the development of natural plant product industry

H. Rodolfo Juliani[1], James E. Simon[1]. [1]New Use Agriculture and Natural Plant Products.Department of Plant Biology and Pathology. 59 Dudley Road. New Brunswick 08901, NJ. E-mail: hjuliani@rci.rutgers.edu

Abstract. The natural product industries in Africa share many common challenges and impediments to growth. One of the most pressing concerns is the limited quality assurance/quality control of products and the small-scale nature of some of the essential oil producers. Several research reports have highlighted the importance of the physical, chemical and biological characterization of several essential oils entering the marketplace. Since many sub-Saharan countries are entering the production of essential oils, this work sought to review the physical and chemical properties of new essential oils with potential for economic development. This presentation will highlight cases studies on the chemistry and quality of essential oils from different African countries (*e.g.* Rwanda, Namibia) in support of commercialization efforts. Several quality characters are important to determine the value of essential oils (aroma, chemical profile, physical properties) though their potential biological activities provide important information for their commercialization. The presentation will discuss the potential new uses and applications of these new volatiles oils as a way to generate interest in these products to ultimately assist rural communities to generate income.

Oral 36.Repellent and larvicidal activity of five different *Agastache* essential oils and their major constituents against *Aedes aegypti*

Nurhayat Tabanca[1], Betul Demirci[2], Mei Wang[1],Eugene K. Blythe[3], Abbas Ali[1], Ulrich R. Bernier[4], Ikhlas A. Khan[1,5,6]. [1]National Center for Natural Products Research, The University of Mississippi, University, MS 38677 USA; [2]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey; [3]Coastal Research and Extension Center, Mississippi State University, South Mississippi Branch Experiment Station, Poplarville, MS 39470 USA; [4]USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608 USA; [5]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677 USA; [6]Department of Pharmacognosy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia, E-mail: ntabanca@olemiss.edu

Abstract. Mosquitoes are vectors of serious human pathogens that cause malaria, Dengue Fever, Yellow Fever, Rift Valley Fever, and Chikungunya. All of these diseases can lead to explosive outbreaks in humans which can cause high rates of morbidity and mortality. There are no vaccines for many diseases transmitted by biting insects, so methods of insect management and personal protection are the primary tools available to reduce disease risk to human populations. There is an urgent need to develop alternative insecticides to supplement pyrethroids for control of a wide variety of insect-vectored diseases. In a research program aimed at identifying natural insecticides, different essential oils have been evaluated as topical repellents against *Aedes aegypti* L. using a “cloth patch assay” test and DEET(*N*,*N*-diethyl-*meta*-toluamide) as the positive control. Based on these screening results *Agastache mexicana* (Kunth) Lint & Epling, *A. pallidiflora* (A. Heller) Rydb.ssp. *neomexicana* (Briq.) Lint & Epling, *A.pallida* (Lindl.)Cory × *mexicana* (Kunth) Lint & Epling), *A. rugosa* (Fisch. & C. A. Mey.)Kuntze,and *A. rupestris* (Greene) Standl. (Lamiaceae) essential oils showed good repellent activity with minimum effective dosages between 0.109-0.312 mg/cm^2. *Agastache* species are commonly known as giant hyssops or hummingbird mints, with someused in herbal tea due to their pleasant aroma. As a folk remedy, the leaves can be rubbed onto skin and are purported to be repellent to insects.An effective integrated mosquito management strategy relies on the control of the larval stages. *Agastache* essential oils were also subjected to high throughput larval bioassays against *Ae. aegypti*. The constituents of the essential oils were analyzed by GC and GC-MS and their major compounds were individually tested with mosquito repellent and larval assays.

Oral 37.Commercially important indian medicinal & aromatic plants

Sathyanarayana Reddy Ganta, Herbal Garden Scheme, Dr. YSR Horticultural University, Rajendranagar, A.P., India. E-mail: gsn2000@rediffmail.com

Abstract: India has a rich tradition in use of medicinal plants for curing diseases and for better health. According to ancient Indian scriptures, medicinal use of plants dates back to more than 3000 years. AYURVEDA: (Ayur=life, Veda=science), a holistic system of medicine in India uses constitutional model and includes food and lifestyle guidance. An estimated 1587 plants are

used in Ayurveda. Around 40,000 compound formulations have been documented in Indian classical texts written in Sanskrit language. The YSR Horticultural University established the Herbal Garden Scheme to maintain germplasm of endangered and commercially popular medicinal plant species, conduct agronomic research, and provide training in agrotechnology to farmers, extension outreach professionals and university students. This Center also provides good quality pure seed and propagules for research and commercial production. The center has a tissue culture lab for research and micro-propagation, field and lab essential oil extraction units, and currently maintains more than 200 medicinal and 33 aromatic plant species. Some endangered and commercially popular species for which agrotechnology has been developed at the center include *Withania somnifera*(for Immunity), *Aloe barbadensis* (used in dermatology and cosmetology), *Ocimum sanctum* (anti-bronchitis), *Plantago ovata* (laxative), *Cassia angustifolia* (laxative), *Cissus quadrangulatris* (heals broken bones), *Andorgraphis paniculata* (anti-hepatic), *Gymnema Sylvestre* (anti-diabetic), *Phyllanthus emblica* (anti-oxidant), *Coleus Forskohlii*(hypertension, asthma, congestive heart failures), *Acorus calamus* (Bronchitis), *Phyllanthus amarus* (hepatic disorders, diuretic), *Rauvolfia Serpentina* (anti-hypertension, CNS disorders), *Terminalia arjuna* (cardiac problems), *Terminalia bellarica* (relieves constipation, cough, and flatulence), *Terminalia chebula* (alleviates indigestion, asthma and cough), *Aegle marmelos* (tonic, astringent, laxative).Essential oil extracted from aromatic plants are extensively used in perfumery industry, aromatherapy and massage therapy. Some important aromatic plants include *Cymbopogon* species (anti-fungus, preservative, pesticide, insect repellant), *Pelargonium graveolens (Insect repellant, food flavoring)* and *Mentha arvensis (*anaesthetic, antispasmodic, influenza and colds). The ethnobotany and medicinal properties of these plants will be discussed.

SESSION 9B.SELECTED CONTRIBUTED PRESENTATIONS

Chair: Jeff Adelberg(Clemson University, SC, USA)

ORAL PRESENTATIONS:

Oral 38. Towards automation of micropropagation using a mist bioreactor

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Abstract. It is challenging to automate micropropagation mainly because of the diversity of leaf morphology. We propose vertical cultivation of plantlets on hanging strips to efficiently use space and to propagate plants having any leaf shape or size. We used a carrot cell suspension as a model to develop a 1-step propagation method from cells to embryos to fully rooted, acclimatized plantlets. Cells were charge attached to coated polypropylene strips that were hung and then sprayed with B5 medium during growth in the mist reactor. Cells attached to the hanging strips developed into rooted plantlets in 20 days. Although use of an increased misting cycle led to an increased ratio in post-heart embryo formation after 14 days, continuous misting did not further increase embryogenesis. Carbon dioxide enrichment on carrot embryogenesis was also investigated. Compared with embryos developed in unvented culture chambers, after

14 days more embryos formed with 3% carbon dioxide enrichment, and after 20 days the average length of rooted embryos was increased by almost 50% compared to ambient air controls. When only a sucrose solution was used during the adhesion step, cell attachment to coated 50 micron nylon, 70 micron nylon and 74 micron polypropylene mesh was 4.4, 3.3 and 2.3 fold, respectively, compared to half strength B5 salts. Almost 90% of the originally attached cells remained on the 50 micron nylon mesh 24 hrs later after spraying with B5 medium in the mist reactor. It is also possible to propagate attached small attached explants of leaves; *Artemisia annua* leaf explants, for example, attached by charge adhesion and by filamentous trichomes to coated polypropylene or nylon strips. Together these results demonstrate that both the mist reactor and our attachment technology may offer new opportunities for at least partial and possibly full automation of micropropagation.

Oral 39.Skin protective effects of some plant extracts (genus Ficus) against induced toxicological insults in rabbits

Muhammad, F[1]., Waheed, M[1]., Javed, I[1]., Akhtar, M[2]., Saleemi, M.K[4]., Khaliq, T[1]., Anwar, M.I.[3]. [1]Department of Physiology and Pharmacology, [2]Department of Parasitology, [4]Department of Pathology, University of Agriculture Faisalabad, Pakistan, [3]Department of Livestock and Dairy Development, Lahore, Government of Punjab, Pakistan. E-mail: Faqir_m1@hotmail.com

Abstract. Chemical induced skin irritation is one of the major skin problems in Pakistan in workers involved with agriculture, chemical and automobile business. Due to financial constraints, these personnel cannot afford costly treatments and look for alternative ways to resolve skin problems. Skin protective effects of plant extracts (*Ficus religiosa, Ficus benghalensis* and *Ficus racemosa*) against known irritants (Sodium Dodecyl Sulfate (SDS), Atrazine and Petrol) were studied in rabbits (n=30). Ethanol extracts of plants were obtained through Soxhlet. All irritants and ficus extracts were topically applied on the backs of rabbits daily for four days while pure ethanol served as control. Skin was examined after 24, 48 and 96 hours for erythema. Skin biopsies were taken on 5th day for microscopic examination. Data was analyzed statistically using ANOVA and DMR. Erythema produced by irritants reduced significantly with the simultaneous application of ficus extracts. Mean ± SEM epidermal thickness (µm) with SDS was 45.40±1.89, *Ficus Religiosa* + SDS 18.60±0.51, *Ficus Benghalensis* + SDS 18.40±0.25, *Ficus Racemosa* + SDS 18.80±0.37 and mixture of three Ficus + SDS 16.80±0.37. Similar findings were revealed after using plant extracts with atrazine and petrol. The mean ± SEM epidermal layer count for SDS was 3.60±0.25, Atrazine 3.40±0.25, Petrol 3.40±0.25, and Ethanol (control) was 1.00±0.20. This count reduced to 1.00±0.20 for three Ficuses + SDS, 1.40±0.25 for Ficuses + Atrazine, 1.40±0.25 for Ficuses + Petrol. This reflects that Ficus species might have the potential to reduce the irritation induced by chemicals in both animals/humans and thus can be a valuable addition to the cosmetic herbal market.

Oral 40.Antioxidant activity, phenolics content, and other nutritional properties of new hot pepper breeding lines

Mohammad Jalaluddin[1] and Shahidul Islam[1]. [1] Department of Agriculture, University of Arkansas at Pine Bluff, Pine Bluff, AR 71601. E-mail: jalaluddinm@uapb.edu

Abstract. Hot peppers (*Capsicum annuum* L.) are popular spicy vegetables used in food preparation worldwide. They also have special nutritional and medicinal qualities. A breeding program at the University of Arkansas at Pine Bluff (UAPB) conducted for about 14 years generated a large number of genotypes expressing a wide array of important agronomic and pigmentation characteristics. Recent phytochemical analyses conducted on 30 elite lines revealed interesting genotypic variability in contents and concentrations of ascorbic acid, flavanoides, capsaicin, phenolics, and antioxidant activity, which are known for their benefits to human health. The objective of this investigation was to select candidate accessions of hot pepper having higher concentrations of active compounds for use as parents in the breeding program to enhance pepper varieties for their health protective qualities as food materials and possible medicines. In 2011, 12 selected experimental lines of pepper from the germplasm stock were field-grown and analyzed for their phytochemical contents. Peppers were harvested at two maturity stages (green and red-ripe) for the study. Samples were freeze-dried for analyses. Dried red-ripe peppers compared with the green harvest had higher levels of bioactive compounds that exhibited significantly higher levels of antioxidant properties (26−80 μmol trolox equivalents/g of dry matter), polyphenols (>2000 mg/100 g of dry matter), and capsaicin (95−437 mg/100 g of dry matter). However, ascorbic acid contents were higher in the green samples than the red-ripe samples. A high correlation was found between total polyphenolics content and the antioxidant activity. The great variability within and among pepper lines for these phytochemicals suggests that these selected accessions and their enhanced progenies may be useful as parents in breeding programs to produce hot peppers with value-added traits and thereby new spicy processed foods for health. Also, this research may open up opportunities for selection of traits assisted by molecular markers in peppers.

Oral 42.The effects of lutein, camptothecin, and curcumin on prostate cancer and the role of miRNAs in the LnCap cancer cell transcriptome

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Abstract: My lab has been studying the anti-neoplastic effects of carotenoids and certain spice agents from the human diet, on prostate cancer cells. In our studies we have used methods of phyto-chemotherapy; combining the best of both natural products with traditional chemotherapy. While strong synergistic effects have been acclaimed, a major problem in cancer treatment and prevention has been the effects of dietary agents conflicting with the effects of chemotherapy. Prostate cancer, is one of the most commonly occurring cancers, and is the second leading cause of cancer deaths in men in the United States. In our studies, the effects of lutein, camptothecin, or curcumin alone or collectively were studied on human prostate cancer cell lines. Results indicate that camptothecin or curcumin or both, work in a manner where low concentrations are more effective than higher concentrations. This is uncommon from the normal paradigm. The anti-neoplastic effects of lutein depicted the classical standard curve of

cell growth. We hypothesize that curcumin might antagonize the antitumor activity of camptothecin. Many chemotherapeutic drugs generate reactive oxygen species and activate c-jun NH2-terminal kinase pathway in the course of inducing apoptosis. Recently it has been revealed that miRNAs possess a variety of crucial regulatory functions related to cell growth, development, and differentiation, and are associated with a wide variety of human diseases and cancer. There are 72 types of miRNAs involved in prostate cancer, 14 of which are found to be involved in the antitumor properties of curcumin. Therefore we propose that miRNAs may be involved in the opposing mechanism of action of camptothecin and curcumin. Future studies of miRNAs in LnCap cell lines with chemotherapeutic drugs and neutraceuticals are necessary to provide strategies for prostate cancer treatment and prevention.

POSTERS ABSTRACTS

1. BIOACTIVES AND HUMAN HEALTH

P1.Anti-proliferative activity of *Rubus parvifolius* L. extract and total saponins against leukemia K562 cells *in vivo* and *in vitro*

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Abstract. *Rubus parvifolius* L. (RP) has long been used in traditional Chinese medicine for treatment of leukemia. In this work, the anti-proliferative activity of RP water extract and RP total saponins (RPTS) against high tumorigenic K562 cell were investigated *in vivo* by using a leukemia-bearing nude mouse model and *in vitro* by semi-solid agar culture and MTT assay. Our results demonstrated that the RP extract effectively inhibited the growth of leukemia; a high tumor inhibition rate of 84.8% was achieved with an administration of 1.0 g of RP extract daily into mice. The semi-solid agar culture indicated a 50.8% and 100% inhibition of the K562 colony formation in the presence of 20% (v/v) of RP medicinal serum and 150 mg/L RPTS, respectively. The same doses of RP medicinal serum and RPTS showed a proliferation inhibition of 31.4% and 86.3%, respectively against the K562 cells in the MTT assay.This research will potentially provide an alternative medicine for the treatment of malignant leukemia.

P2. Antimalarial evaluation of hairy root culture of *Bixa orellana*

Bo Zhai[1,2,3], Fatima Rivas[3], Michele Connelly[3], Julie Clark[2], Maria Ferrand[1], Luis Nopo[1], Fabricio Medina-Bolivar[1,2,]. [1]Arkansas Biosciences Institute, [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401 and [3]Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN. E-mail: fmedinabolivar@astate.edu

Abstract. *Bixa orellana* (*B. orellana)* is a tropical plant native to South America. Its seeds are heavily used by the food industry for coloring purposes, but in Peru, stems and roots are used to treat a wide range of ailments particularly bacterial and viral infections as well as malaria. In an effort to interrogate the responsible components of its alleged antimalaria properties, we evaluated *B. orellana*'s hairy roots chemical composition. Hairy roots of *B. orellana* were cultured for 16-20 days, stressed (24hrs) with known elicitor, Methyl Jasmonate (MeJA) and the culture medium was extracted with ethyl acetate. Hairy root tissue was extracted with isopropanol under refluxing conditions. The crude materials were fractionated to provide several known compounds and each compound was tested against malaria parasite strains 3D7 and K1. The culture medium contained ishwarane, MEJA, methyl 2-((1*R*,2*R*,3*R*)-3-hydroxy-2-((*Z*)-pent-2-en-1-yl)cyclopentyl)acetate, Jasmonic acid (JA) and inositol. Root tissue contained primarily steroidal sapogenins mainly stigmasterol, oleanolic acid, maslinic acid, traces of tocopherols (mostly δ-tocotrienol) and tannins. Herein, we discussed our results which indicate that despite *B. orellana's* popular use for the treatment of malaria, these compounds showed poor activity against strains 3D7 and K1.

P3. Effect of 2 weeks oral Echinacea supplementation on leukocyte responses

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Abstract. *Echinacea purpurea* is an herbal supplement derived from a North American perennial plant (Purple Coneflower) that is primarily used as a non-specific immunostimulant. Anecdotal evidence suggests that supplementation with Echinacea can shorten the duration and/or severity of the common cold. However, the prospective mechanisms related to the possible immune-enhancing effects of Echinacea remain to be identified. The purpose of this study was to determine whether two weeks of Echinacea supplementation altered resting leukocyte responses. Twenty-four apparently healthy and recreationally active males (age 24.9 ± 4.2 yrs, height 178.9 ± 7.9 cm, weight 87.9 ± 14.6 kg and 19.3 ± 6.5 % body fat) were randomly assigned to either an Echinacea (ECH; n=12) or a placebo (PLA; n=12) group. Subjects were required to be free of any symptoms of upper respiratory tract infections (URTI) for seven days prior to starting the study. Participants were supplemented with 8 $g \cdot day^{-1}$ of ECH or PLA (wheat flour) for 14 consecutive days. ANCOVA was used to determine significant differences with significance set alpha ≤ 0.05. There were no significant differences between ECH and PLA for WBC, neutrophils, monocytes or eosinophils at any time. ECH induced an 11.4% decrease in monocytes at wk 2 (0.44 ± 0.03 x $10^6 \cdot mL^{-1}$) vs. baseline (0.49 ± 0.05 x $10^6 \cdot mL^{-1}$, $P<0.05$) and wk 1 (0.49 ± 0.06 x $10^6 \cdot mL^{-1}$, $P<0.05$), and a 21.7% decrease in eosinophils at wk 1 (0.25 ± .07 x $10^6 \cdot mL^{-1}$, $P<0.01$) and wk 2 (0.23 ± 0.06 x $10^6 \cdot mL^{-1}$, $P<0.05$), respectively. No subjects in either ECH or PLA reported any symptoms of URTI or other changes in health status during their two week study period. These data suggest that oral supplementation of 8 $g \cdot day^{-1}$ of Echinacea for 14 days did not induce clinically relevant alterations in leukocytes at rest.

P4.Anxiolytic-like activity of (*R*)-(+)-limonene inhalation and GC-MS analysis

Damião P. de Sousa[1], Naiana G. P. B. Lima[2], Flávia Cristina F. Pimenta[2], Mateus F. Alves[2], Fábio S. De Souza[2], Rui O. Macedo[2], Margareth de Fátima F. Melo Diniz[2], Reinaldo Nóbrega de Almeida[3]. [1]Dept. of Physiology, Federal University of Sergipe, CEP 49100-000, Aracaju, Sergipe; [2]Dept. of Pharmaceutical Sciences, [3]Dept. of Physiology and Pathology, Federal University of Paraíba; Brazil. E-mail: damiao_desousa@yahoo.com.br

Abstract. The medicinal properties of essential oils are used by people over thousands of years to induce the well-being and healing of various diseases. Its use as a psychic drug represents an alternative source for the treatment of behavioral changes, which undertakes the health of humans, such as anxiety.However there are few scientific studies aboutthese oilsto prove itstherapeutic efficacy.(+)-Limonene isachemical constituentof variousbioactiveessential oils. Thisworkpresentstheanxiolytic-like activityof(+)-limonene on theparametersfromanimalmodel ofelevatedplusmaze.(+)-Limonene administered byinhalationin micesignificantlymodifiedalltheobserved parametersin theelevatedplusmazetestat0.5and1.0% concentration. Pharmacologicaleffectofinhaled(+)-limonene (1%) wasnotblockedbyflumazenil. Analysis of(+)-limonene inGC-MS showedthat its volatilityishigh. Thedata indicate that(+)-limonene canbeusedinaromatherapyasantianxietyagent.

P5.Anti-diabetic activity of fourteen natural compounds

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Abstract. Fourteen naturally occurring compounds and two derivatives of naturally occurring compounds were tested for inhibitory activity with the enzyme α-glucosidase. These compounds included five anthocyanins, five polyphenols, four thiol derivatives, and cinnamic acid. Acarbose was used as the standard. Both total inhibition and kinetic measurements were made in this study. All of the anthocyanins tested showed inhibitory activity. None of the thiol derivatives showed inhibitory activity. Of the polyphenols tested, all were active except for caffeic acid. Cinnamic acid was also weakly active. Inhibition type varied between compounds. Ferulic acid, rutin, cyanidin, malvidin, and delphinidin showed competitive inhibition behavior. However, tannic acid and pelargonidin showed noncompetitive behavior. Quercetin and cinnamic acid showed mixed inhibition behavior. All compounds containing at least one ether or glycosidic link show competitive behavior. Cyanidin and delphinidin do not have any ether or glycosidic links but still displayed competitive kinetic behavior. Whether this is true competitive inhibition or allosteric competitive inhibition needs to be determined by further experimentation.

P6.Variability of antioxidant activity of orange fleshed sweetpotatoes in relation to polyphenolic contents

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Abstract. Sweetpotatoes (*Ipomoea batatas* L.) are popular root vegetables grown worldwide. Studies have been done which compared the antioxidant content of white, orange, pink, red, and purple-fleshed sweet potatoes. These studies indicated that purple fleshed sweetpotatoes had the highest overall antioxidant content, and color intensity was indicative of antioxidant capacity. A study was conducted to investigate the variability of antioxidant activity and phenolic content within orange-fleshed sweet potato lines. Three sweet potato lines were tested for antioxidant capacity by 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC). Phenolic content was measured using the Folin-Ciocalteau assay. It was found that the antioxidant activityof sweetpotato extracts in hydrophilic fraction have a significant antioxidant effect when tested by each method. There was a relationship between total polyphenol content and antioxidant function in case of ABTS (r= 0.59) and ORAC *(r* = 0.35). The hydrophilic ABTS values correlate significantly with the hydrophilic DPPH values (r= 0.84) and the hydrophilic ORAC values correlate reasonably well with the hydrophilic ABTS values (r = 0.85). In case of the hydrophilic DPPH values and hydrophilic ORAC values also showed a strong correlation (r = 0.87). Results showed sweetpotato genotypes had significant antioxidant activity as well as polyphenolic contents. The phenolic content of all genotypes was ranged 3.5-4.8 mg TAE/g dry weight. Among the methods examined, ABTS proved the best for antioxidant determination in orange-fleshed sweetpotatoes followed by ORAC method. The information provided by this research will also facilitate the genetic and chemical breeding study for improvement of the desired quality criteria of orange fleshed sweetpotatoes as well as other produces.

P7.Assessing the ability of tomato fruit extracts to prevent oxidative damage to DNA

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Abstract. Fruits are major components of the human diet, contributing a large portion of vitamins, minerals, antioxidants, and fiber. Tomato is a major source of several health-promoting phytonutrients, including ascorbate, carotenoids, and flavonoids. In addition to being one of the most popular vegetables worldwide, the cultivated tomato (*Solanum lycopersicon*) is an important model species for fruit physiology and development, genetics, and plant breeding. In this study we measured levels of selected metabolites in three varieties of tomato (Moneymaker, Ailsa Craig, and Manapal), as well as two mutant varieties (*hp*-2^{dg} and *aw*) with altered photomorphogenic responses. Levels of chlorogenic acid and rutin, the major phenolic compounds in tomato, were measured by HPLC. The total antioxidant activity of methanol fruit extracts was determined by the Oxygen Radical Antioxidant Capacity (ORAC) and Trolox Equivalent Antioxidant Capacity (TEAC) radical scavenging assays. We also assayed the ability of tomato fruit extracts to prevent DNA strand breaks caused by peroxynitrite, a powerful oxidizing agent produced by inflammatory cells *in vivo* that has been linked to several chronic degenerative diseases. This study aims to determine if differences in antioxidant content measured by traditional chemical methods correlates with the ability to prevent oxidative damage to biological targets such as DNA. This knowledge will provide a better understanding of the biological activity of tomato phytonutrients in an effort to produce healthier foods.

P8.Comparison of anti-cancer agent curcuminoid and essential oil composition in turmeric (*Curcuma longa*)

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Abstract. Turmeric (*Curcuma longa*) is grown primarily for its rhizome, with the greatest amount produced in India and South Asia. Curcuminoids, the major active constituents of turmeric and the source of its distinctive yellow color, have exhibited anticancer, antioxidant, and anti-inflammatory properties and have been extensively researched for additional health benefits.
The objective of this study was to determine the curcuminoid and essential oil content of different plant parts using different turmeric genetic resources from India, Korea, and South Asia.
The curcuminoid content differs by genetic resource, plant part and the use of cloned plant material, with turmeric grown in the greenhouse containing more than field-grown plants. Three of the major compounds in turmeric are curcumin and its analogs bisdemethoxycurcumin and demethoxycurcumin, also known as curcuminoids. The amount of each compound in turmeric from India and South Asia was quantified in the main rhizome, branch roots, buds, and leaves using HPLC. It was also quantified in the epidermis, intermediate, and core of the branch roots from India, Korea, and South Asia. The main rhizome of Indian turmeric was found to have the highest curcumin concentration at 52.04 mg/g dry weight, with the lowest amount present in the leaves at trace levels.
Essential oil constituents were compared in the leaves and rhizome of all three genetic resources using hydrodistillation and GC. The major compounds found were ar?-tumerone, tumerone and α-tumerone in the rhizome, and limonene and terpinolene in the leaves. In the rhizome, ar?-tumerone ranged from 22.4% (Korea) to 35.5% (South Asia), tumerone ranged from 18.5% (South Asia) to 20.9% (India), and α-tumerone ranged from 8.25% (Korea) to 13.8% (India). The leaves have a different composition of essential oils than the rhizome, with limonene ranging from 1.73% (Korea) to 51.56% (India) and terpinolene ranging from 14.75% (India) to 24.48% (South Asia).

P9.Microfluidic chips for proteomic biomarker profiling in cancer cells

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Abstract. We report the first demonstration of a fully integrated microfluidic microchip incorporating all the components of a benchtop liquid chromatography system: pump, valve system, buffer gradient and mixing features, separation column, and electrospray ionization

(ESI), interfaced to a mass spectrometer (MS), allowing the comparative analysis of a complex cellular extract. Differential protein expression was determined by comparing stable isotope iTRAQ-labeled peptides from treated or untreated breast cancer cells that were loaded and separated on the microfluidic microchips and analyzed by MS/MS.Microfluidic chips are a disposable, low cost, high throughput alternative to bench scale LC systems and may potentially reduce analysis times and sample and solvent volumes (sample volumes are < 1 ul and < 1 ug protein), thereby addressing a traditional problem of limited amounts of protein for analysis, particularly in cancer research with limited availability of diseased samples. A key feature differentiating microfluidic microchips from traditional LC and other lab on a chip systems is the use of a voltage potential to produce electroosmotic flow (EOF) of the ions in the solvent, which drives the movement of solvent instead of a traditional LC pumping system and generates ESI for MS detection. Microfluidic chips can thus be interfaced with MS instruments without proprietary equipment. Microchip performance was assessed by demonstrating the stability of EOF flow for consistent electrospray ionization and the potential application of microchips as tools for quantitative proteomic profiling was established by demonstrating reliable quantitation for ≥ twofold changes in protein expression levels. Microfluidic microchips appear to be a promising analytical tool for automated, multiplexed and high throughput analyses with no sample carryover, for applications such as large scale cancer biomarker profiling and therapeutic agent screening.

P10.Anti-asthmatic activities of *Opuntia humifusa*extract in BALB/c mice

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Abstract. This study aimed to evaluate the anti-asthmatic effects of *Opuntia humifusa*extract (CANSE) using in BALB/c Mice. Asthmatic mice model was conducted by repeated challenge of OVA and CS using BALB/c mice. Each group was treated CANSE (242mg/kg) extract or cyclosporin A (10 mg/kg) for the later 8 weeks, Penh (plethysmography), Anti-OVA-IgE in BALF, WBC, neutrophils and eosinophils was analyzed. Administration of CANSE significantly decreased Penh levels comparing to control group. WBC, neutrophils, eosinophils and Anti-OVA-IgE level in BALF were significantly decreased by CANSE treatment too. These results strongly suggest that CANSE may be useful in the treatment of asthma.

P11.Inositol polyphosphates play crucial roles in mediating cellular apoptosis

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Abstract. Inositol polyphosphate (InsP) metabolites constitute important signaling molecules both in plants and animal cells and regulate diverse cellular processes including calcium

homeostasis, vesicular trafficking, chromatin remodeling and nuclear export of mRNA. Recent studies have shown that increased cellular levels of higher InsPs such as $InsP_6$ (phytic acid) and diphospho-inositol pentakisphosphate ($InsP_7$) mediate apoptotic mechanisms. We have previously analyzed changes in cellular levels of lower InsPs ($InsP_1$- $InsP_4$) during apoptosis. In this study, we have confirmed our previous findings and show that cellular levels of $InsP_1$ and $InsP_2$ increase while levels of $InsP_3$ and $InsP_4$ decrease significantly upon induction of apoptosis by dichloroacetic acid (DCA). DCA is a mitochondrial pyruvate dehydrogenase Kinase (PDK) inhibitor that shifts aerobic glycolysis to oxidative-phosphorylation thus correcting mitochondrial dysfunction found in cancerous cells. Aerobic glycolysis, also known as "Warburg Effect", is a common problem in most cancers. Therefore, DCA can be potentially exploited for cancer chemotherapy. Additionally, attempts were made to detect higher InsPs by polyacryamide gel electrophoresis under apoptotic conditions. Changes in InsPs during apoptosis were correlated with markers of apoptosis and cell proliferation as measured by MTT assay.

P12.Light quality as a means of increasing concentration of bioactive compounds in red leaf lettuce

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Abstract. This research was carried out to investigate the hypothesis that blue (440 nm) light can be used to increase the production of antioxidant phytochemicals in red leaf lettuce cv. Outredgeous. Lettuce contains bioactive compounds which have been shown to be effective in protection against cardiovascular disease and to a lesser extent, cancer. Four light treatments were applied with light emitting diode (LED) arrays at 300 µmol m^{-2} s^{-1} of photosynthetically active radiation with an 18 h-light/ 6 h-dark photoperiod for 28 days. Two treatments applied blue light during either early or late stage of development. The treatments were: (a) red 640 nm (290 µmol m^{-2} s^{-1}) + blue 440 nm (10 µmol m^{-2} s^{-1}) with blue light removed at 21 days after planting (DAP); (b) red 640 nm light (300 µmol m^{-2} s^{-1}) with blue light being added at 21 DAP. Control treatments were: (c) red and blue light for the 28 day cycle and (d) red light only for the 28 day growth cycle. Environmental conditions were maintained at 23 ˚C, 65% relative humidity and ambient CO_2 for the duration of the experiment.
The phytochemical content of the leaves was determined before and after changes in blue (440 nm) light treatments using LCMS analysis of 60% methanol extracts of the leaves. Preliminary research has identified chicoric, tartaric and chlorogenic acid as major components of the methanolic extracts. An increase in the concentration of cyanidin glucosides and quercetin glucosides was also observed upon the introduction of blue light during the final week of growth. This suggests that spectral quality can be optimised to increase the production of these bioactive phytochemicals. Work is ongoing to identify the key steps for blue light regulation of the biosynthetic pathway resulting in the increased production of these compounds in red leaf lettuce.

P13.Antimicrobial and antioxidant effects of compounds in sweetgum bark (*Liquidambar styraciflua* L.)

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Abstract. Sweetgum trees grow freely in the Southeastern U.S. and are often considered a nuisance to the lumber industry. They often grow in pine forests and must be removed before the pine trees can be harvested. To make use of this otherwise wasted biomass, sweetgum could be converted into cellulosic ethanol through dilute acid pretreatment, enzymatic hydrolysis, and fermentation. If value added compounds can be identified, the value of sweetgum as a potential biofuel-destined feedstock will increase.

The goal of this project is to determine if sweetgum bark contains compounds that display antioxidant activities. In this study, 65°C water was used to extract phytochemicals from sweetgum bark prior to dilute acid pretreatment. The crude sweetgum extracts were tested for antioxidant properties. Sweetgum bark yielded 1.7 mg g^{-1} of shikimic acid. Sweetgum bark also contained gallic acid, which is a documented antioxidant. Therefore, the gallic acid reference standard and the crude sweetgum bark extract were further analyzed for their antioxidant effect using the *in-vitro* Cu^{2+} induced low density lipoprotein oxidation (LDL) thiobarbituric reactive substance (TBARS) assay. Crude sweetgum bark extract, 12.5 mg ml^{-1}, and gallic acid, 75 µM, inhibited LDL oxidation by at least 90%. These results indicated that sweetgum bark extracts contain compounds, of which gallic acid is certainly contributing to, that display potent antioxidant effects.

The carbohydrate recovery in bark material was also tested. Including the 65°C water-based extraction step prior to 0.98% H_2SO_4 pretreatment at 130°C for 50 min, resulted in a 21% increase in xylose recovery from the bark, as compared to direct pretreatment. Thus, in addition to recovering phytochemicals, the 65°C wash step also increases xylose recovery. These results demonstrate that it could be possible to devise a biorefinery operation that, in addition to producing biobased fuels or chemicals, also recovers high value phytochemicals from feedstock.

P14. Effect of individual soyasaponins on human colon cancer cells

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Abstract. Group B saponins, the predominant form of saponins in heat-treated soy products, have been shown to possess hypocholesterolemic, antimutagenic, and anticarcinogenic properties. Previous studies have evaluated crude mixtures of soyasaponins, but studies evaluating a single purified soyasaponin as an anticarcinogenic agent are limited. The objective of this study is to examine the effects of purified soyasaponins I and III as well as their aglycone form, soyasapogenol B, as anticarcinogenic agents on the human colon cancer cell line Caco-2. Experiments were conducted to determine the effects of purified soyasaponins on cell proliferation, Protein Kinase C (PKC) activity, and cell morphology in cultures of Caco-2 cells. Our results showed that treatment of cells with soyasaponins I and III at concentrations of 300–

900 ppm significantly reduced viable cell numbers after 48 and 72 hours of exposure by 10-35% ($p<0.05$). Soyasapogenol B at a concentration of 100 and 150 ppm significantly reduced viable cell numbers after 24 hours by 15 and 62%, respectively ($p<0.05$). Cell morphology changes demonstrated that as concentrations and lipophilicity of soyasaponins increased, cell membranes became rougher and more irregular. These results indicate that purified soyasaponins I, III and soyasapogenol B, at physiologically relevant doses, can suppress Caco-2 colon cancer cell proliferation. These findings suggest that purified group B soyasaponins and their final metabolite soyasapogenol B may be a colon-cancer suppressive component of soy that warrants further examination as a potential nutraceutical or functional food.

P16.Production and accumulation of isoflavonoids in hydroponically grown Red clover *(Trifolium pratens)*: effects of environmental growth conditions

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Abstract. Isoflavonoids are bio-actives compounds associated with secondary metabolism, which have become a staple of the human diet in recent years, being a large component of soy-based foods. Their phytohormonal activity has numerous biological benefits to both human nutrition, due to their relationship with estrogen, including but not limited to cardiovascular diseases and cancer. However, commercial isoflavonoid-based products such as extracts and supplements don't give specifics as to the quality or quantity of isoflavonoids present. By growing Red clover hydroponically under varying environmental conditions, the environmental effects on isoflavonoid accumulation and production can be optimized and up-scaled for potential commercial growth result in viable and clearly identifiable products. Red clover seedlings were grown for 28 days under different light qualities (cool white fluorescence and high intensity discharge-high pressure sodium (HID-HPS)) as well as different light quantities ranging from 150 - 1000 µmol m^{-2} sec^{-1} with concurrent changes in ambient growth temperatures ranging from 11 - 23 ºC. Isoflavonoid concentrations (daidzin, genistin, biochanin A and formononetin) in leaf, stem and root tissues of the seedlings were analysed. Exploration into isoflavonoid extraction methods was carried out using direct solvent extraction on fresh and freeze-dried tissue and by means of super-critical fluid extraction. Preliminary result show that clover biomass increases with increasing light up to 300 µmol m^{-2} sec^{-1}, at 450 µmol m^{-2} sec^{-1} evidence of photooxidation was present hindering plant growth. Preliminary results also show that isoflavonoid accumulation differs between each of the three tissue types, leaves, stems and roots. Also, isoflavonoid extract concentrations were higher when using direct solvent extraction with freeze-dried tissue.

P17.Stable coexpression of vitamin C enhancing genes for improved production of a recombinant therapeutic protein, hIL12, in *Arabidopsis thaliana*

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Abstract. Human interleukin-12 (hIL-12) has been studied as a potential therapeutic factor in the immune response against viral disease and cancer. Plants are a promising platform for the production of complex mammalian proteins, as they can produce large amounts of functional polypeptides free of animal pathogens, and protein production can be increased to agricultural scale in a relatively short period of time. The presence of vitamin C (L-ascorbic acid, AsA) has been reported to have a positive effect on recombinant protein recovery. In a previous study we have demonstrated the favorable impact of the exogenous addition of AsA on the accumulation and recovery of hIL-12 synthesized in a *Nicotiana benthamiana*-based transient expression system. Based on these results, we hypothesized that the stable transformation of two key genes encoding proteins that will lead to enhanced levels of AsA and hIL-12 production will lead to plants that will produce and accumulate higher levels of hIL-12. To prove our hypothesis, stable *Arabidopsis thaliana* plants derived from crosses of plants over-expressing hIL-12 and AsA enhancing genes (*myo*-inositol oxygenase or *At*MIOX4, and glucuronate reductase or *At*GlcUR) were generated and characterized. Our results demonstrate a positive impact of elevated AsA content on hIL-12 production and recovery as the crosses (*At*MIOX4 x hIL-12) accumulated 70% more hIL-12 compared to the parent line solely expressing this cytokine.

P18. Medicinal plants in dentistry

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Abstract. Herbal extracts are used in dentistry for the prevention of caries and periodontal disease, due primarily to their antimicrobial and anti-inflammatory activity. The purpose of this study is to provide a review for the use of medicinal plants in dentistry.
Periodontal diseases affect more than 50% of the population and are potential risk factors for systemic diseases, including diabetes, cardiovascular and respiratory diseases. Ingredients such as menthol, thymol, and eucalyptol are commonly used in mouthrinses or dentifrices for their antimicrobial properties. In addition, extracts from Commiphora myrrha, Mentha camomilla, *Echinacea purpurea* and *Sanguinaria canadenis* inhibit periodontal bacteria (Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum) in clinical studies. Herbal extracts (*Vernonia amyglalina, Massularia acuminata*) also demonstrate antibacterial activity *in vitro*. Herbal extracts from *Centella asiatica* and *Punica granatum* formulated in biodegradable chips, when applied subgingivally, reduce periodontal disease significantly. Non-dialyzable constituent of cranberry juice inhibits the adhesion of cariogenic and periodontopathogenic bacteria and disrupts oral biofilm formation and bacterial activity. Herbal extracts with anti-inflammatory activity may also contribute in the management of periodontal inflammation. *M. chamomilla, M. laevigata* and *Plumeria acuminate* all inhibit edema in rat models.

Dental caries are the primary cause of tooth loss and are five times more common than asthma in children in the United States. Extracts from Cranberry, *Azadirachta indica, Mikania glomerata, Mikania laevigata, Myristica fragrans* (nutmeg) and *Camellia sinensis* demonstrate antibacterial activity against major cariogenic bacteria, including *Streptococcus mutans*, *S. salivarius*, *Lactobacillus acidophilus* and L. casei.
Other uses in the dental practice include anxiolytic activity from *Valeriana officinalis*, *Passiflora incarnata* and *Piper methysticum* extracts. Limited side effects and adverse reactions have been reported related to the use of medicinal plants in dentistry. Most importantly, the use of sanguinarine (Viadent®) has been associated with leukoplakia in the maxillary vestibule, a potentially precancerous lesion.

P19. Antimicrobial activity of selected medicinal plants extracted in different ethanol concentrations against human pathogens

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Abstract. Ethanol extraction is widely used to obtain crude plant extracts in the herbal medicine industry for therapeutic applications. Different plants may require different concentration of ethanol to achieve maximum recovery of bioactive components.In this study, three concentrations (50%, 70% and 90%) of ethanol were used for extraction of eight medicinal plants to determine which ethanol concentration would yield the optimal antibacterial activity. The ethanolic extracts of boldo leaf, buchu leaf, *Echinacea angustifolia* root, hops strobile, licorice root, Oregon grape root, usnea lichen and yerba mansa root were screened for antibacterial activity using standard well assay and micro-broth dilution method. The plant extracts showed strong antibacterial action against Gram-positive bacteria; *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and *Streptococcus pyogenes*, while there was negligible to no inhibitory action towards Gram-negative bacteria; *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella enteritidis.* Among the plant extracts, boldo, hops, licorice and yerba mansa exhibited a very strong antibacterial action at all three levels of ethanol concentrations. Hops showed the strongest activity at 90% ethanol. *Echinacea angustifolia* showed no or negligible antibacterial activity, while usnea showed strong activity only at 90% against *S. epidermis.* Except *Echinacea angustifolia* and usnea, the plant extracts were strongly inhibitory towards the MRSA strain. Buchu, yerba mansa and Oregon grape showed higher activity at 50 or 70% against MRSA. Minimum bactericidal concentrations varied from 1/4 to >1/256 dilution levels and were in agreement with well assay results. The results suggest that the extracts of boldo, hops, licorice and yerba mansa could be considered as potentially effective antibacterial agents against Gram-positive bacteria. The activity of hops, buchu, Oregon grape and usnea is dependent on the concentration of ethanol. The ratio of ethanol/water mixture used for extraction of medicinal plants is an important factor to obtain optimum antibacterial activity.

P20. Natural prenylated resveratrol analogs arachidin-1 and arachidin-3: Altered glucuronidation could lead to enhanced bioavailability

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Abstract. *trans*-Resveratrol (tRes) is known to have protective effects against cardiovascular disease, ageing, and cancer. However, the therapeutic promise of tRes is limited by its rapid metabolism, which leads to poor bioavailability making analogs of tRes possessing enhanced bioavailability an important area of study. We hypothesize that two peanut hairy root-derived isoprenylated analogs of tRes – *trans*-arachidin-1 and -3 (tA1 and tA3) – will exhibit slower metabolism/enhanced bioavailability relative to their non-prenylated parent compounds *trans*-piceatannol (tPice) and tRes, respectively. The glucuronidation activities of 9 human recombinant UGTs towards these compounds were evaluated using HPLC and LCMS/MS. A single monoglucuronide was observed for tA3 while two glucuronides each of tPice and tA1 were produced. Importantly, an additional isoprenyl and/or hydroxylgroup in tA1 and tA3 slowed overall glucuronidation and completely blocked 3-O-glucuronide formation in tA3. The greatest activity was observed for extrahepatic UGT1A10 and -1A7, followed by hepatic -1A1 and -1A9, and these isoforms were used for further characterization. UGT1A7 showed Michaelis–Menten kinetics with K_m values ranging from 27–56 μM. UGT1A10 demonstrated atypical kinetics toward all three compounds suggesting the presence of multiple binding sites. Studies on the cytotoxic effects of tA1 and tA3 in MCF7 and Panc1 cells are in progress. Our results indicate, for the first time, that areodigestive tract expressed UGT1A7 and intestinal UGT1A10 may contribute significantly to the first pass metabolism of these stilbenoids, which can potentially be developed into alternatives to resveratrol therapy due to their likely increased bioavailability, thus overcoming a major obstacle to the pharmaceutical use of resveratrol. (NIH-GM075893 to AR-P; NSF-EPSCoR EPS-0701890 to FM-B).

P21. Gambogic acid and its analogues are novel substrates for human UDP-glucuronosyltransferases and bind cannabinoid receptors CB1 and CB2

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Abstract. Gambogic acid (GA) is the principle pigment and main active factor of gamboge, a resin from the *Carcinia hanburyi* plant, which has a long history of medicinal use in Southeast Asia. The anti-tumor activity of this compound has been reported, and considerable potential for a new drug has also been described. However, there is no information currently available on the Phase II metabolism of this compound in humans. Due to the presence of carboxyl and hydroxyl functions in this compound, we theorized that GA and its analogs – dihydro-GA, acetyl-iso-GA, tetrahydro-GA, and garcinolic acid – would be substrates for human UDP-

glucuronosyltransferases (UGTs). This hypothesis will be tested using a set of human recombinant UGT isoforms and hepatic and intestinal microsomes. Preliminary experiments with a panel of human liver and intestinal microsomal preparations showed the involvement of hepatic and intestinal metabolism of each of these compounds. The formation of glucuronides was identified using HPLC and β-glucuronidase hydrolysis. We have also shown that all of these compounds bind to cannabinoid receptors (CBRs) in the low micromolar range, with approximately 2-5 fold selectivity for CB2 relative to CB1Rs. Based on this data, we hypothesize that CB1 and CB2Rs may play an important role in the molecular mechanism of action of GA. Interestingly, the modifications to the GA scaffold found in the selected analogs, appear to significantly decrease the binding affinity to CB1Rs, while producing much less reduction in the binding affinity to CB2Rs. Studies on the cytotoxic effects of these compounds in MCF7 and Panc1 cells are in progress. Based on this data, we hypothesize that GA and its derivatives may serve as novel templates for innovative drug development, leading to identification of highly selective and efficacious therapeutic analogs. (NIH-GM075893 and DoD-X81XWH-11-1-0795 funded by USAMRMC to AR-P).

2. BIOPRODUCTION AND PURIFICATION

P22. Elicitation and secretion of specialized metabolites in *Scutellaria lateriflora* hairy root cultures treated with cyclodextrin and methyl jasmonate

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Abstract. *Scutellaria lateriflora* is a plant in the mint family which produces biologically active compounds exhibiting antioxidant and anti-cancer properties. In order to develop a bioproduction system for these bioactive compounds, we developed hairy root cultures of *S. lateriflora* using *Agrobacterium rhizogenes* and hairy root line SL-4 was selected for further studies due to its growth characteristics in liquid medium. In the current work, we studied the effect of methyl jasmonate in combination with a methylated cyclodextrin on production of specialized metabolites in hairy root cultures line SL-4. Thirty-day-old hairy root cultures were treated with 0.75, 7.5 or 15 mM of cyclodextrin alone or combined with 100 □M of methyl jasmonate (MeJA). As controls ethanol (solvent of MeJA) and MeJA alone were used. After 24 hours of treatment, the roots and culture medium were collected and the metabolites were extracted with ethyl acetate. The extracts were further analyzed by HPLC. Whereas the levels of the known *Scutellaria* flavonoids baicalin, baicalein and wogonin did not vary significantly in the roots or medium upon the above treatments, at least 10 novel compounds were induced and secreted into the culture medium upon treatment with MeJA and cyclodextrin. Our results suggest that this strategy could be used to identify novel bioactive compounds in this important medicinal plant.

P23.Biochemical technologies for plant biomass processing and generating value-added bioproducts in Arkansas

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Abstract. Economic viability of agricultural processing requires efficient processing and effective use of processing residues. Sugar beets are identified as an advanced biofuel crop in the AR Delta to complement sweet sorghum for industrial sucrose production for fermentation. The resulting pulp residue is targeted for biorefining to functional polysaccharides and component monosaccharides. Similarly, the non-cellulosic polysaccharide components in rice bran and hull generated from AR rice processing may be better utilized for improved nutritional and feed products. Research objectives in the ABI Protein Chemistry Laboratory have focused on producing, identifying and characterizing, and evaluating enzyme systems for their utility in biochemical processing of plant residues relevant to agriculture in AR. This presentation will highlight preparation of reagent fungal enzymes active towards polysaccharide cell wall components in beet pulp and rice bran. It will also highlight associated program outcomes including integration of teaching and training activities using the P3 Center MALDI-TOF mass spectrometer and *Pichia* (yeast) protein expression system.

P24.Industrial technology to produce plant-based anticancer molecules

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Abstract. Plant roots are one of the rich sources of anticancer molecules with a wide array of small molecule metabolisms. Industrial-scale production of plant-based high-value anticancer molecules, either from cultivation or wild in all countries with all seasons are challenging due to different weather, dormancy, pesticides, etc. Western pharmaceutical companies are importing different raw medicinal plant materials from different parts of the world for natural or semi-synthetic drug manufacturing which has several limitations *e.g*., quality control, chemical profiles, import and export policies, endangered issues etc. In addition, the molecular mechanisms underlying anticancer molecules biosynthesis are unpredictable in *in vivo* due to different agro-climatic conditions and pests. To overcome these limitations, we established a unique bioreactor technology for high-quality important anticancer molecules production via plant root factory with competitive cost as well as up-regulating metabolic pathway gene expression by a key regulator in elicitor signal. We will present our commercially successful industrial technology for new generations of plant-based health-molecules and recent progresses in potential unique root-based anticancer molecules production.

P25. Bioproduction of omega-3-fatty acid from *Stichococcus bacillaris*strain Siva2011: A bioreactor approach

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Abstract. Omega-3-fatty acid is called an essential polyunsaturated fatty acid and cannot be synthesized by human because we lack the enzymes required to introduce double bonds after C_9. Vegetable oil or fish oil are our primary source of omega-3-fatty acid. Omega-3-fatty acid lowers the risk of cardiovascular problems and plays an important role in normal brain development and function. In addition, it acts as an anti- inflammatory, anti-oxidative etc. Medicinal microalgae are an alternative source of natural omega-3-fatty acid that synthesize and accumulate significant levels within few days. Therefore, we established a quick bioproduction system to produce the omega-3-fatty acid from *Stichococcus bacillaris* strain siva2011. We will present the unique and cost-effective bioreactor production strategies to produce omega-3-fatty acid and boost the accumulation level in *S. bacillaris* strain siva2011 by different elicitors. This bioreactor production approach is a promising high-throughput industrial technology for the development of natural omega-3-fatty acid based drug therapeutics.

P26.*Stichococcus bacillaris* strain Siva2011 made *RRR*-α-tocopherol: Bioprocess and bioassay

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Abstract. The natural *RRR*-α-tocopherol and synthetic *all-rac*-tocopherol are of different chemical existence. In other words, the bioactivity and the relative safety are not same because human proteins such as enzymes and receptors usually exhibit high stereospecificity, normally exit only as a single stereoisomer. Therefore, the natural *RRR*-α-tocopherol is more bioactive than the synthetic form. Our microalgae, *Stichococcus bacillaris* strain siva2011 is capable of producing significant amounts of bioactive vitamin E within a few days (3-6 days) and, more importantly, only biosynthesizes the natural bioactive form of *RRR*-α-tocopherol. Therefore, we scaled-up this strain in a different capacity unique airlift balloon type bioreactor system for pharmaceutical applications. We will present the bioprocess and bioassay data of *S. bacillaris* strain siva2011.

P27. Analysis and fermentation of juice from Arkansas energy beets with a self-flocculating yeast

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Abstract. Energy beets have been identified as a viable alternative crop for advanced biofuel production in the Mid-South Delta region. Compared with corn and starch-based ethanol production, energy beets can produce better than 2X higher ethanol yields per acres with lower nitrogen and water inputs. Raw juice from energy beets contain about 175 g/L sucrose, providing an additional benefit of direct conversion by yeast to ethanol, eliminating costs from saccharifying enzymes. Innovative processing technologies will be need for processing beet roots grown in this region, rather than traditional processing used for food grade crystal sugar. Here we present our initial efforts to develop cost-effective technologies to efficiently covert beet juice to fuel ethanol. For this we are using a novel self-flocculating yeast SPSC01, which is a fusant of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. This stain can ferment at high sucrose gravity and tolerate high ethanol accumulation. We will present progress in evaluating fermentation efficiency in shake flasks prior to trials in a traditional fermenter. We have also developed a fast, highly-sensitive microplate assay based on the bicinchonic acid reagent to quantify reducing sugars (monosaccharides) levels and sucrose (after efficient acid hydrolysis) in juice feedstock and during the course of fermentation. Validation of the assay and its application for sugar analysis will be summarized in the presentation.

P28. Self-flocculation of microalgae in anaerobic digester effluents

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Abstract. Biodiesel and jet fuels produced from green algae is regarded as third-generation biofuels that present one of the most attractive energy sources to resolve the worldwide energy shortage crisis. However, one of the major challenges to commercializing biofuel production from microalgae is the costly biomass harvesting. Various methods have been investigated for microalgae harvesting, including centrifugation, filtration, chemical flocculation and bioflocculation induced by environmental stress or bacteria, but they are all considered as unreliable on a commercial scale. Here, we report a recent finding of self-flocculation of an oil-rich microalgae species grown in diluted Anaerobic Digester Effluent (ADE) solution. Complete cell-medium separation was achieved within 2 min for the self-flocculated algae in contrast to more than 24 hr required for the single-cell algae. The self-flocculated algae could increase biomass by 15-fold within 6 days of culture in diluted ADE solution and accumulate more than 50% of neutral lipids on a dry weight basis. This study might provide a simple and low-cost technology of microalgae harvesting, though the flocculating mechanism remains to be elucidated.

P29.Induction of stilbenoids in hairy root cultures of peanut and muscadine grape treated with methyl jasmonate and hydrogen peroxide and determination of the antioxidant capacity of selected stilbenoids by the ABTS assay

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Abstract. Stilbenoids are polyphenolic compounds with important biological properties. These natural products are synthesized by species from unrelated plant families like the Fabaceae and Vitaceae. In order to study the biochemical and molecular regulation of stilbenoid biosynthesis, hairy roots of muscadine grape (*Vitis rotundifolia*) and peanut (*Arachis hygogaea*) were produced by infecting leaves with *Agrobacterium rhizogenes* strain ATCC 15354. Several hairy root lines were obtained from both species and lines 3A (grape) and Hull-3 (peanut) were selected based on their sustained growth in liquid medium. Kinetics analysis for the grape hairy root line showed an exponential growth between 6 and 24 days in a modified Murashige and Skoog (MSV) medium. To induce the production of stilbenoids, the hairy root cultures were treated with 100 mM methyl jasmonate and 10 mM H_2O_2 separately during a time course that lasted up to 96 h. HPLC analysis of ethyl acetate extracts from the media and tissue of the elicited cultures showed the presence of resveratrol, arachidin-1, and arachidin-3 in peanut while piceid, piceatannol, resveratrol, and ε-viniferin were found in the grape. Additionally, we determined the antioxidant capacity of seven stilbenoids: piceatannol, piceid, resveratrol, □-viniferin, arachidin-1, arachidin-3, and pterostilbene by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay in efforts to understand the antioxidant capacity of the hairy root extracts.

3. METABOLIC ENGINEERING & GENOMICS/TRANSCRIPTOMICS

P30. Metabolic engineering of flavonoid biosynthesis in *Scutellaria lateriflora* hairy roots by ectopic expression of the AtMYB12 transcription factor

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Abstract. American skullcap(*Scutellaria lateriflora*), aperennial herb native to America,is rich in specialized metabolites that have shown various biological activities.In particular, the flavonoids baicalein, baicalin and wogonin have proven anticancer properties *in vitro.* However, the regulatory mechanisms and biosynthetic steps leading to these specialized metabolites have not been elucidated. To address this issue, hairy root cultures of *S. lateriflora* are being established using an engineered *Agrobacterium rhizogenes* strain 15834 containing the flavonoid-specific transcription factor AtMYB12 under the control of either the constitutive 35S promoter or the superP:TEV promoter system which shows preferential expression in roots. Several hairy root lines were developed with the superP:TEV:AtMYB12 construct and are currently investigated to test the effect of the transcription factor on expression of genes involved in flavonoid biosynthesis and accumulation baicalein, baicalin, wogonin or other flavone derivatives.

P31. Heterologous production of plant phenylpropanoid compounds caffeic acid and resveratrol in cyanobacteria *Synechocystis* sp. PCC 6803

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Abstract. Caffeic Acid and Trans-Resveratrol are polyphenol compounds, produced by plant as secondary metabolites under stress conditions. They are known to exert beneficial effects on human health for their anticancer, anti-toxic and anti-inflammatory functions. Both Caffeic Acid and Resveratrol can be synthesized either from phenylalanine or from tyrosine by plants. Cyanobacteria *Synechocystis* is photoautotroph which can be used as a 'photo-bioreactor' for biosynthesis of nutriceuticals after genetic modifications.
C3H (p- coumarate 3- hydroxylase) is the enzyme converting p-coumarate into caffeic acid. *ref8* gene (coding for C3H) was cloned and integrated into the genome of *Synechocystis* sp. PCC 6803. PCR was done to verify the insertion of *ref8* gene. Expression of *ref8* in *Synechocystis* sp. PCC 6803 was confrimed by SDS-PAGE and Western Blot. Upon feeding of p-coumarate to the culture, production of caffeic acid was detacted by HPLC and LC/MS.
Three enzymes are involved in the pathway that converts tyrosine to resveratrol: TAL (Tyrosine ammonia-lyase), 4CL (Coumaroyl-CoA ligase), and STS (Stilbene synthase). Codons of these genes (*TAL* from *Saccharothrix espanaensis*, *4CL* from *Nicotiana tabacum* and *STS* from *Vitis vinifera*) were optimized to enhance their expression in *Synechocystis* sp. PCC 6803. Modified TAL, 4CL and STS genes were assembled together into an expression plasmid pACYCDuet-1, which was transformed into *E. coli* BL21 DE3 strain. Resveratrol was detected by HPLC and verified by LC/MS from culture medium of *E.coli* growing in 2xYT medium without adding any substrates. Formation of p-coumaric acid, the immediate precursor for resveratrol biosynthesis, was also found, and its amount decreased as biosnthesis of resveratrol proceeds. Construction of *Synechocystis* sp. PCC 6803 mutant expressing these three genes is in progress.

4. TRADITIONAL MEDICINE / ETHNOBOTANY

P32. Recent advances in formal botanical education at Arkansas State University: An eye to the future

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Abstract. The Bachelor of Science in Biology: Botany Emphasis degree program at Arkansas State University (ASU) recently has undergone significant improvement to better prepare students for graduate school and professional careers as botanists. For example, the curriculum was restructured to include mandatory courses of Evolution, Wetland Plant Ecology, and Plant Systematics in addition to the previously required Plant Physiology and Microbiology. In addition to these five Botany "core" courses, students must now choose one each of a pair of Entomology courses and three Mycology courses. These changes to the curriculum emphasize biological diversity and the interrelatedness of plants and the environment. A new section of Honors Biology of Plants (Spring 2012) brings together the most prepared and committed new students in Biological Sciences, and as part of their coursework these students submit contributing content to the Encyclopedia of Arkansas History and Culture. In the Biology of

Plants Laboratory, students now study the tenets of the scientific method and are able to explore their creativity in hypothesis development and implementation. Recently, the Plant Systematics course has implemented a writing development component that applies knowledge of plant families to medicinal or wildlife uses. A new upper-level elective course in Dendrology was recently developed as well. We are increasingly offering undergraduate students independent research opportunities within our department and other departments across the ASU campus, including the Arkansas Biosciences Institute (ABI). Increased student interest in medicinal uses of plants and the pharmacological and nutraceutical research at the ABI has opened the possibility for an emphasis or major in Ethnobotany. Plant sciences at ASU have a long and important history, and we are working to ensure that this legacy continues long into the future.

P33.Ragwort: A medicinal weed??

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Abstract. Ragwort, (*Senecio jacobaea*), has long been identified as a threat to Irish horse breeders and farmers due to the toxic effects suffered by livestock when ingested. Legislation (Noxious Weeds (Thistle, Ragwort and Dock) Order, 1937) is in place enforcing the removal of all traces of ragwort from fields where livestock are grazing. Severe penalties can be handed down to any farmers and/or breeders who allow ragwort to flourish.
However from a medicinal point of view; folk remedies suggest the use of ragwort extract as an external treatment for ulcers and wounds as well as a rinse for throat infections indicating an antimicrobial aspect to some compound(s) found in the plant (MacCoitir, 2008). The question is, are the medicinal properties of this plant due to a single one compound or family of compounds?
New research conducted at the Limerick Institute of Technology has shown that ragwort has the potential to become an economically viable commodity. Wild ragwort has been found to contain large quantities of antioxidants, specifically polyphenols, which provide a wide variety of medicinal properties.
Studies using theOxygen Radical Absorbance Capacity (ORAC) assay, and a series of different extraction solvents, have shown the ragwort contains between 18 and 85μM Trolox equivalents per gram (μM TE/g) of fresh plant material. Upon further analysis of these samples, it was found that these samples contained large quantities of polyphenols in the ranges of 280-2900 Gallic acid equivalents per gram of fresh plant material. These figures suggest that ragwort contains similar antioxidant activities to that of common vegetables e.g. peas (19 μM TE/g), carrot (60μM TE/g), white cabbage (61μM TE/g), tomato (67 μM TE/g), snap bean (79 μM TE/g) and white onion (85 μM TE/g) (Ou et al., 2002).

5. BIOACTIVES AND ANIMAL HEALTH

P34. Plant-based bioproduction of an immune-modulating cytokine for improving fish health in aquaculture

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Abstract. Aquaculture has rapidly emerged a key production platform in meeting global demands for high quality protein. Such market pressures have driven production practices towards maximal pond productivity which often result in increased fish disease outbreaks. Current restrictions on antibiotic usage in aquaculture, limited commercially-available vaccines for many fish diseases and the potential catastrophic use of low efficacy vaccines that compromises pond health and profitability, necessitates new and creative solutions in tackling this important agricultural problem. A recent study has shown strong positive correlation between viral protection and expression of an immune system cytokine, interleukin-22 (IL-22) in fish gills suggesting a similar role to the mammalian IL-22 homolog that enhances innate immunity and promotes antimicrobial activities at mucosal surfaces. Therefore, with a goal of utilizing plants as a scalable, low cost production platform for promoting fish mucosal immunity and health, we expressed a number of his-tagged trout IL-22 constructs using a transient agroinfiltration plant system. Constructs were compared for maximizing fish IL-22 protein stability and accumulation *in planta* while maintaining functional activity of the recombinant product. Data developing the use of a trout gill cell line (RTgill W1) for *in vitro* bioassessment of plant-expressed fish IL-22 will be discussed. Outcomes from this study will contribute to new approaches for improved fish health and enhanced current vaccine performance in providing better control of fish diseases in the aquaculture setting.

6. CHARACTERIZATION AND STRUCTURE-ACTIVITY OPTIMIZATION

7. CROP IMPROVEMENT, MICROPROPAGATION & GERMPLASM CONSERVATION

P36.*In vitro* growth of turmeric in responses to five mineral elements and their interactions in a fed-batch culture system over 22 weeks

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Abstract. Using D-optimal criteria and response surface methods, the effects of buds/vessel, P, Ca, Mg, and KNO_3 for biomass, propagation, and microrhizome development were studied with long-term (22-week) liquid cultures of turmeric. A fed-batch culture was used to maintain set point by sucrose measured below 1%, and sucrose solution was added to return volume to 200 ml and sucrose to 5% in non-fertilized group. In the fertilizer group, the concentration of nutrients in spent medium was used to determine the amount of additional 2x treatment media, to be added with sucrose solution to restore set points. The growth and shoot multiplication were greatest in fertilized treatments and rhizome growth greatest in some non-fertilized treatments. The different media formulations and process have been identified. Maximized rhizome dry mass, 25.7 g/vessels, was from non-fertilizer process with a 13 buds/vessel, 6.25 mM P, 9 mM Ca, 4.5 mM Mg, 40 K mM, and 40 N mM. The optimized leaf mass, propagation

rate, and total plant mass are with fertilizer process; plant multiplication media would maximize the plant ratio to 10.87 by using 6 buds/vessel, 4.86 mM P, 5.90 mM Ca, 1.5 mM Mg, 38.715 mM K, and 38.715 mM N. The medium to optimize leaf mass to 207.75 g/vessel would be 18 buds/vessel, 6.25 mM P, 3 mM Ca, 4.5 mM Mg, 54.015 mM K, and 54.015 mM N. Maximized plant mass to 379.8 g/vessel by using 18 buds/vessel, 5.79 mM P, 3.96 mM Ca, 4.5 mM Mg, 52.765 mM K, and 52.765 mM N. Rhizome mass was significantly affected by the interaction of P with KNO_3 under non-fertilizer process, while minerals have no significant effects on rhizome mass in fertilizer process.

P37. *In vitro*germplasm maintenance growth, yield and quality of Assiut-1,broccoli (*Brassica oleracea var. italica*) cultivar

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Abstract. Broccoli is highly nutritious, and has been deemed as a vegetable with potential anti-cancer activity due to high levels of glucoraphanin, which can hydrolyses to form sulphoraphane, an isothiocyanate. Assiut-1 is a synthetic cultivar that was produced at the Department of Horticulture, Assiut University by Damarany and Aboul-Nasr (2000). The original parents of this genotype were namely Parma, Atlantic, Walthon-29 and Toro. A mass selection was conducted for twelve years to get a late flowering broccoli genotype under Assiut conditions. Several research papers were done about this cultivar concerning its yield, head quality, chemical components, storage quality, seed oil contents and quality. Assiut -1 gave the heaviest plant fresh weight, number of leaves per plant, head height, high yield, and good quality oil and high quality chemical components as compared to other cultivars or hybrids. Cultivar Assiut-1 gave the highest value of sulphur percentage, while it gave the lowest value of vitamin C percentage. Recently we are doing in vitro study to produce haploid plants through anther culture. Pre- results about germplasm maintenance will be discussed.

P38. Thin cell layer (TCL) culture and genetic transformation studies on *Bacopa monnieri*

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Abstract. *Bacopa monnieri* is traditionally used as a brain tonic to enhance memory development, learning and concentration. It is also used to treat asthma, spleen enlargement, rheumatism, leprosy, eczema and ring worm, and as a diuretic, and cardiotonic. It has been reported that *Bacopa* extracts may have anticancer properties, possibly due to its inhibition of DNA replication in cancer cell lines in vitro. Bacosides, the bioactive phytochemical in *Bacopa*, aid in the repair of damaged neurons by enhancing kinase activity, neuronal synthesis, restoration of synaptic activity, and ultimately nerve impulse transmission.Explants derived from leaf and internode tissues of the in vitro cultured plants were cultured on the MS and Gamborg's B5 medium supplemented with various concentrations and combination of cytokinins and auxins to study regeneration and somatic embryogenesis. As an explant, thin cell layers derived from leaf and internodal segments (0.3-0.5 mm thick) were used. A comparative study was done to study callus and morphogenic responses in TCLs and traditional explants.

Agrobacterium mediated genetic transformation protocols using explants mentioned above was also developed. *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35SGR containing the neomycin phosphotransferase (nptII) and β-glucuronidase (GUS) fusion gene, and an enhanced green fluorescent protein gene (reporter) were used to optimize transformation process. Kanamycin at 30 mg L^{-1} was used for selecting transformed shoots.

P39. Micropropagation and genetic transformation of *Scutellaria Ocmulgee*

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Abstract. In vitro results clearly indicate that *S. ocmulgee* extracts contain significant antiproliferative activity. Ocmulgee skullcap is a medicinal plant with anti-tumor property and requires immediate conservation efforts due to its threatened status. Further, it is confined to a few counties in the state of Georgia only. We present successful use of leaf explants for rapid induction of shoot buds, elongation and generation of complete hardened plants. Murashige and Skoog (MS) medium supplemented with 0.5 or 2.5μM benzyl amino purine (BAP) with 0.5μM α-naphthalene acetic acid (NAA) induced highest number of shoot buds in comparison to other treatments. Kinetin (0.5, 2.5, 5.0, and 10.0μM) and thidiazuron (TDZ) (0.5, 2.5, 5.0, and 10.0μM) with 0.5μM NAA exhibited similar patterns but less number of shoot buds were produced in comparison to BAP. Elongated microshoots rooted easily in MS basal or MS media containing 5.0μM indole butyric acid (IBA). Using this method, 10-30 rooted plants were generated from a single leaf explant in 14-18 weeks. Regenerated plants were hardened in a mist chamber in the greenhouse and then potted in containers. An *Agrobacterium*-mediated genetic transformation protocol using leaf explantswas also developed. *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35SGR containing the neomycin phosphotransferase (nptII) and β-glucuronidase (GUS) fusion gene, and an enhanced green fluorescent protein gene (reporter) were used to optimize transformation process. Kanamycin at 40 mg L^{-1} was used for selecting transformed shoots.

P40.The effect of pyroligneous liquid treatment on leaf tissue and growth of ginseng

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Abstract. To explore the possibility of using pyroligneous liquid for environmentally friendly ginseng farming, this study observed samples of ginseng whose shoots were treated with pyroligneous liquid sprays beginning in mid June, which is after foliation stage, for two years. The results show that the spotting disease incidence rate dropped most in the samples treated with pyroligneous liquid at 500x dilution, and that the spongy tissue structure got thickened from triple layers to quadruple layers with the pyroligneous liquid treatment regardless of the concentration. The upper and lower epidermis of the leaves as well as the leaf mesopyll cells also became thicker. Compared to the no-treatment group, the overall growth and development of ginseng shoots and roots treated with pyroligneous liquid were excellent. Accordingly, it is

believed that pyroligneous liquid can be an environmentally friendly alternative to conventional agro-chemicals applied to ginseng that can be used to facilitate the growth and development of ginseng and strengthen ginseng's resistance against diseases.

P41. Bioprospecting for podophyllotoxin in the Big Horn Mountains, Wyoming

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Abstract. Podophyllotoxin is an anticancer compound and the precursor of the semi-synthetic anticancer drugs etoposide, teniposide and ethopophos. Currently, podophyllotoxin is commercially obtained from Himalayan mayapple (*Podophyllum emodi* Wall.). The American mayapple (*P. peltatum* L.), was suggested as an alternative source for podophyllotoxin. However, despite numerous studies in the US and in other countries, American mayapple was never domesticated in the US or elsewhere. Juniperus species, such as *J. virginiana*, are another possible source for podophyllotoxin. However, not much is known about the concentration of podophyllotoxin in other Juniperus species, especially in the Western US. The objective of this study was to evaluate variations in podophyllotoxin concentrations in Juniperus species found in the Big Horn Mountains in Wyoming. Big Horn Mountains is a sister range of the Rocky Mountains, and includes the Big Horn National Forests, 189,000 acres of wilderness. We found that Juniperus species in the Big Horn Mountains included three species; *J. communis*, *J. horizontalis*, and *J. scopulorum*. Of these species, noneof the 36 accessions of *J. communis* contained any podophyllotoxin. All accessions of *J. scopulorum* but one contained podophyllotoxin. Podophyllotoxin concentration in *J. horizontalis* and *J. scopulorum* did not correlate to the elevation of the selection sites. The concentration of podophyllotoxin in *J. scopulorum* ranged from 0 to 0.4%, while the concentration of podophyllotoxin in in *J. horizontalis* ranged from 0.27 to 0.7%. Overall, *J. horizontalis* accessions in the Big Horn Mountains showed higher concentrations of podophyllotoxin than the *J. scopulorum*. The range of concentrations in podophyllotoxin in *J. horizontalis* and *J. scopulorum* in the Big Horn Mountains was within the range or higher than the ones reported previously for *J. virginiana*. This study demonstrated that Juniperus species in the Big Horn Mountains have a potential to be used as a source for podophyllotoxin.

P42*. In Vitro* propagation of *Aloysia gratissima*, a South American medicinal plant

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Medicinal and aromatic plants constitute an important group of plants with economic value because of their therapeutic properties. In the past years, there has been increased in the

demand of native medicinal plants. In Argentina, very few native plants are currently cultivated and most of them are directly collected from wild populations. *Aloysia gratissima* (Verbenaceae) is an aromatic shrub that occurs in Argentina and it is used in popular medicine as digestive, to alleviate stomach problems and to treat minor wounds. The objective of this work is to develop a micropropagation protocol for the rapid propagation of this species, as means of collecting germplasm and introducing this species into cultivation.
Direct organogenesis without callus formation was induced by culturing node segments on Murashige and Skoog (MS) medium supplemented with 3% sucrose with different combinations of Benzyladenine (BA), Kinetin and Zeatin. Cultures were incubated for four weeks under continuous cool white fluorescent light at 25 °C. A high number of healthy shoots was induced in MS media supplemented with 4.4 μM BA. For rooting, shoots longer than 1.5 cm were transferred to MS media containing naphthaleneacetic acid indolebutyric acid or indoleacetic acid (IAA). Rooting of regenerated shoots was achieved on MS media supplemented with 0.57 μM IAA. Rooted plantlets were successfully acclimatized to soil.

P43.Response of basil (*Ocimum tenuflorum*) to nutrient stress

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Abstract. Holy basil [*Ocimum tenuflorum*], belonging to the Lamiaceae (Mint) family is used as a medicinal herb in African, Asian and South American cultures. Medicinal properties of holy basil are attributable to a wide range of secondary metabolites present mainly in its leaves, and to some extent in other plant parts. Secondary metabolites have been reported to accumulate due to stress caused by drought, low or high temperatures, lack of nutrients, etc. The species, variety, and type of stress, its intensity and duration influence(s) the amount and type of secondary metabolites produced. A field experiment was conducted to study the effects of nutrient stress on two variants of holy basil, one a green-leaved “Sri tulsi” and the other a purple-leaved “Krishna tulsi”. Five greenhouse grown six-week old seedlings of each variant were transplanted on to raised seed beds covered with plastic with drip tape underneath. Plots were arranged in a randomized block design with three replications. Half the plots of each variant received Peters soluble fertilizer 20:20:20 @ 290 ml at 10-day intervals while the other half did not receive any fertilizer. A week before predicted freeze, two plants from each plot were harvested, fresh weights recorded and separated into leaves, stems, and inflorescences. There was a difference between the two variants in their response to treatments. The Sri tulsi plants produced more branches and had greater stem and leaf weight and thus a 50% more biomass without fertilizer compared to the plants that received fertilizer,whereas the Krishna tulsi plants produced 12% more biomass with fertilizer than without. The changes in the leaf chemical profile and accumulation of heavy metals in plant parts with and without fertilizer will be discussed.

8. VOLATILE OILS

P46. Linking education, research and development: Volatile and non-volatile components of *Piper guineense* from Liberia

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Abstract: Herbs, spices and medicinal plants are important sources of income for local producers around the world. *Piper guineense* is a vine that grows in the Guinea forest of West Africa. In Liberia, *P. guineense* is known as West African Black Pepper and is sold in local markets and is used as a flavoring agent in foods. The volatile and non-volatile components of spices are responsible for the aroma and taste of these products. The objectives of this work are: a) to determine the chemical composition of essential oils and pungent principles of *P. guineense* collected in Liberia, and b) to develop trade standards for the commercialization. This work was used as a vehicle to involve undergraduate students in research and development activities. Seven samples of *P. guineense* were obtained from local markets in Liberia during the years 2009 to 2011. The essential oils were extracted by hydrodistillation and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). The pungent principle piperine was extracted with ethanol and analyzed by High Pressure Liquid Chromatography (HPLC). The essential oil content varied from 0.5% to 1.3%, the oils were dominated by high levels of linalool. The oil content in the commercial black pepper (*P. nigrum*) was 0.9% and contained high levels of □-pinene and (E) caryophyllene. The piperine content showed high levels of variation ranging from 0.4 to 3%, with an average value of 1.8% (3.3% in *P. nigrum*). These results suggest that *P. guineense* samples from Liberia showed a unique profile of essential oils though with lower levels of the pungent principle piperine.

9. NOVEL TOOLS IN PLANT BIOTECHNOLOGY

P47.Meta-analysis of wheat qtl associated with heat and drought tolerance

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Abstract. Heat and drought are the two most important environmental constraints to wheat production globally, are often present simultaneously and will become more severe with global climate change. This presents a unique challenge to wheat scientists who must work to develop wheat cultivars that are productive and adapted to future environmental conditions. Understanding and improving complex traits requires an approach that integrates traditional breeding with advanced genetic and physiological techniques. A number of recent studies have reported QTL associated with heat and drought tolerance, as well as QTL for stress adaptive traits such as the availability of stem carbohydrates or crop canopy temperature. While biparental mapping provides insight into the genetic control of a trait, the importance of the detected regions in additional genetic backgrounds is often unknown. The goal of this study was to conduct a QTL meta-analysis of wheat genome regions associated with heat and

drought stress tolerance. This was done in three steps; 1) Develop a database containing QTL profiles of 26 recent studies targeted specifically at heat and drought tolerance and/or adaptive physiological traits, 2) Project QTL locations and confidence intervals onto the consensus genetic map developed by Somers et al. (2004) and, 3) Identify conserved QTL regions and molecular markers associated with heat and drought tolerance. In total, 852 QTL were characterized for 84 different traits. Co-localization of agronomic and physiological adaptive traits, overlap between heat and drought tolerance QTL and the potential of marker assisted selection for abiotic stress tolerance will be discussed.

P48.Responses to copper excess in *Synechocystis* PCC 6803

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Abstract. The pollution of the natural environment by heavy metals has become a serious problem, not only in industrialized countries, but also in developing countries in Southeast Asia. Many studies have been completed at different scales confirming the high probability of cyanobacterial species for removing nutrients that support the growth of cyanobacteria.
In this study, Synechocystis cells were cultured in BG-11 liquid medium supplemented with various concentrations of CuCl2 (0.1, 0.2, 0.3, and 0.5 mg/l) for six days in normal light (50 μ mol photo m^{-2} s^{-2}) and high light (400 μ mole photon m^{-2} s^{-2}). The growth and pigment composition of the cells were analyzed. A light and time dependent increase in toxicity of Cu^{2+} to cyanobacterial cells was observed upon exposure to Cu^{2+} at concentrations higher than 0.3 mg/l. The effects of Cu^{2+} on cell growth differ in normal light and high light. In normal light Cu^{2+} appears to be a nutrient and stimulates the cell growth of cyanobacteria at concentrations lower than 0.3 mg/l, while Cu^{2+} toxicity was observed upon exposure of Synechocystis to Cu^{2+} at 0.5 mg/l. In high light Cu^{2+} appears to be toxic to Synechocystis at concentrations higher than 0.3 mg /l. The chlorophyll and carotenoid contents of the cells at normal light were reduced upon exposure to Cu^{2+} at concentrations higher than 0.3 mg/l. The chlorophyll and carotenoid contents were affected more severely in high light than in low light upon exposure of cyanobacterial cells to copper at concentrations higher than 0.3 mg/l. Clearly, high light aggravates Cu^{2+} toxicity and vice versa. The sensitivity of photosystem I and II to copper treatments appears to be different, and the PS II to PS I ratio appear to be reduced by Cu^{2+} (0.3 mg/l) treatment for 24 hours. Future the study will focus on dissecting Cu^{2+} toxicity to cells and using cyanobateria to remove heavy metals from sewage water and as sensor to detect the pollution by heavy metals.

P49. Promoter study of the HRGP gene from B73 corn

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Abstract. In maize (*Zea mays* L.), the primary cell wall contains several structural proteins, the most abundant being hydroxyproline-rich glycoprotein (HRGP), or extensin. Its main function is

to give strength and flexibility to the plant cell wall, but it also takes part in secondary defense mechanisms against abiotic and biotic stresses. This protein is highly expressed in reproductive tissues such as silk and pericarp. DNA hybridization experiments using a conserved region of the extensin gene showed that the B73 genome contains one copy of this gene. Bioinformatics analysis of the maize genome from the B73 inbred line also showed one copy of the gene on chromosome number 2. Previous studies showed that transgenic maize expressing the GUS reporter gene (beta-glucuronidase) fused with a region of the promoter of an extensin gene showed positive GUS staining in maize epidermal cells and pollen. Our hypothesis is that the entire extensin promoter region in B73 corn (2Kb) contains three repetitive regions which can be responsible for the expression of this gene in different parts of the plant. A BAC (bacterial artificial chromosome) containing the genome region of the extensin gene and promoter was purchased from BACPAC Resources Center. Promoter specific primers were designed and the promoter was amplified by PCR and cloned into a construct driving the expression of the GUS gene. Corn plants are being genetically transformed with EHA 101 carrying this construct and plants analyzed for GUS staining. Results of these studies will help determine the function of this extended promoter in expressing this gene in multiple tissues at various times of growth and development.

P50.Optimization of virus–induced gene silencing (VIGS) conditions for functional analysis of genes involved in biotic stress signaling in tomato plants

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Abstract. Medicinal plants have always been considered a source of wide spectrum pharmacological products. Productivity of medicinal plants can be greatly reduced in response to different environmental factors including biotic stress. Our ultimate goal is to increase stress tolerance of medicinal plants using approaches of functional genomics. Virus induced gene silencing (VIGS) is a powerful tool for transient gene silencing in plant functional genomics studies. In this work we are using VIGS to regulate/disrupt the *in planta* response to a biotic stress by selectively silencing several genes that could be involved in sensitivity to a crude phytotoxin from culture filtrate of *Rhizoctonia solani*. Candidate genes involved in this process have been identified from previous studies on resistance to rice sheath blight disease caused by virulent *R. solani* isolates. We searched and identified in the tomato genome homologues of some of these genes that will be targeted for silencing. Through infiltration tests we have identified tomato cultivars that are sensitive to the *R. solani* phytotoxin and exhibit necrotic symptoms that are similar to those observed in sheath blight infected rice plants. Additionally, we have optimized conditions of VIGS methodology for the selected phytotoxin-sensitive tomato cultivars and determined suitable concentrations of *R. solani* culture filtrate to perform a screening procedure. Using tomato plants as a model system and VIGS methodology as a tool we are starting to elucidate the functions of several selected tomato genes involved in toxin recognition and necrosis inducing response.

P51. Genetic reduction of $InsP_3$ as new strategy for activation of secondary metabolism in plants

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Abstract. Almost one-quarter of all pharmaceuticals contain plant derived components. Understanding the regulatory mechanisms for biosynthetic pathways of important plant compounds is a major focus in planning genetic engineering strategies to increase production of valuable secondary metabolites by plants. The phosphoinositol pathway is one of the major eukaryotic signaling pathways. The metabolite of the phosphoinositol pathway, inositol-(1, 4, 5) trisphosphate ($InsP_3$), is a regulator of plant responses to a wide variety of stresses. Recently it was shown that reduction of $InsP_3$ in tomato plants expressing human inositol phosphate 5-phosphatase resulted in increased fruit lycopene contents, biomass and drought tolerance (Khodakovskaya *et al.*, 2008). All these properties of transgenic tomato lines have economic importance. However, the molecular mechanism by which $InsP_3$ affects production of secondary metabolites was not clarified. We hypothesized that activation of biosynthesis of lycopene observed in transgenic lines are based on the role of $InsP_3$ in mediating light-regulated processes in plants. To understand the molecular links between the activation of different branches of plant metabolism and $InsP_3$ reduction in tomato fruits, the expression of transcription factors known to be involved in light signaling was analyzed by real-time RT-PCR. The expression of *LeHY5*; a positive regulator of fruit pigmentation, and also *SIMYB12*, and *LeELIP* was found to be higher in fruits expressing InsP 5-ptase with reduced level of $InsP_3$. Analysis of secondary metabolites in mature tomato fruits from different lines (WT, Empty vector EV, and transgenic lines L6, L7) indicated significant increase of two major flavonoids (chlorogenic acid and rutin) and ascorbic acid in transgenic fruits expressing human inositol phosphate 5-phosphatase. The enhanced accumulation of these metabolites in transgenic tomato plants was in direct correspondence with the observed up-regulation of the genes that express the key enzymes of phenylpropanoid metabolism (*CHS1; HCT*) and biosynthetic pathway of ascorbic acid (*MIOX, GLDH*). These results proved that genetic reduction of $InsP_3$ have a biotechnological potential for activation of secondary metabolism.

P52. Early stages of cotton leaf expansion: a biological set point?

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Abstract. We have observed in field-grown cotton that early in the growing season (when the majority of the canopy consists of juvenile leaves), young leaves are warmer (under high irradiance) and more thermotolerant when compared with fully expanded, mature leaves. This conclusion is based on observed differences in leaf stage photosynthesis as measured by chlorophyll *a* fluorescence under varying regimes of temperature and light intensity. We are currently working on modeling the impact of differing thermal sensitivity of leaf stages on overall canopy photosynthesis using STELLA software. We are also investigating the hypothesis that

the differences in thermotolerance are because young leaves alter the unsaturation level of membrane fatty acids (18:3 fatty acids decrease and 16:0 and 18:2 increase as temperatures rise) through the differential expression of membrane-bound fatty acid desaturase (FAD) genes. We suggest that events occurring during early leaf expansion have a major impact on the survival and subsequent productivity of crop and other plants, particularly when grown under stressful conditions.

P53. Strategies for eliciting cell-mediated immunity in plant-made vaccines

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Abstract. Inducing strong cell-mediated immunity (CMI) remains challenging for vaccines that are delivered transmucosally (e.g., orally) or those comprised of protein subunits rather that whole organisms or virus-like particles. For example, CMI responses are considered critical for subunit vaccine strategies targeting 'universal" flu vaccines in response to emerging H1N1 and H5N1 influenza strains. We are targeting two strategies for enhancing CMI and Th-1 type responses for plant-made vaccines. First, we have used plant to produce the complex heterodimeric glycoprotein cytokine, interleukin-12 (IL-12), a key immunomodulator that directs CMI responses. We have produced mouse, human, chicken, and porcine IL-12s in plants and demonstrated bioactivity in both cell and animal systems. Our second strategy exploits lectin-mediated cell trafficking to direct antigen delivery and selective presentation either to the major histocompatibility complex I (MHC-I; enhancing CMI) or to the MHC II (enhancing antibody-mediated immunity). Both strategies show significant promise for enhancing overall immunogenicity of vaccines and may be enabling for various plant-based vaccine strategies, especially those that target agricultural or veterinary diseases.

P54. Optimizing recombinant protein yield in an A*grobacterium*-mediated transient expression system

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Abstract. Plant-based transient expression systems facilitate the production of large amount of recombinant protein in very short time compared to stable transgenic approaches. These systems typically involve vacuum infiltration of *Agrobacterium tumefaciens* containing the "gene of interest" into leaves of intact *Nicotiana benthamiana* plants. Leaves are then harvested for protein or chemical product recovery after 2-4 days of additional incubation. In this study, we evaluated different factors that may influence the expression/yields of different proteins in *N. benthamiana*, selecting variables designed to impact DNA template levels; transcription and transcript stability; protein synthesis, folding and trafficking; and product stability. Both plant and

animal recombinant proteins were tested ranging in size from 30 to 70 kD. Factors that were evaluated included viral-enhanced vectors, co-expression with the P19 suppressor of gene silencing, sequence optimization for codon use and transcript stability, and use of plant versus native signal peptides. Protocols altering the conditions of *Agro*-infiltration, addressing *Agrobacterium* growth and induction conditions, infiltration media, and bacterial concentration, were evaluated for product yields, quality, and kinetics of expression. Selective amendments to the infiltration solution with the potential to reduce oxidative stress (e.g., vitamin C; vitamin E) or proteinase activity (e.g., BSA) were tested for their potential to stabilize the protein product and increase yield. Factors or conditions were identified that increased yields of specific recombinant proteins by 4 to > 10 fold. However, results indicate that different factors affect expression of particular proteins differently leading to the development of a strategic matrix for optimizing yields of a new candidate protein.

P55. Differential expression of glyoxalase I-like genes and their potential role in salt tolerance in *Medicago truncatula*

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Abstract. Transcriptome profiling has been a valuable tool in identifying differentially expressed genes in closely related individuals. In this study, Affymetrix gene chips were used to profile the transcriptomes of *Medicago truncatula* calcium oxalate-deficient (*cod*) mutants. Transcripts annotated as encoding two members of the glyoxalase I (GLXI) family of enzymes were co-regulated, and they are expressed in inverse patterns in the *cod5* and *cod6* mutant lines. Genes previously annotated as *glxI*, referred to by tentative consensus numbers TC122307 and TC123769, were both up-regulated in the *cod6* mutant and down-regulated in *cod5* as compared to the wildtype. However, the predicted proteins lack a critical Zn-binding motif found in GLXI, and sequence alignments indicate that another *M. truncatula* gene likely encodes the authentic GLXI. The glyoxalase pathway is a ubiquitous system involving GLXI and glyoxalase II (GLXII), which in the presence of reduced glutathione, catalyze the detoxification of methylglyoxal and other harmful by-products of metabolism. Previous studies showed that overexpression of GLXI and GLXII in transgenic plants improved tolerance to salt and methylglyoxal. Salt tolerance was therefore evaluated in the *cod* mutants, and *cod6* plants were significantly more tolerant of sodium chloride than the wildtype plants. Likewise, *cod5*, which does not accumulate the GLXI-like transcripts, was more sensitive to salt treatment. Transcript levels of TC122307 and TC123769 did not change in leaves following sodium chloride treatment. Moreover, the three genotypes were equally sensitive to toxic levels of methylglyoxal, the substrate of GLXI. Although it seems that TC122307 and TC123769 do not encode a functional GLXI, they potentially contribute to salt tolerance in *cod*6 and encode members of the vicinal oxygen chelate enzyme superfamily. Therefore, these genes are excellent candidates for further study as tools to enhance salt tolerance in crop plants.

P56. Identification of interacting partners for drought resistance gene HYR in rice

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Abstract. Drought is one of the most serious challenges to production worldwide for the major cereals such as rice (*Oryza sativa*), affecting half the world rice production. Many approaches have been proposed to boost intrinsic yield, such as modification in plant architecture, enhancement of growth rate, increase in photosynthetic rate and capacity. Members of the plant superfamily of AP2/ERF transcription factors have been shown to act either in developmental programs, such as flower development, or as mediators in the plant responses to various environmental stresses. The rice HYR gene was identified as a drought responsive gene in vegetative and reproductive tissues. Overexpression of HYR in rice confers an increase in biomass, water use efficiency, root growth, photosynthesis, sugars, drought resistance and yield under normal and drought conditions. Analysis of regulated pathways shows that the HYR transcription factor is involved in the regulation and maintenance of photosynthetic carbon metabolism under drought, providing us a system to identify all genes regulated in photosynthetic carbon metabolism involved in the grain yield components. In order to identify the interacting pathways and proteins in HYR function, we are generating rice plants containing TAP-tagged HYR proteins (TAP: Tandem Affinity Purification) to identify the interacting/bound DNA by ChIP-Seq (chromatin immunoprecipitation followed by next generation sequencing of bound DNA) and interacting proteins by MASS spectrometry. These approaches will provide us direct interacting partners for HYR function, among the genes regulated by HYR expression in rice, and thus the cascade of gene regulatory networks involved in yield and drought resistance in rice.

P57. Drought stress response of rice T-DNA/Tos17 insertional lines

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Abstract. Drought, together with other environmental stresses like salinity, high or low temperature, can negatively impact plant growth and productivity. The effect of water scarcity is more on crops like rice which consumes 30% of all the fresh water used in agriculture. In our previous study towards understanding the drought stress responses in rice, we have analysed the drought transcriptome of rice at seedling, vegetative and reproductive stages. Processes and regulatory elements involved in common and stage specific responses were identified. Although this analysis will give insight into drought-regulated gene expression in rice, it is important to experimentally prove the involvement of a gene in drought stress response through functional characterization. With the availability of complete genome sequence in rice, several national programs have generated mutant resources towards discovering the function of all rice genes and the site of insertion has been identified. Analysis of the knock-out mutant is an effective way to establish the gene function as it provides direct biological function of the gene under the study condition. In this direction, we have identified T-DNA or *Tos17* retrotransposon insertion lines for 165 regulatory genes in rice which are up-regulated or down-regulated in either one of the vegetative stage and also at reproduction stage. Water-deficit stress will be imposed to homozygous mutant lines following gravimetric approach at high light intensity

mimicking the field situation and the primary response will be studied physiologically by measuring photosynthetic rates and fluorescence parameters.

P58. EBook reader: a software tool for knowledge discovery from bioscience publications

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Abstract. With the development of computer technology and Internet, the electric publication can do more than only emulate the printed publication in cheaper and more transportable forms like web pages and electric files. Electronic publications can be for any discipline such as Biology, and also have the potential to enhance the reading process itself through the identification of new ways to retrieve, index, and search information throughout the entire article. This could entail for example: the rereading, searching, updating, content categorization of different interests, and knowledge discovery in the related text such as characteristics of different botanical plants. Moreover, with the development of Internet, people want to keep information up-to-date all the time, write down notes and make comments easily. It is our intention that these improvements in eBook reader will make the reading and learning process more convenient, more efficient and more personalized for any discipline such as Biosciences. This paper discussed the difference between electric and printed reading materials, the technology which can be used to implement those functions, and experimental results. For this research work, there are many details that we can continue to work on in the future.

P59. Plant hairy roots as a unique platform to study plant hydroxyproline-*O*-glycosylation process

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Abstract. Hydroxyproline-*O*-glycosylation involves post-translational hydroxylation of proline to hydroxyproline (Hyp) and subsequent glycosylation, a modification that is unique to plants and green algae. Our earlier work with synthetic genes expressed in tobacco BY-2 cells showed that peptide sequence directs Hyp-*O*-glycosylation of Hyp-rich glycoproteins (HRGPs) in the plant cell wall. Furthermore, Hyp-*O*-glycosylation facilitates secretion and improves stability of recombinant proteins expressed in plant cell cultures. However, the precise process of Hyp-*O*-glycosylation in plants has not been elucidated so far. Plant hairy root culture is regarded as a unique platform to study Hyp-*O*-glycosylation process. Hairy roots are generated by infection of plants with the bacterium *Agrobacterium rhizogenes.* Hairy roots are fully differentiated plant tissues that can be propagated rapidly in liquid media at comparable rates as plant cell suspension cultures. In this work, tobacco hairy roots expressing peptide backbones of two major types of HRGPs, extensin consisting of contiguous Hyp $(Ser\text{-}Hyp\text{-}Hyp\text{-}Hyp\text{-}Hyp)_{15}$ and arabinogalactan protein (AGP) consisting of non-contiguous Hyp $(Ser\text{-}Hyp)_{32}$ were created. A

reporter protein, enhanced green fluorescence protein (EGFP) was engineered to the C-terminus of each peptide backbone to facilitate protein detection and purification. These hairy roots were grown in different liquid media (MS, SH and B5) and the secreted protein yields and the glycosylation of each peptide backbone inside and outside cells were characterized. Treatment of the cultured hairy roots with brefeldin A (BFA) that blocks protein transport from the ER to the Golgi apparatus was also conducted to track the Hyp-*O*-glycosylation process in hairy roots.

P60. Expansin synergy with cellulases

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Abstract. Bio-fuel production is a hot issue because of the price of oil and because of global warming. One of its biggest limitations in production is the difficulty of breaking down complex plant materials into the sugars from which we can make biofuels. The low activity of cellulases, enzymes that break down the plant materials,is the biggest problem. One solution to these sluggish enzymes is to improve their activity. Expansin is a cell wall loosening protein and it increases the activity of cellulase and facilitates the conversion of lignocellulosic (plant) materials to sugars by its synergistic activity. The synergistic effectfrom combination of expansins and cellulases will lower the required amount of cellulases by ≥ 2 fold on lignocellulosic biomass and therefore, decrease the ultimate cost for ethanol production. To study expansin's synergistic activity with cellulase, a fast and efficient assay for its activity is highly desirable. Currently expansin activity assays are cumbersome and require complex preparation steps. Moreover, current assays work only with purified expansin. In this study, 1) we developed a high throughput expansin activity assay which can be directly applied to crude expansin solutions without any further purification, 2) we screened transgenic corn seeds with this novel expansin assay to identify high expressing lines that could be used for ethanol production and 3) synergistic activity of transgenic expansin from corn was characterized in a larger scale system to determine the best condition for degradation of pre-treated cellulosic material.

P61.Preparation and evaluation of arabinan-hydrolyzing enzymes for generating biobased products from sugar beet pulp polysaccharides

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Abstract. This presentation summarizes our efforts to prepare a diverse toolbox of polysaccharide-active enzymes and evaluate their suitability for generating valuable biobased products from sugar beet pulp polysaccharides. Well-defined monocomponent enzymes can be

used as a high resolution analytical tool to decipher fine structure of cell wall polysaccharides, to evaluate structure-function relationships, and to modify functional properties of isolated polysaccharides. We are currently producing complementary suites of enzymes previously cloned from *Aspergillus nidulans* (Bauer *et al*., PNAS 103:11417–11422, 2006) by recombinant over-expression in the yeast *Pichia pastoris*. Our current objectives are to produce gram quantities of enzymes, define biochemical characteristics, and demonstrate activities on defined polysaccharide substrates. This project provides a vehicle for training and research participation by students in biochemical technologies for protein bioproduction and enzyme conversion of plant cell wall polysaccharides to value-added products.

10. PHYTOCHEMISTRY

P62. New hydroquinone melissifolanes from *Lindera melissifolia* (Lauraceae)

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Abstract. Opportunistic bacterial and fungal infections represent a significant health threat worldwide, especially in immune-compromised individuals. The search for antimicrobial agents struggles forward as increasing drug resistance and reduced financial support for antimicrobial discovery looms. It now becomes increasing important to focus on underutilized resources for new chemical entities to treat these infections. Globally at least 13 % of known flora are endangered or threatened and recently the USDA claimed that there are now over 780 endangered or threatened species of plants in the United States and its territories. There are a handful of reports in the literature that investigate endangered U.S. plants for useful natural products but this number is insignificant when compared with the number of endangered U.S. species. *Lindera melissifolia*(Walt.) Blume (Lauraceae), commonly called pondberry, is an aromatic and rhizomatous shrub and populates the edges of lakes and ponds in the Southeastern U.S. region. The ethyl acetate extract of the *L. melissifolia* drupes showed significant anti-microbial potency. Bioassay-guided fractionation of the active extract afforded 10 known butanolides and 4 new hydroquinone terpenoids entitled melissifolanes A-D.The butanolides exerted significant anti-infective action on *Cryptococcus neoformans* and MRSA. The assignment of relative configuration for melissifolanes was achieved using a combination of ^{1}H-^{1}H-NOESY correlations with (^{1}H-^{1}H) homonuclear and (^{1}H-^{13}C) heteronuclear *J*-based coupling analysis. The absolute configuration of melissifolane A was assigned by comparison of theoretical and empirical circular dichroism spectra. Melissifolane B was isolated as a racemic mixture based on optical rotation and chiral chromatography. Utilization of these metabolites or others obtained from endangered or threatened plant species as treatments or applications to control infectious disease would support their conservation and commercial development.

P63.Metabolomic analysis of fruit natural products in the high pigment (*hp*-1, *hp*-2^{dg}) photomorphogenic mutants of tomato

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Abstract. Tomato is one of the most popular vegetables worldwide and is an excellent source of several health-promoting phytonutrients. In this study we compared LCMS-based metabolite profiles in three tomato cultivars (Moneymaker, Ailsa Craig, and Manapal), an anthocyaninless mutant (*aw*), and two *high pigment* mutants (*hp*-1 and *hp*-2^{dg}) that exhibit altered photomorphogenic responses. Untargeted metabolic profiling can reveal fundamental biosynthetic relationships in metabolic networks and demonstrate how developmental, genetic, and environmental factors can affect the secondary metabolite content of fruit. As in previous studies, we found that green fruits contain substantially higher levels of the common phenolic metabolites chlorogenic acid (CGA) and rutin than red fruits. We also observed a correlation between CGA and rutin levels across all genotypes suggesting that synthesis of hydroxycinnamic acids and flavonoids is coordinately regulated. The *hp*-2^{dg} mutant contained significantly higher levels of CGA and rutin than the other cultivars, particularly in green fruits, suggesting that alteration of light signaling pathways has pronounced effects on phenylpropanoid metabolism. LCMS-based metabolic profiling generated a peak list of over 60 known and currently unidentified metabolites that vary across genotypes. Preliminary analysis suggests the presence of CGA (5-caffeoylquinic acid) and its isomer (3-caffeoylquinic acid) in red fruits, while green fruits contain only the main CGA isomer. Also, the amount of naringenin chalcone (*m/z* 271) appears to vary extraordinarily across genotypes and is completely absent in the *aw*mutant of Ailsa Craig. Correlation and discriminant function analysis will be used to identify metabolic relationships among different antioxidant classes in tomato. This study provides a framework for future studies focused on engineering a more nutritious tomato through selective breeding, genetic engineering, and optimal growth conditions. Funding for this project was provided by the Arkansas Center for Plant Powered Production (P3 Center) through NSF EPSCoR (EPS-1003970).

P64. P3 Center Instrumentation at ASU: Biomolecule analysis by MALDI-TOF mass spectrometry

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Abstract.A Waters-Micromass MALDI micro MX bench-top matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) was purchased through the Arkansas ASSET NSF EPSCoR P3 Center grant. It is located in the Protein Chemistry Laboratory in the Arkansas Biosciences Institute at ASU in Jonesboro. This instrument provides new capabilities in biomolecule analysis to support research capacity development at ASU. The MicroMX is fully operational and detailed sample processing and instrument protocols have

been established for its operation. Various resources have been leveraged to support training of MBS graduate students (i.e., through Techniques in MBS instruction), associated ABI staff, and visitors to ASU in its application and operation. It has now been used to support various research publications and new grant funding applications. Although the instrument primarily finds application for unequivocal structure-based identification of recombinant proteins, we have demonstrated the instrument's versatility in application to a broad range of biomolecules of interest in the P3 Program. These are summarized in this presentation.

P65.Expression of a mammalian polyphosphate 5-phosphatase in tomato hairy roots affects root growth and antioxidant levels.

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Previous work has shown that transgenic tomato plants harboring a human polyphosphate 5-phosphatase (InsP 5-ptase) show increased drought tolerance, biomass and lycopene content. To further understand the mechanism of action behind these observations, we established hairy roots from the wild type and transgenic tomato plants. When cultured in liquid medium, the transgenic root cultures exhibited significantly lower biomass than the wild type cultures. To address if auxin signaling was affected in the transgenic lines, hairy roots from wild type and transgenic tomato were cultured in solid medium with or without supplementation of IBA. No growth difference was observed in the presence of IBA. To study the impact of manipulating $InsP_3$ pathway on specialized metabolism, 15-day cultures were treated with 100 μM methyl jasmonate (MeJA) for 24 hours. HPLC analyses showed a different profile of compounds in the medium and root tissue following elicitation. Whilst MeJA induced production of non-polar compounds that secreted in the medium, more polar putative phenolics were induced and retained in the tissue. More significantly, much higher levels of these latter compounds were found in the root tissue of the transgenic hairy roots. In addition, the ABTS assay showed that the antioxidant capacity of root extracts from the transgenic lines was higher than the wild type lines, suggesting that the manipulation of $InsP_3$ levels in the hairy roots affected specilized metabolism.