Young Scientists Meeting

9th Young Scientists Meeting 2016

9th – 11th November
in Quedlinburg

- Abstracts -

Berichte aus dem Julius Kühn-Institut

186

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Bundesforschungsinstitut für Kulturpflanzen

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Greetings from the President

Dear Young Scientists,

Welcome to the 9th Young Scientists Meeting of the Julius Kühn-Institut at the JKI headquarters in Quedlinburg.

The meeting has already a quite long and eventful history and it has become an important part and parcel at the JKI. At the same time it developed to a longed for and looked forward event for our young scientists. For this year, 58 participants applied which is 34% more than the last year.

During this year's meeting you will for the first time have the opportunity to propose and discuss your own ideas and solutions to set up key research aspects and prospected challenges of the JKI. For this, there will be a workshop where you can first discuss and dispute your ideas within a group of you. In a following panel discussion I cordially invite you to present your group results and discuss them in the big round together with the research coordinators and the press office. The final results and consensus of this round will be integrated in the strategic discussions about research at the JKI and thus give you the direct possibility to participate in the process of designing the scientific future of the JKI.

Furthermore, three keynote lectures will enrich the meeting. Christian Kohl, a newly appointed scientist from the Institute for Biosafety in Plant Biotechnology, gives an overview about evidence synthesis and decision making and presents a new software tool developed at the JKI. Klaus Humbeck from the Institute for Biology of the Martin-Luther-University Halle-Wittenberg talks about epigenetic control of normal and stress-induced leaf senescence presenting the novel project - the Barley Epigenome Platform (BEP). Finally, Anja Hühnlein from the Information Centre and Library of the JKI gives a talk about interesting news and facts to copyright, open access and licenses. This is the first talk of an upcoming series "Open Research at the JKI – You make it possible, we make it visible" informing you about your opportunities to publish and visualize your work.

I am convinced that all participants will personally benefit from the YSM by expanding their knowledge and by increasing their presentation skills. In addition, you get the chance to get to know each other during the joint evening events and expand your personal network, an important base for your future professional life.
I hope you all enjoy your stay at Quedlinburg and return home enriched by the experience and inspired both scientifically and socially.

Quedlinburg, November 2016

Dr. Georg F. Backhaus
President of the JKI

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Keynotes

I`m my own expert: Evidence synthesis and decision making

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Evidence-based decision making processes are depending on targeted scientific information, whereas the presented format should provide a precise and reliable answer to a question under debate. In case evidence pertinent to the post question does exist in form of published studies, it might be synthesized by the performance of a literature review. Reviews of the scientific literature vary considerably in how they are conducted and if they do not follow an *a priori* defined and documented procedure that employs explicit means to identify, critically appraise, and evaluate included studies they are usually referred to as "traditional" or "narrative" reviews. In contrast, systematic reviews represent powerful tools to identify, collect, synthesize, and evaluate primary research data on specific research questions in a highly standardized and reproducible manner. They enable the defensible synthesis of outcomes by increasing precision and minimizing bias whilst ensuring transparency of the methods used. Although seen as a "gold standard" for synthesizing primary research data, systematic reviews are not without limitations as they are often cost, labor and time intensive. In order to increase the efficiency of systematic review performance, an online-tool called CADIMA was developed at JKI to 1) guide review authors through the evidence synthesis process, 2) ease steps with a considerable workload and 3) assure for its thorough documentation.

Open Research at the JKI – You make it possible, we make it visible

Part 1: Copyright, Open Access and Licenses

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Authors submitting their manuscripts to subscription based journals are often compelled to assign exclusive rights of use to the publisher. Unfortunately, some authors do not consider the consequences associated with such a copyright transfer agreement. If no additional convention guarantees rights to reuse the article, the authors are not allowed to share the content with colleagues or to post/archive an electronic version on a personal website or a repository, not even the manuscript version prior to acceptance (pre-print). Therefore, it is very important to read the copyright transfer agreement carefully and to choose journals offering their authors considerable permissions for re-use. Meanwhile, most of the scientific journals allow their authors at least an archiving of the pre-print version. Sometimes, archiving is also allowed for the final draft post-refereeing (post-print), although often subjected to a six or twelve month embargo. Authors should also be so confident to cross out unfavorable wording or to add parts to the agreement resulting in an assignment only of non-exclusive rights.

Next to the archiving of pre- or post-prints (green open access), authors can also publish in open access journals (gold open access). However, authors should be cautious, if the journal promises very fast review periods, asks for submission instead of publication fees or publishes articles with fundamental errors even in the titles and abstracts. These "predatory journals" enter the market and impair the trust in science. On the other hand, some popular publishers have increased the fees for open access publishing dramatically, which leads to a tremendous increase of costs for the scientific community paying for journal subscriptions and for open access publications in parallel.

A good alternative is to submit a manuscript to universities or non-university research institutes who self-publish journals or monographs, mostly with open access and no or less article processing charges (APCs). That way authors can be sure that their manuscripts are subjected to a reliable review process and that the published items are properly archived and indexed in a broad range of databases, such as Web of Science, Scopus or PubMed. A misconception, however, is that the term open access would lead to considerable rights of use. This is not the case. In general open access means that an article is free to read or to download from a website. The reader is not automatically allowed to derivate, remix or distribute the work. The latter is allowed, if the work is licensed, e. g. under a creative commons attribution license. With these licenses authors are able to determine themselves whether and how their work can be re-used.

BEP - the Barley Epigenome Platform of the ScienceCampus Halle

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Plant development and responses to environmental stresses are based on coordinated reprogramming of gene expression. Recent findings show that epigenetic control levels play an important role in this process. Epigenetic control via dynamic and specific histone and DNA modifications affects chromatin structure at certain genes and thereby influences expression. The talk will focus on epigenetic control of normal and stress-induced leaf senescence as a major developmental step, which determines yield in crop plants. Techniques to analyze local and global histone and DNA modifications will be presented. A novel project, the establishment of the Barley Epigenome Platform (BEP) at Martin-Luther University in cooperation with IPK Gatersleben, for analyses of genome-wide epigenetic mechanisms in crops will be presented. This project is funded by the ScienceCampus Halle.

Session 1

Pests, Viruses and Bacteria

Chemical cues for oviposition site acceptance of grapevine moth

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The European grape berry moth, *Eupoecilia ambiguella* and the grapevine moth *Lobesia botrana* are the most serious pests in European vineyards. The larvae developed from the second generation eggs damage the grapes enabling bacteria and fungi, especially grey mould *Botrytis cinerea*, to develop more rapidly.

For oviposition site acceptance several sequences have to be fulfilled in turn or at the same time. Firstly, the gravid females get guided to the suitable environment, such as the vineyard, mostly through olfactory cues. Once the moth has landed on the plant, olfactory as well as tactile, visual and contact-chemosensory cues are contributing to the decision to oviposit.

To unravel this puzzle, the volatiles of grapes at different phenological stages have been analyzed and identified by GC-MS. The perception of potential attractive compounds by *L. botrana* and *E. ambiguella* has been checked via elektroanntennography (EAG). The structure of the egg laying substrate as well as the composition of non volatile compounds on the substrate (e.g. wax layer) facilitates oviposition. The preferred egg laying substrates as well as attractive wax extracts could be identified.

Currently sex pheromone traps are used for determining the activity of male grapevine moths but they do not generate reliable information on egg laying behavior. Unraveling the cues for oviposition site acceptance could be used in developing an oviposition monitoring tool appropriate as decision support system for exact timing of insecticide spraying and may help to reduce insecticide treatments in vineyards.

Variety-depending susceptibility of cherries to *Drosophila suzukii* according to fruit firmness and other ripening parameters

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The invasive pest species Spotted Wing Drosophila (SWD), *Drosophila suzukii*, was first recorded in Germany in 2011. *D. suzukii* is an extremely polyphagous pest and can reproduce on a large number of cultivated and wild fruits. With their serrated ovipositor females lay eggs in undamaged ripening and ripe fruits. During larval development infested fruits collapse rapidly and become unmarketable. *D. suzukii* can cause tremendous economic crop losses, especially in cherries and soft berries.

Characteristics of the fruit, especially fruit firmness and skin parameters, can influence oviposition behaviour. Hence, cherry varieties were analysed during the ripening phase: Firstly, with a texture measuring apparatus which indicates the force and energy needed to puncture the cherry skin; secondly by measuring the impedance, i.e. the resistance of the skin, in different positions (top, side and bottom) and thirdly, skin thickness (wax layer and subjacent cell layers) will be determined by microscopy. These data were correlated with the natural infestation level in the field. In addition, oviposition tests were carried out in the laboratory with cherries from the corresponding sampling dates. For this study, the sweet cherry varieties 'Hedelfinger' and 'Regina' and the sour cherry variety 'Schattenmorelle' were chosen. During the ripening process samples were taken from the field at different ripening stages determined by the colour of the fruits.

Texture analysis shows a decline of penetration force and energy during the ripening process. The same tendency can be observed for the impedance. However, variations were noted depending on the tested positions. The quantitative determination of the cherry skin thickness is in process and statistical analyses have not been finalized, yet.

First results show that the skin characteristics and the firmness of different cherry varieties have an effect on the oviposition preference of *D. suzukii.* With decrease of fruit firmness and skin resistance connected with the ripening process, oviposition increases.

These findings should be confirmed with a larger selection of cherry varieties over several growing seasons. This study provides initial indication for the possibility of managing *D. suzukii* in cherries by developing new cherry varieties with more resistant fruit skin.

Landscape-level movements and a molecular approach to analyze the diet of *Drosophila suzukii*

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The invasive pest species Spotted Wing Drosophila (SWD), *Drosophila suzukii*, has been first recorded in the USA and Southern Europe in 2008. Since 2011 it is also known from Germany. *D. suzukii* is an extremely polyphagous pest species. It feeds and reproduces on more than 120 cultivated and wild fruits from 20 plant families. Infested fruits quickly collapse and become unmarketable. *D. suzukii* overwinters as adult in a reproductive diapause at sheltered sites, especially in forests and hedges. Since this invasive pest is active at mild days in winter and spring it needs nutritional resources.

To investigate which resources are used, monitoring adult activity, laboratory bioassays and molecular techniques were applied to identify wild host plants sustaining *D. suzukii* during winter and spring. The occurrence of *D. suzukii* at landscape level and its remigration into orchards was observed all year round. Further, an automatic trap was developed to examine the diurnal activity of *D. suzukii*. No-choice assays with mistletoe berries were established to investigate egg laying, feeding activity and survival of *D. suzukii*. For investigating the attraction of *D. suzukii* to trees parasitized with mistletoe, *Viscum album*, we analyzed the volatile organic compounds (VOCs) of berries by GC–MS. Feeding experiments (FEX) were established to examine the digestion time and to develop a molecular approach to identify food sources during winter and spring. Additionally, bleaching experiments (BEX) were conducted in order to decontaminate the body surface which should not negatively affect the DNA of the gut content.

During fall and early winter, catches in monitoring traps indicated a shift of fly activity from orchards towards forests and hedges. Significantly higher numbers of flies were captured in the canopy of *Pinus sylvestris* parasitized with *V. album* compared to non-parasitized trees. From April onwards we found females with mature eggs coinciding with ripe berries of *V. album*. Under laboratory conditions eight adult individuals emerged from 1.100 field-collected berries in 2015. The odor spectrum identified from the berries was comparable to common berry odors. Further, we succeeded in identifying ingested chloroplast-DNA in the gut content *D. suzukii*. First results of the FEX show that females ingest more DNA than males. Following, the results of the BEX show that the body surface was decontaminated whereas the chloroplast DNA of the gut content stays unaffected.

The combination of the presented field studies and laboratory assays will provide important information to identify the nutritional resources of *D. suzukii* in winter and spring, and to get a better understanding of *D. suzukii* behavior on host plants in the course of a day.

Insights into the *Nanovirus*-legume-aphid interactions

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Legumes are important crops with high nutritional value for both human and animal consumption. They are susceptible to many viral diseases. The last season showed a high incidence of viral diseases on leguminous crops in Germany and Austria. Analyses of symptomatic plant samples revealed that infections were resulting mainly from pea enation mosaic virus (PEMV) and pea necrotic yellow dwarf virus (PNYDV).

Nanoviruses such as PNYDV, are multicomponent circular ssDNA viruses. They are transmitted by aphids in a circulative, persistent manner and infect mainly legumes. Interestingly, this transmission requires a viral helper factor (HF).

In this study, we aim to investigate the interactions between *Nanoviruses*, their hosts and their aphid vectors. In particular we want to investigate the role of the HF during aphid transmission as well as studying the aphid feeding behaviour on infected and healthy plants. Currently we are characterising the genetic variation of legume viruses in Germany using next-generation sequencing technologies.

Novel *Cydia pomonella* granulovirus isolates break virus resistance in codling moth

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Cydia pomonella granulovirus (CpGV) (family *Baculoviridae*) is an effective viral agent to control codling moth (CM, Cydia pomonella L.) populations in apple and pear plantations. Since 2005, resistance of CM field populations against CpGV products has been documented in more than 40 orchards and two different types of resistance of CM (sex-linked and autosomal inheritance) had been successively found in Europe. Most resistant CM populations can be controlled by newly registered, resistance breaking CpGV isolates but some resistant CM populations are still difficult to be controlled. Therefore, searching for other resistance breaking CpGV isolates is of urgent need.

We determined the efficacy of seven Chinese CpGV isolates on different CM laboratory strains, a sensitive and two resistant CM strains, in bioassays. Among them, two isolates (CpGV-JQ, CpGV-ZY2) showed virulence comparable to already described CpGV isolates. Interestingly, two isolates (CpGV-ZY, CpGV-WW) were more infective to the autosomal inherited resistant CM strain than to the sex-linked resistant strain and differed in this regard from any previously recorded CpGV isolate. All isolates were sequenced using next generation sequencing and were grouped into previously reported CpGV genome types A to E. In depth genome sequence comparisons are going on to identify the genetic factors determining the CpGV virulence in different CM strains. These studies will allow us to unveil the partial resistance mechanism and point the way to improve novel strategies for resistance management.

Survival of *Salmonella* in agricultural soil – attack from the underground

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In the last years, salmonellosis outbreaks were increasingly associated with contaminated fruits and vegetables. This indicates that plants are suitable vectors for *Salmonella enterica*. Contamination of produce can occur along the whole production chain also, for instance, during plant growth. The survival of *Salmonella* in soil is an essential precondition for the colonization of plants. However, so far the knowledge about factors influencing its persistence in soil and in plant environment is scarce, and the question whether *Salmonella* uses plants as opportunistic bacterium or if it behaves as a plant pathogen is still controversially discussed.

We analyzed the influence of soil fertilization and soil sterilization on the survival of *Salmonella*. We observed an adaption of *Salmonella* inoculated into soil with reduced diversity that leads to enhanced persistence and survival in the plant environment. While fertilization with pig manure had a positive effect on the survival of *Salmonella* in soil, chicken manure had no distinct influence on the survival. Usually, sterilization of soil by autoclaving does not lead to a sterile soil but to a drastic reduction of the abundance and diversity of soil bacteria. *Salmonella* was able to survive in this soil for a relatively long time (monitored up to 6 months) and seemed to adapt to this environment. This adaption led to a change in the persistence in the presence of plants. Despite an initial decline, our data indicated a long-term survival of *Salmonella* in agricultural soil. The presence of the indigenous soil microbial community reduced its survival, most likely due to competition for resources.

Together, our results indicate that *Salmonella* can persist in soil for extended times and that adaptation to the soil environment enhances the risk of contamination of produce in the agricultural environment. The fact that *Salmonella* uses plants as alternative hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than estimated so far.

Session 2

Breeding and Resistance

Identification of genes for resistance against *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* employing genome-wide association studies

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Pyrenophora teres f. *teres* (PTT) and *Cochliobolus sativus* (CS) are the causal agents of the net type of net blotch and spot blotch in barley, respectively. Both fungal pathogens are widely spread and cause high yield losses. The most cost effective and environment-friendly way to prevent and control these pathogens is growing resistant cultivars. In order to identify sources of resistance, in a first step more than 10,000 barley accessions including landraces and commercial cultivars were screened for resistance to PTT and CS under greenhouse and field conditions. Out of these, 450 barley accessions derived from the centres of barley diversity, and expressing different levels of resistance to respective pathogens were selected.

Next, greenhouse experiments were conducted with these 450 accessions with two PTT and CS isolates, respectively. Three week old plantlets were inoculated with a spore suspension of 5000 spores/mL and assessed for symptom expression 14 dpi. Additionally, field trials were conducted in Russia, Belarus and Germany. The disease severity was scored three times during the growing season to calculate the area under disease progress curve (AUDPC).In parallel respective genotypes were genotyped with the Barley 9k iSelect chip. Markers with a minor allele frequency (MAF) <5%, missing data >10% and heterozygosity >12.5% were removed prior to conducting genome-wide association studies (GWAS). The population structure was calculated based on 508 markers with high PIC (polymorphism information content) values covering the whole genome at an average distance of about 2 cM. GWAS was carried out using the software TASSEL 5 and a mixed linear model (MLM) including population structure and kinship and a false discovery rate of FDR=0.1.

In summary six regions associated with PTT resistance on chromosomes 1H, 2H, 3H, 5H, 6H and 7H and four regions associated with CS resistance on chromosomes 1H, 2H, 5H and 7H were detected. In the next step, additional field and greenhouse trials will be conducted to broaden the base of the phenotypic data and respective associations will be subsequently validated in different DH-populations.

Introducing a herbicide tolerance into the Russian Dandelion by means of genome editing

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The Russian Dandelion *Taraxacum koksaghyz* (*Tks*) is in the focus of scientists as well as industry being a new source for natural rubber (cis-1,4-polyisoprene). Presently that is almost solely delivered by the rubber tree *Hevea brasiliensis*. Due to a fungal affection threatening that population, the supply for the world's rubber market is endangered. Therefore, alternatives are urgently needed, which are able to provide a rubber with similar chemical and physical characteristics. *Tks* is able to deliver such a rubber.

Tks is a diploid *Asteraceae* that naturally grows under sparse conditions in the Tian Shan Mountains, enabling cultivation on marginal acreages in a broad climatic area. The rubber itself is stored in the roots, facilitating the cultivation of *Tks* as a perennial crop. However, a lot of issues have to be solved to make *Tks* a quantitative source for natural rubber. Besides the problems with germination on the field and the slow early growth, the crop still requires a lot of manual labour because an effective strategy against weed as well as automated equipment for harvesting do not exist yet.

To overcome one of those problems, a herbicide tolerance shall be introduced to enable a better competitiveness of *Tks* on the field. This is done in the framework of the "EVITA" project. The target for this approach is the acetohydroxyacid synthase (AHAS), an enzyme indispensable in the metabolism of essential branched-chain amino acids in plants. By single nucleotide substitutions, *Tks* can become tolerant to AHAS-specific herbicides like sulfonylurea or triazolopyrimidine. The unspecific mutagenesis by ethyl methanesulfonate as well as the site specific nuclease system CRISPR/Cas9 are used therefore. Herbicide treatment of seedlings and regenerates, respectively, is used for selection.

From other plants and *Asteraceae* it is known that they can have up to three AHAS homologues. However, according to our results from RACE (rapid amplification of cDNA ends)-experiments and transcriptome data, *Tks* harbours presumably only one AHAS gene. To finally confirm this, a knock-out approach is running. Here non-viable plants are being expected if a homozygous knock-out of the essential AHAS gene occurs.

Resistances to *Erysiphe necator Ren3* and *Ren9* are encoded on chromosome 15 from 'Regent' in close vicinity

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The obligate biotrophic pathogen *Erisyphe necator* (Ascomycete) causing powdery mildew (PM) on grapevine was first introduced to Europe from America around 1845. Since then it has spread and is now present in every winegrowing region worldwide. PM is one of the most devastating fungal diseases known to *Vitis vinifera* L. and is responsible for a considerable annual yield loss if no counteractions such as the application of fungicides are taken.

Breeding programs nowadays aim at combining different resistances against a certain pathogen (pyramiding). This strategy pursues the goal that pathogens will not easily overcome resistances of newly bred cultivars. For this approach of resistance breeding a combination of different resistance mechanisms is highly beneficial. For most of the loci conferring resistance to *E. necator* a hypersensitive response (HR) upon infection is described but for only one of them it is known which gene mediates this reaction. Molecular characterization of these loci is therefore a crucial step to combine different resistance mediating genes.

For the resistance locus *Ren3* only the location on chromosome 15 was published in 2004 and confirmed over the following years. This locus was found in a cross population of 'Regent' x 'Lemberger' and was recently confirmed in 'Regent' x 'Cabernet Sauvignon'. Detailed analysis of this resistance locus involving fine mapping of chromosome 15 and analysis of *Ren3* recombinant genotypes we could narrow the locus down to an interval of around 200kb. The identified region includes four genes of the NBS-LRR (nucleotide binding site – leucin rich repeat) type which resemble typical resistance mediating genes known from *Run1* (from *Vitis rotundifolia*) and *Ren1* (from *Vitis vinifera* 'Kismisch Vatkana'). Microscopy showed that *Ren3* carrying cultivars react with HR upon infection with *E. necator* and therefore can restrict the growth of the pathogen.

During the characterization of *Ren3* we observed a shift of the QTL for resistance to *E. necator* to the anterior part of chromosome 15 early in the epidemic. Analysis of further recombinant plants delimited the two QTL regions and indicated resistance response of the HR type for both loci. The new locus was named *Ren9* and could be delimited to around 1,8 Mb. Screening of the 'Regent' BAC-library is currently done to obtain the sequence information for this locus which will allow a search for possible candidate genes.

Breeding of Russian dandelion (*Taraxacum koksaghyz*) – From the wild type to a new resource for a sustainable rubber production

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Russian dandelion (*Taraxacum koksaghyz*) has the ability to produce and store high quality rubber in its roots. Due to the lack of alternative sources for natural rubber next to the Para rubber tree (*Hevea brasiliensis*), whose cultivation is problematic due to economical and ecological reasons, Russian dandelion provides an interesting new sustainable resource for natural rubber demanding industries.

Since Russian dandelion shows high diversity and relatively weak growth, it is still considered as wild type. This work aims to gain insights into the genetic background of this plant and provides important information for promising breeding programs in order to develop a new rubber producing crop.

As part of a network of different research institutions and private companies, the Institute for Breeding Research of the Julius Kühn-Institute is part of a value chain from breeding up to the finished product made of dandelion rubber. In close cooperation with a breeding partner, the comprehensive genetic variability of Russian dandelion shall be used for the development of new varieties with high level and quality of rubber. On that account, different agronomic traits, such as the formation of a large, clear taproot with high contents of rubber, early and uniform flowering time, improved tillering in the first year of cultivation, as well as different disease resistances have been defined as breeding objectives.

These objectives will be supported by genetic analysis of different accessions of Russian dandelion and the development of a genetic map based on a defined mapping population. In order to develop sufficient SNP-markers for genetic mapping, a Genotyping-by-Sequencing (GBS) approach was applied. In combination with AFLP (Amplified Fragment Length Polymorphism)-markers, this high density genetic map provides a useful tool for mapping quantitative trait loci (QTL) related to rubber content and other important traits with regard to the development of selection markers for marker-assisted breeding (MAS).

Genetic dissection of two wild emmer QTLs conferring drought tolerance

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Drought, one of the major factors limiting global wheat (*Triticum* spp.) production, is expected to increase in severity and frequency in the future, as a result of climate change. The genetic diversity concerning genes responsible for tolerance to drought or other abiotic and biotic stresses has been depleted due to domestication and modern wheat breeding. Therefore, wild relatives offer a valuable source for improving drought tolerance in domesticated wheat.

In previous work QTL regions conferring drought tolerance in wild emmer on chromosome 2BS and 7AS (*T. diccocoides*) have been identified and were transferred into elite wheat cultivars. These near isogenic lines were shown to be more tolerant to drought than their recurrent parents but suffer from linkage drag.

The main aim of this ongoing project is to narrow down the size of these QTL-regions and to re-introgress the shortest fragments bearing drought tolerance into Israeli and German elite wheat cultivars. For that purpose 151 F_7 plants of the original F_6 mapping population were genotyped with the 15k i-Select chip, a high resolution map with 4118 polymorphic marker was constructed and validation of both QTL-regions conducted.

Few candidate genes for both QTL-regions were identified. QTL-region 2BS shows synteny to a genomic region in wheat that is known to contain genes involved in ABA perception and calcium signaling. 15.67 and 26.02 cM intervalls of QTL-regions on chromosome 2BS and 7AS were selected for fine mapping. iSelect-SNP markers mapping in the regions of the QTL-intervals were converted into different types of PCR based molecular markers, such as kompetitive allele specific PCR markers (KASP), cleaved amplified polymorphic PCR-markers (CAPS) and simple sequence repeats (SSR).

Currently 82 and 159 heterozygous segmental recombinant F_2 inbred lines of QTL-region 2BS and 7AS, respectively, are subjected to genotyping. Specific F_2 heterozygote recombinants, showing recombination events in the targeted intervals are going to be selected and F_3 progenies of these plants will be screened to identify homozygous recombinant plants. Next, F_4 progenies of these plants will be phenotyped and the QTL interval reduced in length.

Mapping QTL for resistance to net blotch (*Pyrenophora teres* f. *teres*) in a wild barley nested association mapping (NAM) population

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Net blotch, caused by the fungus *Pyrenophora teres* f. *teres,* is an important foliar disease of barley causing high yield losses. The identification of QTLs and underlying genes conferring resistance to this fungus is the basis for targeted and sustainable breeding approaches aiming to improve net blotch resistance in modern barley cultivars and to broaden the genetic base of resistance. Therefore, a SNP-based nested association mapping (NAM) approach was used to map QTL for resistance derived from *H. spontaneum* or *H. agriocrithon.* To achieve this, the barley nested association population (HEB-25) comprising 1420 BC_1S_3 lines in 25 families originating from a cross of 25 wild barley accessions (*H. spontaneum* and *H. agriocrithon*) with the cultivar Barke was screened for resistance in two-years field trials using a summer-hill-design. Best linear unbiased estimates (BLUES) for average ordinate (AO) and reaction type (RT) were calculated. Using these and 5702 informative SNPs obtained from the 9k iSelect barley chip nested association mapping was conducted.

Results indicate a high variability in net blotch resistance between and within families of the NAM-population. A high correlation between AO and RT data was observed. In summary, SNPs highly associated to net blotch resistance were detected on all seven chromosomes of which some map to chromosome regions previously identified to be linked to net blotch resistance. In a next step, identified QTL will be analyzed in more detail to identify potential candidate genes.

Session 3

Pathogens and Nematodes

Apple Replant Disease soil effects on the microbial community: a split-root experiment.

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Apple Replant Disease (ARD) occurs when young apple trees of the same or closely related species are planted repeatedly at the same site leading to decreased growth and root browning with subsequent losses in fruit yield and quality. Causal agents of ARD have yet not been defined, but biotic factors are presumably responsible.

A split-root experiment was performed to give insights into the causal agents of ARD and to determine how ARD soil shapes the apple rhizosphere microbiota. Therefore, young apple trees were grown in rhizoboxes with two separated compartments consisting of different combinations of ARD soil (+ARD), gamma treated ARD soil (-ARD) and soil never planted with apple (Control). Plants were grown in a climate chamber for five weeks with continuous root growth monitoring. Samples were taken from several spheres (rhizoplane, rhizosphere and bulk soil) and total microbial community DNA was extracted. Microbial community structure and function was studied by quantitative PCR, DNA fingerprinting by DGGE (Denaturing Gradient Gel Electrophoresis) and Southern blot hybridization.

Plant root growth was substantially lower in +ARD soil than in -ARD and control soils. DGGE of bacterial 16S rRNA and fungal ITS (internal transcribed spacer) amplicons exhibited several responders to ARD. Permutation tests based on similarities of DGGE fingerprints showed significant differences in the bacterial and fungal community structure between +ARD vs. control, control vs. -ARD and +ARD vs. -ARD soils at all three spheres, especially at the rhizoplane. Gamma-irradiation led to severe shifts in bacterial and fungal communities in rhizosphere and bulk soil. The ARD effect was most pronounced in the bulk soil and rhizoplane compared to control soil. By group-specific PCR-DGGE fingerprinting, strongest responders to ARD could be affiliated to *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria*. Southern blot hybridization of functional genes and of plasmids (IncP-9, IncP-1) involved in degradation of aromatic compounds, which are common exudates of apple roots, displayed differences between soils, treatments and within spheres, with higher abundance of IncP-9 plasmids in the rhizoplane and rhizosphere in all soil treatments whilst IncP-1 was only present in the rhizoplane of the control and gamma-treated soils.

In summary, our results indicate a strong effect of ARD soil on root growth and plant performance accompanied by shifts in the microbial community composition which might contribute to ARD. To which extent microorganisms are causal agents or responders to plant symptoms is currently studied by Illumina MiSeq sequencing of 16S rRNA gene and ITS amplicons.

Suppression of infective stages of phytonematodes by associated microbiomes

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The role of soil-borne microbiomes in suppression of plant diseases caused by plant-parasitic nematodes (PPN) has become a focus of many studies and search for putative microbial suppressors is essential in this regard. Our project is based on the assumption that specific microbes attach to migrating infective stages of PPN in soil and trigger induced systemic resistance in plants, rather than directly antagonizing nematodes.

To better understand this paradigm of nematode-plant-microbe interactions we have designed a greenhouse experiment where we tested three different soils for their suppression against root-knot nematode *Meloidogyne hapla*. Infective second-stage juveniles (J2) of *M. hapla* were baited in soil suspension of respective soils and inoculated in the pots with tomato plants. After one week we stained the roots and found a significant reduction in number of invading J2 in two soils compared to the control, and a reproduction rate is yet to be determined. In order to detect and isolate microbes that are responsible for this suppression, we aim to apply culture-dependent and culture-independent methods, including the next generation sequencing.

As hypothesized that soil microbes specifically attached to J2 induce systemic resistance in plants, we established protocols for RNA extraction from roots and RT-PCR to analyse the expression of defence genes. In this case surface-sterilized nematodes are baited in soil suspensions and applied to aseptic tomato plants. In addition, we have succeeded to produce aseptic tomato plants via callus in order to avoid the interference of nematode-attached microbes with plant endophytes when assessing the induced systemic resistance in plants.

Relying on our work to date, we are aiming to expand our study and include some other nematode species and different populations of *M. hapla* so we could see if the same story applies to all of them and/or which differences are involved.

Biological control of PPN has gained an important place in successful agriculture systems and our aim to widen knowledge in nematode suppression by specifically attached soil microbes will be the basis for including a soil microbiome management in integrated control strategies.

Differentiation of field populations of the sugar beet cyst nematode based on a pathogenicity gene

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An improved investigation of intra- and interpopulation genetic variation is required to follow the epidemiology of the important sugar beet cyst nematode *Heterodera schachtii*, and design an effective control management with respect to specific properties of local populations.

The venom allergen like protein gene, *vap1*, is an essential pathogenicity gene of *H. schachtii* which is expressed during the initial period of root penetration and migration. The secreted effector protein interacts with the immune system of the host plant and thus is probably under strong selective pressure, so that the *vap1* gene is expected to exhibit high genetic variation among populations of *H. schachtii*.

In our study we aimed to develop and apply the genetic fingerprinting technique PCR-DGGE to resolve gene variants of *vap1*. From each individual of *H. schachtii* up to six variants of the gene were amplified by PCR which differed in DNA sequence and appeared as separate bands in DGGE. PCR-DGGE profiles from multiple cysts from a field reflected the relative distribution of *vap1* variants in the population. Populations from distant fields significantly differed in *vap1* allele frequencies. Significantly different *vap1* patterns among populations from selected sugar beet regions in Germany were detected. The concomitant differences in aggressiveness towards host plants will be investigated. Conclusions of our results with respect to spread of populations and selection of *vap1* gene variants will be discussed.

Nematodes contributing to apple replant disease, and as indicators of soil quality

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Understanding the etiology of apple replant disease (ARD) is challenging owing to the fact that many agents including fungi, bacteria and nematodes play adverse roles in the development of the disease. Nematodes occupy a key position in the ecosystem and as such are involved in ecosystem functioning as well as being indicators for soil quality. This study seeks to elucidate the contribution of nematodes to ARD by investigating the long term soil decline in apple fields, to examine the changes in nematode community structure and how they influence the plant-associated microbial community. Two samplings per year will be carried out from three different apple fields, each with four replicate ARD plots and control plots. In the ARD plots apple was planted yearly since 2009. The control plots were covered by grass and have been switched to apple in 2016. We will utilize both morphological and molecular techniques such as PCR-DGGE, qPCR and amplicon NGS on the samples to compare nematode communities among ARD and control soils, and to correlate ARD severity to nematode species abundance. The NGS approach using Illumina Miseq 18S rRNA gene sequencing will be combined with SMRT-CCS sequencing of large fragments to resolve the nematode diversity in both ARD and control plots. This will enable us to assess soil quality based on ecological indices, to find indicator species and their associated microbes that may contribute to ARD. A preliminary sampling in Ellerhoop in December 2015 revealed that *Paratylenchus spp*. were the most abundant nematodes in the ARD plots while *Tylenchorynchus spp*. were mostly associated with control plots. These species feed on root surfaces and may not pose serious economic damage to apple plants as single entities. However, their potential synergistic role in the disease will be investigated. A colonizer-persister analysis on the free-living nematodes in both ARD and grass fields suggested a gradual shift from a stable environment with N enriched, conducive environment to more C enriched, less stable (stressed) environment with time. Additional sampling data coupled with molecular analysis may give us better understanding about the role of nematodes in ARD.

Ti Host status of different cover crops for *Pratylenchus penetrans*

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Cover crops that are poor or non-hosts are effective in reducing nematode population densities below damaging levels. Greenhouse experiments were conducted to evaluate the host status of nine cover crops to *Pratylenchus penetrans*. The average number of nematodes per root systems and the nematode multiplication rate (ratio of final population density to initial population density = Pf/Pi) were determined 10 weeks after inoculation with 745 mixed stage nematodes of *P. penetrans*. The cover crops tested were common bird's foot (*Ornithopus sativus*), forage rape (*Brassica napus*), rape seed (summer oil type) (*Brassica napus*), Italian ryegrass cv. Tetraflorum (*Lolium italicum*), common vetch (*Vicuña sativa* subsp. nigra), Sun flower (*Helianthus annuum*), lentil (*Lens culinaris*), buckwheat (*Fagopyrum esculentum*), fodder radish RSAS1037 (*Raphanus sativus*). Maize (*Zea mays*) and French marigold (*Tagetes patula*) were included as susceptible and non-host controls respectively. The results indicated that the susceptible control maize supported only a low level of nematode reproduction which was not expected. However, most of the cover crops tested supported significant levels of nematode reproduction. The highest nematode multiplication rate was obtained in lentil (Pf/Pi = 45.9) followed by common vetch (Pf/Pi = 19) and rape seed (Pf/Pi = 5.8) and the lowest was recorded in Italian ryegrass (Pf/Pi = 0.6). The Pf/Pi value of the other cover crops tested ranged from 2.6 to 3.9. Among all the cover crops tested only Italian ryegrass reduced the reproduction of *P. penetrans*.

Session 4

Application technique and Crop production

High precision weed control by a direct injection system

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Often agricultural tank mixtures with several plant protection products (PPP) are used, whereby a site-specific application of individual pesticides is impossible. With direct injection systems, a site-specific use of single PPP on the other hand is possible. Direct injection systems dose pesticides and water from separate containers in real-time with immediate mixing before application, without having incurred residues. The technical implementation in practical devices represents a major challenge. A prototype of a field sprayer with direct injection system was developed and used in first practical tests in cooperation of Herbert Dammann GmbH and the Julius Kühn-Institute.

Precision Farming requires a site-specific application of plant-protection products. Without the direct injection in crop protection site specific application cannot be implemented in agriculture. The aim of the project is the intensive testing of the direct injection system in the field in order to evaluate the reliability of the system and the effects of site-specific treatment. An important question in this context is how to optimize the handling of the prototypes, the electronics and the direct injection system itself. An important role for precision farming is the handling of residues and the creation of application maps. For the market maturity and the consequent implementation into commercial use this is of fundamental importance.

To verify the accuracy of the direct injection system, different plots were calibrated in a field test on farmland with GPS to create an application map. Based on this map, the prototype treats the entire area and the selected parcels with various herbicides. The plots were designed to get different section widths for use with different plot sizes.

The effectiveness of the treatment to the appropriate plots confirmed the metering accuracy of the direct inject system. The switching at the beginning or end of each plot showed that the developed field sprayer works without delay with direct injection. The investigations have shown that the sprayer prototype with direct injection is able to apply liquid formulated pesticides site-specific and without delay times. In a further development step, the operation and cleaning of the prototype will be simplified. The initial experiences with the sprayer prototype show that practical systems for delay-free direct injection can be realized and a site-specific application of various pesticides is possible.

Market maturity for sprayer equipped with sensor technology for gap detection?

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Due to social and political objectives, the sprayer industry is requested to develop sprayers, which enable the reduction of potential input into the environment. Simultaneously, the reduction of the amount of plant protection products (PPP) and the minimization of drift are in focus. Following these requirements, different sensor systems e.g. ultrasonic and infrared sensors, camera systems and laser-sensor were developed and used by researcher but up to now no system achieved market maturity.

First and foremost, in a prior project "LADUS", a sprayer with radial fan was equipped with a harmonized number of sensors (IR01) and nozzles for an optimized gap detection.

In the year 2015, field experiments in Jork (fruit growing region "Altes Land") have shown that the avoidable specific fluid volume in the gap area of apple trees can be reduced to a minimum of 10 % compared to a full application with 100 %. At the same time the savings of PPP can be increased significantly. In general, the savings potential is depending on orchard structure and age of the trees.

Presently, in the project "OLSVA" in total three different sprayer types were equipped with a different number of infrared sensors and nozzles for optimized gap detection usable in different cultivars. All sprayers should be tested in diverse climatic conditions and fruit growing regions of Germany for practicability.

As an objective of the project, the achievement of market maturity for the sprayers with different fans and a retrofitting kit for sprayers in use is in focus.

Crop Production of the future – possible without a rethinking?

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The current production systems in arable farming have reached their limits. Sizes of machinery are continuously increasing. Compaction and limits on the road are the consequences. Production-related restrictions like nitrogen pressure and development of resistances against plant protection products are further problems. Last but not least, the sociopolitical acceptance of crop production is questioned in public opinion. Due to these circumstances the question arises if the system of crop farming which has been adapted to the machinery available on the market is the right strategy for the future. Why not going the other way round and decide what a plant production system has to look like to be at an optimum and then decide what kind of machinery is needed to cultivate? Following this idea the plants must be in the focus.

The demand of plants to a variety of factors is the origin for their growth. Local parameters, like the sufficient access of light, water or nutrients are some examples. Furthermore, different soils and the plant health are factors with high impact on best possible growth. Moreover, the available growing space is another parameter. An optimum would be in a triangulated growing system where all plants have the same distance to their neighbors. At the moment neither the typical seed drilling nor single-seeding is achieving this optimized, homogenous and equal plant sharing. As a result of this triangulated system, the sowing density can hardly be reduced. Suitable wheat plant densities can be reduced by approximately 60-70%. Among various environmental factors, location and soil requirements of cultures are the most important ones. There are cultures existing like rye with low demands concerning location parameters and soil quality or ones with high demands like sugar beets.

The current practice is using soil mapping for agricultural areas. By using a map overlay on the basis of soil mapping and corresponding yield maps one can deviate so called potential-maps of a field. That permits to draw conclusions regarding the location heterogeneity.

With this location heterogeneity, it enables the opportunity to adapt different cultures to single soil properties so that there is more than one crop on a field. Several site-specific crop rotations might be adjusted to the location heterogeneity.

With this approach guaranteeing an optimum growing space and adapting crops and crop rotations to partial surfaces, the present situation in production systems in arable farming mentioned above can be prevented. Thus an essential element in forthcoming, sustainable and efficient crop production can be set.

Session 5

Viticulture

Studies on flowering time control in grapevine

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Viticulture is impaired by effects of biotic and abiotic stress at the same time. Apart from resistance research, studies of phenological traits such as flowering and ripening are becoming increasingly important due to climatic change. Especially high light intensities and temperature promote an early development and hence negative effects like e.g. late frost danger during spring time. To counteract this problem breeding programs aim at selecting genetically determined late flowering within new breeding lines. However, knowledge about which genetic loci are involved in the complex flowering time control network of grapevine is still limited.

Phenotyping of flowering time was carried out during 10 years on a mapping population derived from a cross of the early-flowering breeding line GF.GA-47-42 and late-flowering cultivar 'Villard Blanc' consisting of 151 F1 individuals ("basic population"). Subsequent QTL analysis showed QTLs for time of full bloom on seven chromosomes, yet some of them span rather large chromosomal regions. Limitation and verification of the detected QTL regions is intended by the refinement of the underlying genetic map and QTL analysis in further populations. For this purpose the "basic population" was expanded to about 1000 F1 individuals ("extended population") and a half-sib population was included in the experiments.

Identification of candidate genes so far was realised by a global approach using sequence information of model organisms, which led to various candidate genes spread over the whole grapevine reference genome. However, for a detailed investigation of the detected QTL regions screening for conserved domains known to be associated with flowering time is in progress.

The analysis for correlation of the allelic constitution in the QTLs with the flowering time phenotype will lead to the identification of genetic markers to be used in marker assisted selection (MAS). This will accelerate the selection process for late flowering breeding lines. First investigations within the basic and extended population led to several potentially suitable markers. Their analysis in a set of related and unrelated cultivars will clarify their general usefulness for the prediction of flowering time phenotype and therefore in MAS.

Influence of the pruning system on the fungal community of grapevine *(Vitis vinifera)*

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The novel grapevine training system called semi minimal pruned hedge (SMPH) is an innovative production system, which is economically beneficial, environmental friendly and climate change adapted. In this system the time and money intensive pruning process, usually done by hand, is accomplished by a mechanical harvester, which tremendously affects the production costs as well as the physiology of the plant.

A grapevine stock trained in SMPH shows more woody canes, a wider leave canopy and more bunches carrying fewer berries, compared to the traditional vertical positioning system (VPS).

Whether and how these physiological changes influence the fungal community of grapevine plants is mostly unknown. For that reason the aim of this three year study is to compare the fungal community of grapevine trained in SMPH and VPS. The main focus is on the susceptibility of the respective training system against fungal grapevine diseases like grey-mould (*Botrytis cinerea*), powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*), but also grapevine trunk diseases (GTD), e.g. Esca. The information which will be collected during this study shall support farmers by adapting their plant protection system to the pathogen situation in vineyards with SMPH trained grapevines.

Another aspect of this work addresses the composition and the temporal development of the fungal endophytic community in grapevine. For this purpose fungi from grapevine branches with different ages (2 months – 8 years) are isolated and identified. The analysis of the fungal communities over the time will help to better understand the role of certain fungi in the grapevine wood, especially of GTD-associated fungi, e.g. *Cadophora luteo-olivacea* or *Phaeomoniella chlamydospora*.

‘Riesling Rot’ and other grapevine berry color mutants from the German-speaking area

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The first mention of ‘Riesling’ goes back to a bill of the Earls of Katzenelnbogen in Rüsselsheim from the year 1435. However, a unified spelling of the name ‘Riesling’ did not exist at that time. The variety was mentioned in the course of the 15th century as among others with the name Rueßelinge, Ruesseling, Rüszlinge or Ruszling. The origin of the name is to this day highly controversial. According to the latest findings by the researcher Prof. Dr. Jürgen Udolph, the name derives from the so called ‘Rußflecken’ (soot stains, lenticels), which become visible at a later ripening stage. As an offspring of ‘Heunisch Weiss’ and a presumed seedling of ‘Traminer’ and *Vitis sylvestris,* it can be assumed that ‘Riesling Weiss’ originated in the Rhine Valley between Karlsruhe and Worms. With an acreage of 23.440 hectares (22.9% of the total German winegrowing area) in the year 2014, ‘Riesling Weiss’ is the most widely planted grapevine variety in Germany.

About the origin of the colored variation ‘Riesling Rot’ it is not much known. However, it can be suggested that the cultivar was already present in the mixed plantings of the late Middle Ages with a low acreage. After the phylloxera crisis, the variety disappeared nearly completely, but survived in collections and was interspersed in a few old vineyards. After the process of clonal selection starting in 1991, ‘Riesling Rot’ is classified for the wine growing region Hessen since 2002. Compared to other color mutants, the variety shows a relatively high back mutation rate to white and due to its world famous relative ‘Riesling Weiss’, the public interest on the color origin of this cultivar is very high.

The anthocyanin biosynthesis in blue/black varieties is controlled by two adjacent MYB-related transcription factor genes, *VvmybA1* and *VvmybA2*, whereas two loss-of-function mutations in both genes, an insertion of a Ty3-gypsy-type retrotransposon (*Gret1*) in the promoter region of *VvmybA1* and two amino acid-changing mutations in the coding sequence of *VvmybA2*, were identified leading in combination to a non-functional white allele. Due to recombination events of the white allele, red-skinned cultivars like ‘Riesling Rot’ should possess at least one functional *myb*-gene variant leading to a weaker anthocyanin formation compared to typical red wine cultivars with blue/black berries.

This work focuses on the molecular analysis of the color recovery in ‘Riesling Rot’ and other color mutants of famous varieties from the German-speaking area like ‘Silvaner Blau’ or ‘Elbling Rot’.

Effects of plant protection intensity and pruning method on functional arthropod biodiversity and the effectiveness of natural pest control in vineyards

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Despite ongoing efforts to reduce pesticide and fungicide use in viticulture, the economic and ecological stability of vineyards is still limited by intensive plant protection measures. One promising approach for a sustainable intensification of viticulture could be growing fungus resistant grape varieties as a semi-minimal pruned hedge (SMPH). This practice can reduce the fungicide-input by up to two thirds. Additionally, in contrast to the traditional trellis system (TS), the SMPH offers a structurally more diverse habitat and a different microclimate, therefore potentially offering new niches for pest and beneficial arthropods.

Using the beat sheet method and baits made from grape berry moth (*Lobesia botrana*) eggs, we investigate the effects of reduced plant protection intensity and the pruning method SMPH on the functional biodiversity in the vineyard and its effects on natural pest control.

Predatory arthropods, particularly spiders and predatory mites, benefit from minimal pruning and reduced plant protection respectively. Consequently, the predation pressure on *L. botrana* eggs is increased. Pest mites follow the opposite pattern, showing an increase following more intense plant protection measures.

The actual predator-prey interactions in this ecosystem have yet to be examined, but it is likely that pest mite populations are directly controlled by predatory mites and therefore depend on their numbers.

Spiders are important predators in all terrestrial ecosystems and are very likely to also play a big role as natural pest control agents in viticulture. Promoting their abundance and biodiversity can contribute to a stable vineyard community that is less susceptible to pest arthropods and arthropod-transmitted diseases.

Poster Session

Priming of soybean to enhance the plant defense against phytonematodes

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In their natural environment plants are continuously exposed to a wide range of abiotic and biotic stresses, for example pathogens. Those can lower the crop productivity and influence our food security. Priming the crop plants for an enhanced defense against pathogens is an environmentally friendly strategy of plant protection. Prior priming of plants by an inducer results in a faster and stronger response of the plant defense to following pathogen attack. In our work we tested whether soybean can be primed for enhanced defense against the phytonematode *Pratylenchus penetrans*, which is a major pest of soybean in Germany. As inducer we used *Sinorhizobium meliloti* strain expR+, producing *N*-acyl homoserine lactones (AHL) as signaling molecules. In a greenhouse experiment, the rhizosphere of soybean plants (cv. Primus) was inoculated with the expR+ strain for two times and consecutively. Two weeks after this inoculation, the plants were exposed to *P. penetrans*. Significantly less nematodes invaded the roots of expR+ treated plants compared to non-primed plants, or compared to plants inoculated with the attM strain, a derivative of expR+, which does not accumulate AHL. This suggested that priming of soybean with AHL might be a useful strategy for plant protection. In the next step we will test whether soybean varieties differ in priming capacity, thus making it a possible target for breeding approaches.

Does the interaction of host species and host's substrate affect the olfactory host search of the larval ectoparasitoid *Holepyris sylvanidis*?

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During the olfactory host search, parasitoids use volatiles that are directly associated with the host (e.g. host pheromones or volatiles released from host faeces) or derived from host's habitat or the host's food plant. Over the last decade, research revealed that the background odour (here: the host's food substrate) can affect a parasitoid's host-finding behaviour by enhancing, masking or neutralizing the attraction to host-specific compounds.

Holepyris sylvanidis is a polyphagous ectoparasitoid on larvae of different coleopteran species. All of its potential host species are major pests in the food processing industry, infesting diverse stored products. So far, only the interaction between *H. sylvanidis* and the confused flour beetle *Tribolium confusum* has been in detail. A previous study showed that *H. sylvanidis* females locate *T. confusum* by using volatiles derived from faeces of *T. confusum* larvae reared on wheat grist. Moreover, volatiles released from the food substrate significantly enhanced the attraction to (*E*)-2-nonenal and 1-pentadecene, two key compounds of the faecal odour.

These results raise several questions: 1) which compounds are used by *H. sylvanidis* to find larvae of alternative host species and 2) do other food substrates (here: host's substrate) have the same effect on the host volatile composition and the parasitoid's host-finding behaviour? Therefore, we started to investigate 1) the acceptance of different host species (three *Tribolium* species and *Oryzaephilus surinamenis*) reared on the same food substrate (wheat grist) and 2) the effect of different host's substrates (sorghum, wheat and rice grist) on the host-odour composition on the standard host *T. confusum*.

Our initial results show that all three *Tribolium* species are accepted as hosts and allow complete parasitoid development. First chemical analyses via GC-MS of the cuticular hydrocarbon (CHC) profiles of the three *Tribolium* species indicated the presence of similar compounds. Of the tested *Tribolium* species, the dark flour beetle *Tribolium destructor* was a not yet described host. In further experiments, we will compare the CHC profiles and larval faeces of the respective host species as well as different host's substrates for qualitative and quantitative differences by GC-MS. In a four-field-olfactometer, we will, furthermore, test if the detected compounds can affect the host-finding behaviour of *H. sylvanidis* females.

Overall, we may thus identify compounds which are present ubiquitously in all host species or host's substrates as well as compounds which are host- or substrate-specific.

Finally, the identified compounds which have been shown to be attractive will be used to develop a suitable monitoring trap for *H. sylvanidis*. Our results might thus not only enhance the understanding of a parasitoid's host-finding behaviour in relation to different host species and host substrates, but also improve the application of *H. sylvanidis* in Integrated Pest Management of stored products.

Development of methods for pre-symptomatic detection of grapevine diseases like esca, phytoplasmoses and viruses using hyperspectral sensors

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In the course of a growing season grapevines have to compete against many different pathogens which is flanked by plant protection treatments. For a number of diseases like powdery and downy mildew since long suitable control strategies are available. In contrast, diseases/ disease complexes like esca, phytoplasmoses and viruses are especially problematic since they cannot be cured by chemical plant protection. The pathogens can be present in vines in a latent mode without showing any symptoms but finally leading to losses in yield as well as quality and eventually resulting in death of infested vines. Therefore, the BigGrape-project focuses on the analysis of these disease complexes. Since esca, phytoplasmoses and viruses can be transferred during grafting dissemination with the young grafted vines cannot be excluded. Hence, early diagnosis is particularly important for viticulture.

The BigGrape-project aims are the development of pre-symptomatic and specific detection methods of the endogen grapevine diseases esca, phytoplasmoses and viruses. These methods serve as the basis for a regional monitoring of vineyards and the development of control strategies. Within the project a phenotyping pipeline for the early detection of these diseases shall be developed using non-invasive air- and ground-based hyperspectral and multispectral analyses. Visual rating, PCR-based methods and chemical analyses are applied to get reference data to be correlated with the data of the spectral analyses.

This new and non-invasive method provides the opportunity to optimize the monitoring of grapevine diseases in viticulture and also the possibility of selection for grapevine propagation and breeding.

Enrichment of IncP-1 plasmid carrying bacteria in the rhizosphere of lettuce and tomato – is there a fitness advantage?

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Plasmids can offer bacteria a wealth of accessory functions enabling the survival and adaptation of a population in heterogeneous conditions or under environmental stresses. IncP-1 plasmids belong to the group of broad host range plasmids which are of great interest due to their ability to shuttle between different members of bacterial communities under various conditions. Furthermore, the plasmids can replicate in a wide range of hosts. This plasmid group was detected in different environments such as sewage, soils and river sediments and its abundance seemed to be correlated with pollutants. They have a conserved plasmid backbone containing e.g. the *korB* gene which is specific for this plasmid group and is used for the detection of IncP-1 plasmids. The backbone contains also hot spots of insertion where accessory elements can be inserted. IncP-1 plasmids can carry multiple antibiotic resistances and could easily enter the food chain via plant-associated bacteria.

An enrichment of IncP-1 plasmids in soil is often found in response to pollution, like the application of manure containing antibiotics. Recently, an enrichment of IncP-1 plasmids could also be shown in the rhizosphere of lettuce plants grown in untreated soil. The result based on the quantification of *korB* in total community DNA and was compared to bulk soil. The enrichment of IncP-1 plasmids in the rhizosphere compared to bulk soil is plant species dependent which could be shown in another study investigating the rhizosphere of tomato and potato. The abundance of IncP-1 plasmids was higher in the rhizosphere of tomato compared to bulk soil, but not in potato rhizosphere.

To further investigate the unexpected high abundance in the lettuce rhizosphere, IncP-1 plasmids were captured from rhizosphere bacteria in a triparental mating using their ability to mobilize an IncQ plasmid. The majority of the transconjugants obtained belonged to the IncP-1β subgroup.

In current experiments, competition experiments are performed to analyze the potential fitness advantage of *Pseudomonas putida* in the rhizosphere given by the plasmids. Therefore, different *P. putida* transconjugants are inoculated together with a non-plasmid bearing strain in a 50/50 ratio into DS soil with lettuce and tomato plants. Rhizosphere samples are taken using the Stomacher method by which the soil directly attaching the root can be washed off. The samples are used for CFU counts and total community DNA extraction. Quantification of *korB* is done by taqman qPCR to analyze a potential enrichment in non-inoculated plants. First results will be presented at the conference.

High resolution mapping of resistance genes against barley mosaic disease and *Barley yellow dwarf virus*

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Barley (*Hordeum vulgare*) is an important crop in Germany and on the world wide level. The crop is hit by many fungal and viral diseases reducing yield and production. Therefore, breeding for resistance is of prime importance. Due to the transmission by the soil-borne plasmodiophorid *Polymyxa graminis*, yield losses caused by *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV) cannot be prevented by chemical measures. Regarding *Barley yellow mosaic virus* several loci conferring resistance to either BaMMV or BaYMV or both are known. Another important virus disease of barley is the aphid transmitted *Barley yellow dwarf virus (*BYDV*).*

Among others, *rym13* located on the long arm of chromosome 4H provides resistance to BaMMV/BaYMV. Regarding BYDV the locus *Ryd3* on chromosome 6HL is known. The aim of this study was to isolate the respective genes using a map based cloning approach.

For both resistance genes a high resolution population was established. Using different high density maps and genomic resources, marker saturation of the intervals of interest was conducted.

Using genotyping by sequencing (GBS) and whole genome sequencing (WGS) *rym13* was located on one BAC comprising different candidate genes.

For *Ryd3* the high resolution mapping population was extended to 7,177 F_2 plants. Due to a low recombination rate in the region of interest cosegregation of markers and phenotype could not be resolved even by analysing more than 14,000 meioses. By mapping these markers to the current physical map of barley it turned out that there are about 228 million bp between flanking markers comprising more than 2,000 annotated genes.

In a next step a mutation programme will be initiated using EMS to create genetic variation and isolate *Ryd3*.

High resolution mapping of leaf rust resistance gene derived from barley landrace MBR1012

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Barley production is affected by a large number of diseases that cause high economic damages. Leaf rust caused by *Puccinia hordei*, is one of the most destructive barley diseases causing high yield losses in susceptible cultivars worldwide. Therefore, durable and broad spectrum resistance to pathogens is of prime importance for sustainable barley production. Resistance gene $Rph_{MBR1012}$ identified in the landrace "MBR1012" exhibiting a hypersensitive reaction to the very virulent *P. hordei* isolate I-80 has been previously mapped on chromosome 1H. To isolate this gene via a map based cloning approach, construction of a high resolution mapping population was undertaken, i.e. extending the resolution of the mapping population, increase marker density and by this approach identify candidate genes. High-resolution mapping resulted in the identification of 433 heterozygous recombinant and 15 homozygous recombinant F_3-families derived from 2663 F_2 plants by analyzing two co-dominant flanking markers, which were originally separated by 8.0 cM. Out of these, 317 segmental homozygous recombinant inbred lines (RILs) were identified up to now. Due to this work the genetic resolution of 0.023 % recombination from previous work was increased to 0.01 % and the genetic distance between flanking markers was estimated at 6.6 cM.

Currently, 17 markers located between the two flanking markers have been genotyped in order to saturate the locus. Homozygous F_4-RILs were in parallel infected with the *P. hordei* isolate I-80 and segregated 139 resistant: 172 susceptible ($X^2_{1:1}$ = 3.525), indicating a monogenic inheritance of resistance.

PRUNI-REPEL: Utilization of host plant volatiles for controlling the vector of '*Candidatus* Phytoplasma prunorum'

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The plum psyllid *Cacopsylla pruni* is a serious pest in fruit production. This phloem-feeding jumping plant louse is the vector of the phytoplasma '*Ca.* Phytoplasma prunorum'. A specialized bacterium located in the phloem tissue of *Prunus* ssp. which causes one of the most severe diseases in stone fruits, the European Stone Fruit Yellows (ESFY). Infected *Prunus* cultivars yield poorly and lead to high economic losses. During feeding on the phloem sap of infected plants the phytopathogenes are acquired by *C. pruni* and spread by transmission feeding of infected psyllids on healthy plants. To lower the possibility of Phytoplasma transmission and reduce the number of new infections innovative control strategies against the vector are required.

Phytophagous insects use different cues for identification of their host plants. In addition to visual stimuli also allelochemicals play an important role for host recognition and acceptance. This circumstance is used to elaborate a push-and-pull strategy against *C. pruni* with synthetic volatile organic compounds. Push-pull is a common technique in pest management. Cultivars are supplied with repellent stimuli to repulse the pests and attractants are used to lure the insects away from the protected resource.

Within one generation *C. pruni* is alternating its host plant two times. After development of nymphal stages on *Prunus* ssp., the young adults, called emigrants, migrate to their overwintering hosts, spruce and other conifers. In early spring they return (remigrants) to reproduce on *Prunus*. This alternation of hosts leads to a change in the preference for olfactory stimuli during the life cycle of *C. pruni*.

Due to the volatile blends emitted from *Prunus* rootstocks, *Prunus* cultivars and spruce, different mixtures of potential repellents against remigrants and emigrants were identified.

In 2015 first successes have become apparent using repellents against *C. pruni* in a field experiment. The number of captured emigrants was reduced by the application of dispensers with a mixture of repellent compounds in attractive *Prunus* rootstocks. In 2016 the ability of repellents to inhibit the attraction of host plants was evaluated by bioassays in a Y-shaped dynamic olfactometer. A new formulation for repellents was tested under laboratory and field conditions.

Pre-infected grapevine planting material as a cause of Esca in vineyards?

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The grapevine trunk disease (GTD) Esca is caused by several wood-inhabiting fungi and can be observed in wine-growing regions all over the world. The fungi *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium aleophilum* (*Pal*) and *Fomitiporia mediterranea* (*Fmed*) are considered to be the main pathogens in Europe. Direct control strategies are very limited and their effectiveness is still questionable. As considerable economic losses may occur and even young vineyards and planting material can be affected, information on biology and epidemiology of the involved pathogens is required in order to develop effective and sustainable control measures. Accordingly, the current project aims at the investigation of epidemiological factors of *Pch*, probably the most important Esca pathogen for early infections of planting material, during nursery grapevine production. Over a period of three years the grapevine production process of three selected nurseries was investigated regarding the occurrence of *Pch* in various substrates, such as grapevine wood, dipping baths, callusing media, air and soil.

Wood samples, in particular rootstock wood, from grafting material, newly grafted vines, grafted vines from nursery fields and vines ready for sale were subjected to visual evaluation of *Pch*-related wood symptoms and detection of the actual presence of the pathogen via culture on potato dextrose agar (PDA) and a very sensitive nested Polymerase chain reaction (PCR) method. In the same way, culture and nested PCR measures were applied in order to detect *Pch* in dipping baths, callusing media and soil from nursery fields. For detection of airborne *Pch* conidia spore traps were installed in the nurseries and tested through nested PCR.

Observations have shown an increase of *Pch*-associated wood symptoms over the year and the highest symptom frequency in vines ready for sale. The pathogen itself was frequently detected in wood, with detection rates being higher after planting of newly grafted vines in the fields, although detection rates were much lower than visual evaluations would have suggested. Regular detections of the pathogen exist for dipping baths and spore traps. In callusing media *Pch* was found only sporadically. No incidence in soil is determined to date. The detection of the Esca pathogen at different stages and substrates during the grapevine production process indicates that potential infection sources do exist. The overall low *Pch* detection rate in wood in relation to the observed frequencies of wood symptoms may point towards the involvement of further fungi, such as species of *Cadophora*, causing similar symptoms in the wood. To this end first investigations were undertaken supporting this assumption.

A transcriptome-based approach for developing breeding lines in *Lolium* sp. with multiple pathogen resistance

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Ryegrass (*Lolium* spec.) is the most important cool-season forage crop in temperate regions. Though, the seed production is considerably affected by several fungal and bacterial obligate biotrophic pathogens. The overall purpose of this study is directed to developing ryegrass cultivars with multiple pathogen resistance and agronomic adaption to Germany's agricultural conditions. This aim shall be achieved by combining genes for resistances to stem rust, crown rust and bacterial wilt. The pyramidisation shall be accomplished by the use of specific molecular markers which will be derived by bulked segregant analysis combined with next generation sequencing based massive analysis of cDNA ends (MACE) transcriptome profiling. RNA was isolated from bulks of infected and non-infected leaf segments from susceptible and resistant genotypes of various full-sibling mapping populations (n ≥ 200) and their respective parental lines for every investigated pathogen. After MACE was performed, bioinformatic analysis detects SNPs and transcripts that were exclusively expressed in the resistant bulk. Thus, 30 molecular markers were genetically mapped to a 50.8 cM spanning region surrounding the stem rust resistance locus *LpPg1*. The development of this high efficient molecular selection tool marks MACE as a fast and reliable method that detects polymorphisms for genetic mapping of candidate genes and obtains to be the method of choice for investigating the molecular and genetic base of resistances to stem rust, crown rust and bacterial wilt.

Establishment of efficient strategies for controlling downy mildew (*Peronospora salviae-officinalis*) and other pathogenic fungi on common sage (*Salvia officinalis*)

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Sage (*Salvia officinalis*) is an important niche culture in Germany providing high revenues through a complex value chain. Since ancient times, sage has been used as a medicinal plant to cure many different diseases and as a spice plant in the kitchen as well. Because sage is a perennial plant, the stands can be used up to 5 years and all plant organs (stem, leaf, flower and root) are used for a variety of medical products. Therefore pathogen control is crucially important to avoid the build-up of pathogen populations.

Currently, sage cultivation in Germany is threatened by emerging diseases: A downy mildew pathogen (*Peronospora salviae-officinalis*) has been spreading through Central European sage cultures in the last few years. This parasite is specific for sage and has only been classified as a species of its own in 2009. Additionally, problems by stem and root diseases caused by *Phoma exigua* var. *exigua* have intensified. Together, the two pathogens have caused yield losses of up to 50% in some areas in Germany recently.

Because downy mildew on sage is a newly emerging disease, hardly anything is known about this pathogen. Therefore, the main aims of the present project are to elucidate the biology and epidemiology of the two pathogens on sage. Specifically, we want to (1) investigate and reproduce the infection process in climate chambers. (2) Develop a specific, sensitive and quantitative method for detecting the pathogens in seed and soil samples using qPCR. (3) Monitor the distribution and spread of the pathogens in sage cultivation throughout Germany. Taken together, better understanding of the epidemiology and infection biology of the two pathogens will help providing advice for pathogen control and sustainable sage production.

Non-invasive phenotyping methods for abiotic and biotic stress in grapevine breeding

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Climate change impacts greatly upon agricultural ecosystems and also viticulture is directly affected by the effects of global warming. As a consequence, changes in frequency and severity of extreme climatic events are projected. Increasing occurrence of heat waves and droughts and following heavy rain events will lead to higher susceptibility of vines to pathogens. Due to their considerably shorter generation time pathogens show usually better adaptability to climatic conditions than plants. This leads to more problems with diseases like powdery mildew and downy mildew in the field. The interactions between plant resistance and the expected simultaneously increasing aggressiveness of the pathogens are a major biotic impact of climate change for European viticulture.

The project Vitismart aims at establishing a resilient and flexible viticulture system, which is able to recover itself quickly from abiotic and biotic stresses. To achieve this, more resilient cultivars should be identified and genetic resources for resistances should be found. In collaboration with project partners, the beneficial use of microorganisms to improve the plant resistance are examined.

As an example of abiotic stress heat stress will be examined. With regard to biotic stress, on the one hand breeding material will be screened for powdery and downy mildew resistance. On the other hand berry bloom, being one of the parameters of Botrytis resilience, will be evaluated.

To validate the resilience of cultivars and breeding material towards abiotic and biotic stress, an objective and time saving phenotyping method will be developed using hyperspectral imaging to screen leaf disc assays, bunches and single berries in the laboratory. The collected sensor data will be correlated with visual inspections using the International Organisation of Vine and Wine (OIV) descriptors for (1) Downy mildew (2) Powdery mildew and (3) Berry bloom, microscopic studies and chemical analysis to establish a phenotyping pipeline.

After establishing the hyperspectral measurements in the laboratory and for a small set of plants, it will be tested if the method is transferable to field conditions. The method finally could update the existing field phenotyping techniques and improve grapevine breeding programs.

Identification and mapping of QTL for *Zymoseptoria tritici* resistance in the winter wheat accession HTRI 1410

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Zymoseptoria tritici, the causal agent of Septoria tritici blotch (STB) causes yield losses up to 30 – 50% in wheat, globally. Growing of resistant cultivars is the most cost effective and environmentally friendly way to avoid these losses. *Zymoseptoria tritici* causing leaf spot can be found worldwide and has gained importance due to changes in wheat cultivation.

Aims of the proposed project are the exploitation of the Zymoseptoria resistance of gene bank accession HTRI 1410 via genetic analyses and development of molecular markers and its utilization in wheat breeding, in order to broaden the genetic basis of *Z. tritici* resistance in wheat.

In extensive screening programmes for resistance, the gene bank accession HTRI 1410 turned out to be resistant in field tests and will be tested in greenhouse tests against 22 STB isolates carrying virulences against the 18 STB genes described up to now. In order to get information on the genetic background of the STB resistance in HTRI 1410, a DH-population consisting of 135 lines derived from crosses of HTRI 1410 to three susceptible cultivars was generated.

The leaf infestation after artificial infection of the DH population was repeatedly estimated the last two years at three different locations in Germany and the area under the disease progress curve determined. Statistically significant phenotypic differences between the DH lines as well as between locations were detected. In addition climate chamber and greenhouse experiments with STB-isolates being avirulent on HTRI 1410 but virulent on the other parental lines will be conducted.

In parallel, this population has already been genotyped by the 90 k iSelect chip. The genotypic data were used for map construction. About 6.100 SNPs turned out to be polymorphic between the resistant cultivar and the susceptible lines. In total 1.118 marker has been mapped on the A genome with an average distance of 3.46 cM, 1.326 marker were mapped on the B genome with an average distance of 2.76 cM and on the D genome 267 markers were mapped with an average distance of 5.69 cM. QTL analyses based on two years phenotypic data is in progress and first results of these studies will be presented.

Feeding behavior of Green peach aphid (*Myzus persicae)* on *Asparagus* spp. susceptible and resistant to *Asparagus virus 1*

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The Green peach aphid (*Myzus persicae* S.) is probably the most important vector of *Asparagus virus 1* for cultivated asparagus (*Asparagus officinalis* L.). Stylet-borne viruses such as *Asparagus virus 1* (AV-1) will be transmitted during brief intracellular punctures of aphid feeding.

The control of *M*. *persicae* has relied almost exclusively on the use of chemical insecticides, but insecticides normally do not kill aphid vectors quickly enough to prevent the transmission of non-persistent viruses. Therefore, a control of this aphid on asparagus and the virus diseases must base on host plant resistance. In the cultivated asparagus there is no AV-1 resistance. Resistance to AV-1 was found in various wild relatives of asparagus.

In this study we compared the acceptance of asparagus species as host plant by *M. persicae* and the cell penetrations for the transmission of non-persistent viruses by using the electrical penetration graph technique (EPG). In basic principle of the EPG plant and aphid are incorporated in an electrical circuit. When the aphid penetrates the leaf with its stylet, they complete the circuit and as EPG output specific patterns are recorded. There are relationships of waveforms to aphid activities and the location of the stylet tips in the plant tissue.

The results suggested that *Asparagus officinalis* has been best accepted as a host plant by *Myzus persicae*. Wild relatives of asparagus revealed a different feeding behavior on *M. persicae*. Lower numbers of intracellular punctures reduce the probability of virus transmission but do not exclude it. Our results show that an aphid resistance (vector resistance) of wild relatives can largely be excluded. A specific relation between intracellular punctures and the AV-1 resistance in wild relatives will be further examined.

Interspecific hybridization between *Aspargus officinalis* and *Asparagus prostratus* to transmit *Asparagus virus 1* resistance

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Interspecific hybridization is an excellent method to increase the genetic variability of *Asparagus officinalis* and to develop new cultivars with biotic and abiotic resistance.

A. officinalis is afflicted with a variety of diseases. The most common one is the *Asparagus virus 1* (AV-1), which is distributed worldwide but no virus-like symptoms have been observed on infected asparagus plants. Detrimental effects on vigor, yield and quality as well as increasing susceptibility to fungal diseases are often reported due to viral pathogens. AV-1 is transmitted in a non-persistent manner by the green peach aphid (*Myzus persicae)* and by mechanically transmission. The infection with AV-1 cannot be prevented by pesticides and up to now no AV-1 resistance is known in asparagus cultivars. Therefore breeding for resistance is the best solution for a sustainable asparagus production. The genetic diversity in *A. officinalis* is limited but the wild relative *A. prostratus* is resistant against the AV-1 virus. *A. prostratus* grows on dunes and other maritime habitats on the Atlantic coast. The plants are very different in appearance and character from cultivated forms.

We successfully developed an interspecific hybrid between *A. officinalis* (2n = 2x = 20) and *A. prostratus* (2n = 4x = 40) by manual crosses. We used embryo rescue to overcome the crossing border. The cytological investigation of the hybrid plants (F1) showed the expected number of 30 chromosomes. These plants were back crossed with diploid *A. officinalis* genotypes and plants of the first backcrossing generation (BC1) contain approximate 26 chromosomes. In the next crossing step the BC1 plants will be again back crossed with diploid *A. officinalis* genotypes.

The F1 and BC1 hybrid plants segregate for AV-1 resistance. To identify molecular markers linked to the AV-1 resistance we performed a bulked segregant analysis. In this we pooled DNA samples of 9 resistant hybrids plants and compared it with a bulk containing DNA of 13 susceptible hybrid plants. 75 SSR marker were tested and amplified 185 bands. 66.5 % of them were polymorphic in the parental generation but none of them was linked to AV-1. In the next step the bulks will be increased with DNA of new BC1 plants and the bulked segregant analysis will be performed using AFLP.

Comparative studies of bunch architecture in grapevine varieties and 'Pinot Noir' clones

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A loose grape cluster is a desirable trait in grapevine breeding, since it reduces the abundance and severity of fungal infections. This is due to an efficient air exchange within the grape cluster. The reduced exposure to high humidity or water acts as a physical barrier against pathogens which are in need of high moisture to infect and proliferate, e.g. *Botryotinia fuckeliana*, teleomorph of *Botrytis cinerea*.

The aim of this study is to identify loci of genes influencing bunch architecture and the development of molecular markers linked to loose cluster architecture to accelerate the selection process in grapevine breeding.

The plant material under study is a segregating population (GF.GA-47-42 x 'Villard blanc'), a set of 'Pinot Noir' clones with loose as well as compact clusters and extremely loose clustered table grapes of the 'Cardinal' family.

Phenotypic characterization uses 150 F1 individuals of the segregating population for QTL analysis. The assessments of eleven sub traits for loose cluster architecture revealed that seven of the sub traits show 22 QTLs during two years. Deduced from QTL calculation performed in 2013 and 2014 the peduncle length and the length of the pedicel exhibit five stable QTLs over three consecutive years. However the QTLs discovered so far extend over wide genomic regions and candidate gene suggestion is therefore hampered. Gene ontology enrichment studies within the confidence interval of the QTL regions on the reference genome may be helpful for the identification of candidate genes.

In a transcriptional profiling approach 'Pinot Noir' clones with loose and compact clusters were compared. In a first step RNA from dormant winter buds and compound buds harvested during the growing period were used in a differential gene expression experiment. RNA sequencing was performed at the Max-Planck-Institute for Plant Breeding. First candidate genes could be validated with quantitative PCR. In a second step RNA of inflorescences was sampled at time points when loose clustered clones grow faster than the compact clones. With the repeated experiment during two consecutive growing seasons the kinetics of candidate genes could be tracked and further candidate genes should be revealed.

For model plants, the literature provides information about genes involved in the regulation of floral meristem formation. The expression of these genes is conserved over genetic distances and has a great impact on the inflorescence and bunch architecture. Based on the grapevine reference genome (PN40024) orthologues of these genes should be detected.

Automated evaluation and comparison of grapevine genotypes by means of grape cluster architecture

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Botrytis cinerea (*B. cinerea*) is a necrotrophic pathogen that causes the widely known grey mold disease. With the ability to infest more than 200 crop species and furthermore a high adaptability to fungicides, *B. cinerea* belongs to the high risk fungi in agriculture.

In viticulture *B. cinerea* is responsible for severe damage during warm and wet periods close to harvest. Neither *Vitis vinifera* nor other species do show resistance reactions against Botrytis. Therefore, resistance breeding in general is difficult. Thus, in viticulture the focus is on physical barriers, as they seem to pay a promising part in Botrytis resilience.

Loose cluster architecture of grapes implements less favorable conditions for *B. cinerea*. Therefore, cluster architecture is defined by different traits e.g. the length of the rachis, length of the pedicels, and the number of berries etc. These traits become important goals for grapevine breeding. However, determining these traits is complicated by slow and manual phenotyping, which requires invasive and time consuming methods in the lab. The total aim of this project is mapping of QTL, the development of marker and identification of candidate genes for a loose grape architecture.

In a segregating population of 150 F1-plants the data of cluster and berry traits are taken manually. At the same time, with the optical sensor *Artec Spider*, 3D point clouds of these grape clusters will be generated. These two data sets will be used for the development and evaluation of a novel automated high throughput phenotyping pipeline for grape clusters.

Furthermore, the dataset will be extended by 1000 F1-plants, with the new phenotyping pipeline. These phenotypic traits are then used for extensive QTL-analysis and fine mapping of loose grape bunch traits.

Identification of *Wheat dwarf virus* (WDV) resistance/tolerance in wheat

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Wheat dwarf virus (WDV) causes high yield losses in wheat and other cereals. WDV is transmitted by the leafhopper *Psammotettix alienus*. Symptoms include yellowing and dwarfing of infected plants along with heavy yield loss. Due to global warming, insect-transmitted viruses become more important. Growing WDV-resistant/tolerant varieties is the most effective and environmentally friendly way to control WDV.

Hence, the aim of our project is to identify WDV resistant/tolerant genotypes by screening gene bank accessions and breeding lines for WDV tolerance/ resistance and to identify quantitative trait loci (QTL) by genome-wide association studies.

A set of 500 genotypes comprising different wheat species and wild relatives was tested by artificial infection in gauze houses and under natural infection in field trials in Žabčice, Czech Republic during the last two growing seasons.

The majority of the tested genotypes turned out to be highly susceptible. The susceptible standard cultivar 'Hybnos' revealed an average infection rate of 75% indicating a high infection pressure. Six accessions were identified with a very low infection rate (0 - 12%) in two year trials. Among these, one cultivar showed no yield reduction after virus infection during the test in 2014/15. Crosses with this cultivar were conducted by the breeding partners and SSD/DH populations will be developed. The most resistant genotypes (42) are tested repeatedly in this year. A subset of 250 genotypes was selected and will be genotyped by the 15k iSelect chip. The identification of QTL for WDV resistance and development of molecular markers are the prerequisite to replace the laborious and time consuming resistance tests with WDV-bearing leafhoppers. This will facilitate the integration of breeding for WDV tolerance/resistance into applied wheat breeding.

Toward molecular differentiation of common bunt and dwarf bunt fungi

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Bunt disease of grasses is characterized by the formation of black, powdery teliospores, which partially or completely replace the ovary and thus the seed of the host plant. The causal agents of these diseases all belong to the smut fungal genus *Tilletia*. Some 140 species of *Tilletia* (Ustilaginomycotina, Basidiomycota) are described from their Poaceae hosts. Three of these species, *T. caries*, *T. controversa* and *T. laevis*, cause bunt diseases of wheat. *T. caries* and *T. laevis* are the causal agents of common bunt and *T. controversa* causes dwarf bunt. Common bunt is present worldwide in wheat-growing areas whereas dwarf bunt is restricted geographically and is an important quarantine species in several countries.

The three species are morphologically distinct and also differ significantly in infection biology. Therefore, differentiation of species is currently done based on morphological and physiological features. However, this is very time-consuming and requires expert knowledge. The main aim of this study is to develop a robust, quick and reliable molecular method for the differentiation of *T. caries*, *T. controversa*, and *T. laevis.*

Initially, analyses of standard phylogenetic markers (nuc rDNA LSU, ITS, rpb2, tef1α, β-tub) were performed to find polymorphies between the species. However, using these gene regions very little variability could be found in total, and the few differences observed were neither consistent within nor between species. Subsequently, random detection of polymorphism was explored using ISSR-PCR (inter simple sequence repeats). ISSR-PCR products were sequenced, however, obtained sequences did not show sufficient polymorphism to differentiate the species. The results of our phylogenetic studies confirm that these three bunt species are very closely related, and cannot be differentiated with standard phylogenetic markers, thus even questioning their species rank or suggesting that these species have split only recently.

Because of the failure of these two approaches and because no more genetic information was publicly available, whole-genome sequencing was initiated using Single-molecule real-time (SMRT) sequencing developed by Pacific Bioscience (PacBio®).

In the meantime genome sequences of *T. caries and T. controversa* have been published (Illumina data) which are currently under investigation for potential genomic regions that can be used for differentiation of the three species. As a result one promising gene region has so far been identified. The specificity of this region is currently tested using 70 specimens collected from different geographical region, collected between 1933 and 2016.

Potential candidates for plant proteins interacting with bacterial quorum sensing (QS) molecules

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During the cultivation of crop plants, priming for enhanced resistance using biocontrol agents is an efficient disease management strategy. It results in robust resistance and higher yield. The beneficial affects of the bacterial QS molecules, e.g. *N*-acyl homoserine lactones (AHLs), on resistance and plant growth has been shown in different crop plants. Similarly, the model plant *Arabidopsis,* if pre-treated with the AHL oxo-C14-HSL was more resistant to *Pseudomonas syringae* pv. *tomato* 3000. Oxo-C14-HSL primed plants exhibited stronger activation of MAP kinases AtMPK3 and AtMPK6, followed by higher expression of defence-associated transcription factors *WRKY22* and *WRKY29* along with the *PR1* gene.

So far, AHLs of different lengths of their lipid moiety ranging from 6 to 14 carbons and substitution with oxo or hydroxyl groups in the γ position have been identified. These modifications of the molecular structure of AHLs have impact on the resulting influence on plants, contributing either to resistance induction (longer lipid chains) or growth promotion (short-chained AHLs). Upon AHL perception, the transcriptional reprogramming of various defence and growth related genes modifies the physiology of primed plants. In bacteria, AHLs are perceived through their cognate receptors, often from the LuxR-type family. In animals, the Peroxisome Proliferator-Activated Receptors PPARγ and PPARβ, members of the nuclear hormone receptor (NHR) family, and the ras GTPase-activating-like protein IQGAP1 were proposed as potential candidates for AHLs receptors. Although AHLs induce modifications in development and changes in gene expression, AHL interacting proteins in plants are not yet reported. However, in order to elucidate the precise impact of AHLs on plant defence responses, identification and characterization of AHL perception mechanism(s) is essential.

In this study, we present a search for the AHL-interacting proteins in *Arabidopsis thaliana*, and demonstrate the difference in expression of defence-related genes in oxo-C14-HSL-primed wild type plants and two mutants in potential candidate genes.

Improved construction of full-length cDNA clones of *Apple chlorotic leaf spot virus* and agroinoculation on woody plants by vacuum infiltration

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Apple chlorotic leaf spot virus (ACLSV) is the type member of the genus *Trichovirus* in the family *Betaflexiviridae*. It is distributed worldwide and mainly infects *Rosaceae* fruit trees. The infection generally is symptomless in most commercial apple cultivars, but causes symptoms of deformation, reduced size, chlorotic leaf spots and ring pattern mosaic on leaves of susceptible cultivars. ACLSV forms flexible filamentous-shaped particles, containing a positive single-stranded RNA genome of ca. 7,500 nucleotides excluding the 3'-poly (A) tail. In the present work we constructed full-length cDNA clones of ACLSV using an improved method of In-Fusion assembly, and infected apple seedlings with the constructed clones using vacuum-assisted agroinoculation.

Total RNA of infected apple leaves were extracted for generating viral cDNA full-length fragments. The low copy plasmid vector of pV297 (Prof. Dr. Edgar Maiss, Hannover) was linearized by PCR. The gel-purified fragments of linear vector and DNA fragments of ACLSV were then ligated using the In-Fusion assembly commercial kit. *Agrobacterium tumefaciens* strain ATHV was used in agroinoculation. Vacuum infiltration wasconducted at 500 hPa for 10 min using the vacuum system consisted of a Büchi V-500 vacuum pump and Büchi vacuum controller B-721 (Sigma-Aldrich GmbH, Munich, Germany) attached to vacuum desiccators.

Four full-length cDNA clones were screened from transformed *E. coli* cells by colony PCR. Two clones were infectious on *Nicotiana occidentalis* 37B. The viruses could be further transmitted to healthy *N. occidentalis* 37B by sap-inoculation. By the vacuum-assisted agroinoculation method, apple seedlings were infected with constructed clones in an infection rate of above 90%. ACLSV in inoculated plants was detected by PCR and transmission electron microscopy.

This was the first description of construction of full-length cDNA clones of ACLSV using In-Fusion assembly method. The method is suitable to study ACLSV and likely related filamentous viruses. Apple seedlings were infected using vacuum-assisted agroinoculation. The method is highly efficient and time-saving.

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