



Nachwuchswissenschaftlerforum
Young Scientists Meeting

Sechstes Nachwuchswissenschaftlerforum 2013

27. - 29. November
in Quedlinburg

- Abstracts -



Berichte aus dem Julius Kühn-Institut

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Kontaktadresse

Anja Hühnlein
Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen
Informationszentrum und Bibliothek
Erwin-Baur-Straße 27
06484 Quedlinburg

Telefon +49 (0)3946 47-123

Telefax +49 (0)3946 47-300

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Greetings from the President

Dear Young Scientists,

I cordially welcome you to the 6th Young Scientists Meeting of the Julius Kühn-Institut, which is being held at its head quarters in Quedlinburg.

Being a higher federal authority and a federal research institution with a broad variety of research areas and scientific topics, the exchange of knowledge within the JKI and between the specialized institutes is a key for successful work.

While the institutes' directors hold their regular meeting in Braunschweig to discuss the overall research direction of our institute during the time of your conference, you have the chance to connect your research to that of young scientists from other JKI institutes. You can discuss scientific details and, in general, explore the depth of research areas covered at the JKI.

During the next three days, you will process the scientific exchange by presenting your results and ambitions in talks and as posters. As last year, all contributions to the "Young Scientists Meeting" will be in English. So it is a practical training for international conferences to come in your future. Two keynote lectures will give you deeper insights into the daily business of science, on the one hand from a scientific point of view by Professor Andreas Graner (managing director at the Leibniz Institute of Plant Genetics and Crop Plant Research) and on the other hand from an administrative point by Klaus Kasprzyk (head of administration at the JKI).



Therefore, I am convinced that all participants will personally benefit from the NWF by expanding their knowledge and by increasing their presentation skills. This is, as you all know, very important for your future professional life in science, development or industries. In addition, you get the chance to get to know each other during two joint evening events and expand your personal network, an important base in your future professional life.

We have a very copious and well-founded knowledge at the JKI. Make good use of this benefit. It is an easy way to improve your professional competence and by doing so you simultaneously improve the research quality of the JKI. It is tradition that the meeting is self-organized by you, the Young Scientists of JKI. Thus, you already contribute to our high research quality.

At this point, I wish to express my gratitude to the members of the organization team, who invested their precious time to organize this year's "Young Scientists Meeting", continuing the excellent tradition for the benefit of us all. I hope you all will enjoy your stay at Quedlinburg and return home enriched by the experience and inspiration, both scientific and social.

Quedlinburg, November 2013

A handwritten signature in black ink, appearing to read 'G. Backhaus', written over a light-colored background.

Dr. Georg F. Backhaus
President of the JKI

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Session 1: Breeding

High-Throughput Phenotyping of yield parameters in the vineyard – first steps

Anna Kicherer¹, Katja Herzog¹, Ribana Roscher², Markus Wieland³, Philipp Ruger⁴, Heiner Kuhlmann³, Hans-Peter Schwarz⁴ and Reinhard Topfer¹

¹Julius Kuhn-Institut, Institute for Grapevine Breeding, Siebeldingen

²Bonn University, Institute of Geodesy and Geoinformation, Department of Photogrammetry

³Bonn University, Institute of Geodesy and Geoinformation, Department of Geodesy

⁴Geisenheim University, Department of Viticultural Engineering

Email of corresponding author: anna.kicherer@jki.bund.de

Complementary to genotyping techniques like Marker-Assisted-Selection (MAS), faster methods for plant phenotyping need to be established permitting high quality, objectivity, and precision in phenotypic data recording. Furthermore, for a woody crop plant like grapevine high throughput-(HT)-phenotyping needs to be developed for applications in vineyards rather than in the lab or greenhouses. Adaptable image based phenotyping techniques will be one way to receive plant features non-invasive and with high-throughput.

Quantifying yield parameters is challenging, particularly when measurements need to be done on large samples. Complex shapes and slight variations between genotypes make it difficult and very time-consuming. Objective manual screenings can be done on small samples but this method is rather vague especially when done by multiple persons or varying descriptive standards.

Therefore, as an intermediate step two high-throughput image interpretation tools for the lab (CAT-Cluster Analysing Tool; BAT-Berry Analysing Tool) were developed.

Building up on a Prototype-Image-Acquisition (PIA) system developed in CROP.SENSE.net, PHENOvines aims at the implementation of a HT-phenotyping platform for field application to be used within a breeding program. Including an improved Image-Acquisition-System which consists of five cameras, RTK-GPS (GPS position accuracy around 2 cm), and an adjusted prototype software to take geo-referenced RGB images.

These phenotyping platform facilitate a non-invasive and contactless detection of phenotypic traits of grapevines. As a first step to detected yield parameters directly in the field a high-throughput image interpretation which enables a fast acquisition of grape berry size from RGB images was tested.

It is anticipated that in future the phenotyping platform will be based on an autonomous robot that drives through experimental and production vine-yards. In its final version pictures shall be taken automatically depending on the GPS position.

Identification of QTLs for drought stress induced leaf senescence in early developmental stages of barley using genome wide association studies

Gwendolin Wehner, Christiane Balko and Frank Ordon

Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

Email of corresponding author: gwendolin.wehner@jki.bund.de

Premature leaf senescence as one important factor of yield loss is often induced by external stress conditions, e.g. drought stress. So tolerance to drought stress has become an important goal in plant breeding. Therefore, the aim of this project is to identify genomic regions involved in drought stress induced leaf senescence in early developmental stages of barley (*Hordeum vulgare* L.) by applying genome wide association studies (GWAS).

In greenhouse pot experiments 156 barley genotypes including 113 German cultivars and 43 accessions of the Spanish Barley Core Collection (SBCC) are tested for their response to early drought stress and induction of leaf senescence under control (70% of maximal soil water capacity) and stress conditions (20% of maximal soil water capacity). At the end of a four weeks stress period physiological senescence parameters chlorophyll content and chlorophyll fluorescence, as well as drought stress parameters e.g. content of free proline, total soluble sugars,

osmotic adjustment and the above-ground biomass production, are determined.

The experiments of two year's trials revealed variability in the parameters representing different and specific adaptation mechanisms to drought stress. Analysis of variance revealed significant genotype and treatment effects for all investigated traits and genotype treatment interactions for biomass production and content of free proline.

In parallel this set of genotypes was analysed with the 9k iSelect SNP-chip available for barley. In summary 6807 SNPs turned out to be polymorphic. Those being mapped and showing a minor allele frequency >5%, were used for GWAS taking into account population structure and kinship. Based on this procedure, significant marker trait associations were observed under stress conditions for chlorophyll content, total content of soluble sugars osmotic adjustment and biomass production.

Initiation of meiotic double strand breaks in plants depends on two different SPO11 proteins

Thorben Sprink and Frank Hartung

Julius Kühn-Institut, Institute for biosafety in Plant Biotechnology, Quedlinburg

Email of corresponding author: thorben.sprink@jki.bund.de

The pairing and balanced distribution of allelic chromosomes is one of the main factors leading to genetic diversity and a successful meiotic outcome in eukaryotes. This process depends in nearly all analyzed eukaryotic organisms on the initiation of double strand breaks (DSBs) by the protein SPO11, an evolutionary conserved meiotic transesterase. SPO11 is introducing these DSBs during early meiotic prophase. Whereas in animals and fungi only a single SPO11 is present, plants have at least two SPO11 proteins which are active and essential in meiosis (SPO11-1 and SPO11-2). Single knockout mutants of Arabidopsis SPO11-1 as well as SPO11-2 are nearly sterile and random chromosome segregation during meiosis occurs.

In all so far sequenced plants genes orthologous to Arabidopsis SPO11-1 and -2 exist. Our aims are to investigate whether the function of these two different SPO11 proteins is species specific or interchangeable between near and far distantly related plants. Furthermore, we want to define which regions of the proteins determine the specificity of the respective SPO11 protein 1 or 2. To analyze the functional conservation we used

orthologous genes from different land plants (*Brassica rapa*, *Carica papaya*, *Oryza sativa* and *Physcomitrella patens*) for heterologous complementation of well characterized SPO11 mutants from Arabidopsis.

To figure out which part of the protein sequence determines the specificity, we interchanged regions between the two SPO11 genes in Arabidopsis and transformed mutant plants with these swap constructs.

Here we will present first results on heterologous complementation with near related land plants as well as first results of interchanged regions between the two SPO11 proteins. We will show chromosome distribution as well as pollen vitality by different microscopic techniques. We will also evaluate if there is a differences in the number of seeds produced by the different complemented plants.

Analysing the results of these experiments, we should be able to answer the questions if there are sequence and/or species specific functions of each SPO11 and which region(s) of the proteins are essential for the initiation of meiotic double strand breaks.

PlantsProFood - New varieties of narrow-leaved lupin for application in human nutrition

Kristin Fischer¹, Brigitte Ruge-Wehling¹, Eicke Rudloff¹, Anne-Kathrin Schmalenberg², Björn Rotter³, Peter Winter³ and Peter Wehling¹

¹Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz

²Saatzucht Steinach GmbH, Bocksee

³GenXPro GmbH, Frankfurt/Main

Email of corresponding author: kristin.fischer@jki.bund.de

The seed protein of narrow-leaved lupin (*Lupinus angustifolius*) is known for its exceptional functional properties as food ingredient and, thus, represents a resource of vegetable protein for human consumption. To take advantage of this potential for a broad range of food applications the regional R&D network 'PlantsProFood' was set up in Northern Germany. Lupins shall be enhanced as a protein resource for food purposes, like ice cream, sausages, bakery products or pasta.

The network consists of four research institutions and ten local companies and aims at processing the value chain from the development of (I) high-yielding varieties, (II) new processing approaches towards (III) innovative and healthy food.

Requirements for these efforts are high and stable yields of narrow-leaved lupins. This may be achieved by plant breeding as far as sufficient genetic variability is available. To expand the genetic variability of advanced lupin breeding material an EMS (ethyl methanesulfonate) mutagenesis of cv.

'Boruta' was performed and the offspring screened for novel phenotypes. Phenotypes with a conspicuous novel growth type, such as vigorous growth or high branching compared to the wildtype 'Boruta', were identified and devised to homozygous and stable mutant (M) lines.

The yield potential of the M lines was evaluated under field conditions and for some lines the potential was confirmed by significant increase in kernel yields. For genetic analyses, crosses of the respective M lines with genebank accessions as well as with the wildtype 'Boruta' were carried out. Segregation analysis of F2 populations indicated a monogenic-recessive inheritance of novel growth types.

Promising M lines are going to be subjected to an analysis of differentially expressed sequences of mutant lines and the wildtype via RNAseq techniques and SNP identification.

Currently, selected SNPs are analyzed *via* high-resolution melt analysis to develop selection tools for marker-assisted selection.

Evaluation of seed ingredients of *Lupinus angustifolius* for the application in food industry

Helene Beyer, Gisela Jansen, Hans-Ulrich Jürgens and Frank Ordon
Julius Kühn-Institut, Institute of Resistance Research and Stress Tolerance, Quedlinburg
Email of corresponding author: helene.beyer@jki.bund.de

In contrast to most crop species, the narrow leafed lupin (*Lupinus angustifolius*) is a very recently domesticated crop. Lupins were first introduced in Germany in 1781 in order to improve the poor sandy soils of Northern Germany. Systematic breeding was initiated in Germany at the beginning of the twentieth century and accelerated in the 1990s with a focus on reducing alkaloid levels, pod dehiscence and improved seed yield.

The seeds of narrow leafed lupins contain high amounts of protein, oil and fibre. Despite their valuable seed composition, the acreage in Germany is still quite limited as well as their use in food industry. To improve the cultivation of *L. angustifolius*, the seed composition, environmental stability and heritability of important seed ingredients, which might be beneficial for lupin breeders as well as end users of sweet narrow leafed lupins, were estimated.

To achieve this, field trials with 50 genotypes of narrow leafed lupins were

carried out in four different locations with two replications, over three years.

The examined seed ingredients comprise content of crude protein, oil, non-starch polysaccharides, raffinose oligosaccharides and alkaloids as well as the composition of amino acids and fatty acids.

The determination of the relative contribution of the genotype and environment to the variation in seed quality traits revealed little genotype x environment interaction in comparison to the influence of the genotype solely, resulting in a quite high heritability for all traits analysed.

The genotypic correlation was significantly negative for oil- and protein content (-0.36*) indicating that simultaneous improvement in seed oil- and protein may be difficult with regard to the negative correlation between those traits.

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

Helge Flüß¹, Brigitte Ruge-Wehling¹, Fred Eickmeyer² and Peter Wehling¹

¹Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz

²AESKULAP GmbH, Steinach

Email of corresponding author: helge.fluess@jki.bund.de

So far, the production of natural rubber has been depending almost entirely on *Hevea brasiliensis*, a tree that is grown in only a few countries. The idea of cultivating Russian dandelion as an alternative rubber resource crop is not really new, but industrial production has never been performed consequently. Now, with various scientific and economical forces, the rubber production of Russian dandelion shall be enhanced and used for a sustainable production of tires and other natural rubber demanding products.

Since *Taraxacum koksaghyz* shows high diversity and relatively weak growth, it is still considered a wild type. In order to get this wild type on its way to a serious rubber producing crop, this work aims to gain insights into the genetic background of Russian dandelion and provide important information for promising breeding programs in a network of different research institutions and a breeding company.

In close cooperation with the breeding partner, the comprehensive genetic variability of *Taraxacum koksaghyz* 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 clear taproot with high contents in rubber and inulin, early and uniform flowering time, tolerance to high planting density, as well as different disease resistances, have been defined as breeding objectives. These objectives will be supported by (I) drafting a dendrogram of different *Taraxacum* species based on AFLP analysis, (II) development of a genetic map of *T. koksaghyz* as a tool for selection markers and (III) a marker-assisted advanced backcross program. These work packages are focused on providing information and genetic tools for marker-assisted breeding of *T. koksaghyz* as a commercial rubber crop.

Session 2: Agricultural Impact on Environment

Effects of neonicotinoids on honeybees, bumblebees and solitary bees in oilseed rape cultivation: Project ABO 2014

Nadine Kunz, Malte Frommberger, Jens Pistorius and Ina Wirtz

Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Braunschweig

Email of corresponding author: nadine.kunz@jki.bund.de

Neonicotinoids such as imidacloprid, clothianidin and thiamethoxam are systemic insecticides that are frequently used as seed treatment in cereals and bee-attractive crops such as oilseed rape. Currently, the effects of these neonicotinoids on pollinators are widely discussed at international level.

In April 2013, the EU commission agreed on a two-year ban of those agents being used as treatments for certain crop types. During this period, new studies are commissioned to assess and clarify the potential threat of these insecticides to pollinators, such as honeybees, bumblebees and solitary bees, caused by residues in nectar and pollen.

To date, the influence of oilseed rape treated with neonicotinoids on the mortality, development and reproduction has been mainly investigated for honeybees. However, for solitary bees and bumblebees, hardly any higher tier studies in semi-field or field conditions are available and validated methods to evaluate potential risks of pesticides are still lacking.

In order to develop a standardized methodology, pilot field trials and semi-field trials were conducted in 2013 using the Western honeybee (*Apis mellifera*), the buff-tailed bumblebee (*Bombus terrestris*) and the red mason bee (*Osmia bicornis*) as model organisms. Based on experiences gained in these trials, follow-up experiments will be conducted in 2014.

These experiments are part of the "ABO 2014" project which is coordinated by the Julius Kühn-Institut in Braunschweig and will be conducted in five federal states in spring 2014. This study aims to evaluate potential risks of neonicotinoids for honeybees and other pollinators, to develop new evaluation methods and to establish new test guidelines and standard procedures. Several variables such as the mortality, development and reproduction of the target organisms as well as residue concentrations of neonicotinoids in pollen and nectar and their lethal and sub-lethal effects on bees will be assessed.

Recovery of common vole populations (*Microtus arvalis*) after rodenticide application

Susanne Hein and Jens Jacob

Julius Kühn-Institut, Institute for Plant Protection in Horticulture and Forests, Münster

Email of corresponding author: susanne.hein@jki.bund.de

The common vole (*Microtus arvalis*) is the most important vertebrate pest species in European agriculture because of its damage potential during outbreaks. Management usually relies on the use of rodenticides which is limited to one application a year. To base such management decisions on relevant and realistic data, extensive and detailed information about common vole ecology and behavior in different habitats and crops is vital.

In the past, many studies have been conducted dealing with population development and population dynamics of common voles in different habitats and considering the effects of various landscape modifications. Although a great number of publications are dealing with this species, there is only little knowledge about population recovery after rodenticide application.

In this project we will investigate recovery time, possible mechanisms of recovery and recolonisation of common vole populations after treatment with zinc phosphide using live trapping and hair tubes. One further objective deals with kinship analysis via PCR and microsatellite analysis using hair samples obtained in the field.

Preliminary studies in enclosures indicated that hair tubes are frequented by voles only if placed on runways. However, using bait in the tubes attracts voles to the tubes even if they are placed away from runways. This suggests that hair tubes may provide a suitable and cheap alternative to live trapping for monitoring common voles.

Session 3: Biological Control

Native parasitoids and their potential for natural control of the invasive fruit pest *Drosophila suzukii* (Diptera: Drosophilidae)

Stefan Alexander Christ^{1,2}, Sarah Dorothea Awater^{1,3} and Annette Herz¹

¹Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Darmstadt

²Technical University Darmstadt, Plant Membrane Biophysics, Institute of Botany

³Georg-August-University Göttingen

Email of corresponding author: stefan_a_christ@freenet.de

The spotted wing drosophila, *Drosophila suzukii* (Matsumura), is a vinegar fly that is closely related to *D. melanogaster* and has expanded its distribution from Asia to America and recently to Europe. It has been reported in most of the Mediterranean countries in Europe and is rapidly spreading towards north and east. In 2012, *D. suzukii* has been identified in southwestern parts of Germany.

This species infests commercial small and stone fruits as opposed to most species of drosophilids that only infest overripe, fallen or rotting fruits. Female *D. suzukii* possess a distinctive serrated ovipositor that allows them to lay eggs successfully into healthy and ripening fruits. After oviposition, the larval stages causes a high risk to divers marketable crops, especially soft or thin-skinned fruits which are collapsing through larval feeding.

We will give a short overview of the actual situation and our monitoring programme of *D. suzukii* in the Rhein-Main-Area. Within the project it turned out that traps with a vinegar-water-mixture as bait at a ratio of 2:3 has been shown a highly efficient quantity of trapped drosophilids. We started the monitoring in April 2013 to investigate the species composition of the different Drosophilidae throughout the year. In

order to found a laboratory rearing for parasitoid-experiments we disposed mush traps that enable oviposition through drosophilids. In cooperation with the *Hessian Plant Protection Service* we started also a small monitoring project of infested fruits by *D. suzukii* at a commercial fruit farm nearby Darmstadt.

First results reveal that there are strong distinctions in the species composition of different native Drosophilidae-communities between rural and urban areas. We also found differences for surrounding trap location conditions, especially those between wood and cropland. Our data confirmed former studies which found that adult animals were not trapped before July. Hence, we presume that the new invasive fruit pest *D. suzukii* could occupy any ecological niche and build stable populations.

Furthermore, we trapped native parasitoids with *Drosophila*-larvae from our laboratory rearing as bait. In further laboratory experiments we hope to estimate the potential of biological control of *D. suzukii* through parasitic wasp-species. This could raise the hope for a prospective natural control strategy against the invasive fruit pest *D. suzukii*.

Insecticidal efficacy of Cameroonian neem seed powder against *Sitophilus zeamais* Motsch. and *Callosobruchus maculatus* Fab. as influenced by drying regime

Katamssadan Haman Tofel^{1,3}, Elias Nchiwan Nukenine² and Cornel Adler¹

¹Julius Kühn-Institut, Institute for Ecological Chemistry, Berlin

²Department of Biological Sciences, University of Ngaoundere, Cameroon

³Department of Biological Sciences, University of Bamenda, Cameroon

Email of corresponding author: katamssadan.tofel@jki.bund.de

The problem caused by insect pests to stored products in Africa is still a challenge and the use of botanicals needs to be intensified. Neem products are known for their bioefficacy which vary across geographical locations. Therefore, it seems unwise to extrapolate the results from one region to another.

Cameroonian neem seed powder from pulverized dehusked and sun-dried kernels (DSD), undehusked and sun-dried (USD), dehusked and shade-dried (DRD), and undehusked and shade-dried (URD) kernels were used. Maize grains and cowpea seeds were coated with the neem seed powders in order to evaluate their insecticidal efficacy on *Sitophilus zeamais* and *Callosobruchus maculatus* using parameters like adult mortality, progeny production, grain damage, population increase and persistence.

All neem powders tested at different doses and exposure periods, showed various bioactivities against *S. zeamais* and *C. maculatus*. Within one day of exposure and at the highest dosage (40g/kg) level, 20 %, 7.5%, 6.25% and 2.5% mortality was caused to *S. zeamais* by DRD, USD, URD, and DSD, respectively, and to *C. maculatus*, no mortality was achieved for all drying

regimes. For *C. maculatus*, maximum mortality (40 g/kg, within 6 days) of 34.28%, 30.46%, 23.75% and 22.76% were achieved for DSD, DRD, USD and URD, respectively. For *S. zeamais*, mortality of 100% was caused by DSD and USD (30g/kg) within 14 days. All neem powder inhibited the production of progeny in the beetles, reduced the percentage of seeds damaged and mass losses for maize and cowpea. The persistence experiment showed that for dosage level of 40 g/kg, adult mortality of *S. zeamais* decreased from 60% (after 15 days storage) to 20% (180 days storage) and for *C. maculatus* from 7.75% (15 days storage) to zero (180 days storage).

Sun-drying of neem seeds which results in relative better insecticidal efficacy could help farmers to easily dry their neem seeds in sun. This drying regime could help them avoid health risks, in regard of the fact that during shade-drying, the seeds are attacked by fungi which may be toxic when admixed to food products. Also, in traditional post-harvest system in northern Cameroon, farmers poor in resources could prepare and use neem seed powder for insect control at cheapest cost.

Influence of arbuscular mycorrhizal fungi on abiotic stress tolerance of wheat

Heike Lehnert, Albrecht Serfling and Frank Ordon

Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

Email of corresponding author: heike.lehnert@jki.bund.de

Wheat (*Triticum aestivum*) is beside rice and maize one of the most important crops worldwide and used mainly for human nutrition as well as for feeding animals. Due to early summer drought and nutrient deficiencies especially in organic farming, yield and quality losses are expected. One of the most promising approaches to reduce the negative impact of abiotic stress is the identification of stress tolerant wheat genotypes.

Due to the increased water and nutrient transport to the plant, arbuscular mycorrhizal fungi (AM) are beneficial for many plant species especially under water or nutrient deficiency. Therefore, the aim of this project was to identify wheat genotypes which show a high AM colonization leading to enhanced abiotic stress tolerance. To achieve this, a set of 103 and a subset of 30 genotypes respectively were investigated under abiotic stress conditions in order to detect genetic differences of their ability to generate mycorrhiza symbiosis and to get more information on the impact of the symbiosis on agronomic traits like grain yield and shoot dry weight.

In a pot trial, the 103 genotypes were grown under glasshouse conditions in a drought stressed and a well watered variant with and without mycorrhization. Each variant was replicated 3 times. A second pot trial was conducted with a subset of 30 geno-

types grown in an optimal phosphorous fertilized variant and under phosphorous deficient conditions with and without mycorrhization in 3 replications and yield parameters were assessed. Furthermore, PCR analyses and an ink vinegar stain of root segments were performed to evaluate the root colonization by the AM *Glomus intraradices*, *Glomus etunicatum* and *Glomus claroideum*.

Quantification of root colonization using light microscopical techniques showed differences in mycorrhization between genotypes in both trials. Significant differences in root colonization were observed between optimal phosphorous fertilized and plants grown under phosphorous deficient conditions. Under both abiotic stress conditions – drought stress and phosphorous deficiency – significant differences of yield parameters were determined between AM inoculated and non-inoculated plants.

Pot trials will be repeated in order to get more information on the benefit of mycorrhizal symbiosis under limited conditions. Furthermore, genotyping using the 90k iSelect SNP-chip will be conducted to identify QTLs which are involved in root colonization and tolerance to abiotic stress via genome wide association studies (GWAS).

Screening repellents for the management of rodent damage to subsurface drip irrigation systems

Sabine Hansen and Jens Jacob

Julius Kühn-Institut, Institute for Plant Protection in Horticulture and Forests, Münster

Email of corresponding author: sabine.hansen@jki.bund.de

Worldwide use of subsurface drip irrigation systems (SDI) in agriculture increases to boost production in semi-arid and arid areas. Because of low water use these subsurface pipe systems ensure effective soil watering in the fields. Rodents can cause extensive damage to the pipes and therefore be responsible for a considerable water loss and extensive repair. However, reported problems of SDI users with rodent damage are increasing.

To identify substances to repel the rodents from the SDI-pipes we ran choice trials with common voles (*Microtus arvalis*) and house mice (*Mus musculus*). Common voles are the major vertebrate pest species in agriculture in Europe and occur in dry regions of southern and western Europe. House mice damage stored goods and are a health concern because they can transmit zoonotic diseases.

Secondly we test the most promising substances from the screening under semi-natural conditions experiments with both pest species.

For these trials small rodent populations were established in enclosures in semi-natural conditions. The rodents were offered buried plastic boxes with attractive food on soil that was either untreated or treated with a potential repellent substance. Through a transponder scanner we recorded how often each animal visited these boxes to identify repellent effects.

Summarizing the results of both trials point out how effective the substances repel the rodents. An effective repellent applied to piping material or in the vicinity of pipes will help to minimize damage to SDI systems and consequently conserve water resources.

Session 4: Resistance Genetics

Transcription factor ERF5 of grapevine is involved in pathogen resistance

Tina Moser¹, Patrick Merz², Jochen Bogs² and Eva Zyprian¹

¹Julius Kühn-Institut, Institute for grapevine breeding, Siebeldingen

²State Education and Research Center of Viticulture and Horticulture, Neustadt

Email of corresponding author: tina.moser@jki.bund.de

Breeding of grapes has a long tradition and is nowadays assisted by molecular markers associated with different traits. Since the introduction of pathogens like *Erysiphe necator* and *Plasmopara viticola* to Europe from North America in the 19th century one important trait is pathogen resistance. Different loci for pathogen defense have been identified through the last years. But it is still unknown which genes in the regions of the loci are relevant and what kind of molecular mechanism of plant defense they mediate. So it is important to acquire knowledge of the plant defense mechanisms involved.

To unravel the molecular mechanisms of plant defense, one approach was the comparison of a resistant and a susceptible genotype after inoculation with *E. necator* by differential gene expression analysis. It could be shown

that ethylene-responsive transcription factor 5 (ERF5) is 2.1-fold upregulated in the resistant genotype.

Currently we found that ERF5 is 4.6-fold upregulated in the resistant genotype after inoculation with *P. viticola* as compared to non-inoculated controls. In the susceptible genotype ERF5 remains unchanged after inoculation with *E. necator* or *P. viticola*. Furthermore, it appears that ERF5 positively regulates the promoter of the pathogen-related protein 10 (PR10). ERF5 encoding genes from a resistant and a susceptible genotype were infiltrated in another susceptible genotype. Leaf cuttings of the infiltrated plants were inoculated with *P. viticola*. After one week specifically infiltrated plants with ERF5 from the resistant genotype showed a significant increase in resistance.

TRANSBULB: Fine mapping of *Rym16^{Hb}* by using novel molecular approaches

Christiane Nöh¹, Neele Wendler², Maria Schmidt¹, Antje Habekuß³, Nils Stein² and Brigitte Ruge-Wehling¹

¹Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz

²Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben

³Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

Email of corresponding author: christiane.noeh@jki.bund.de

Breeding of resistant barley varieties can help to minimize yield losses caused by plant diseases. The wild *Hordeum* species *H. bulbosum* makes up the secondary gene pool of barley and is a new source of resistance genes for barley breeding. Interspecific crosses of *H. vulgare* (*Hv*) and *H. bulbosum* (*Hb*) led to introgressions of *Hb* chromatin carrying novel disease resistance genes in an *Hv* genetic background. One of them is *Rym16^{Hb}* which was introgressed to barley chromosome 2HL and confers resistance to the soil-borne BaMMV/BaYMV-1/-2 virus complex.

The present study aims at the use of innovative and efficient molecular strategies based on next generation sequencing and SNP genotyping for (I) fine mapping of the resistance locus, (II) reduction of the introgression size via

homoeologous recombination and (III) the development of selection tools for plant breeders.

A total of 76 markers were mapped on the 2HL introgression, some of these were derived as TC markers based on the orthology of barley chromosome 2HL and rice chromosome Os4. Thirty-two recombinants were identified which carried *Rym16^{Hb}* on *Hb* introgressions of reduced sizes relative to the original introgression. Closely flanking markers are available for marker-assisted breeding programmes.

Field tests are underway to examine the influence of individual introgressions on crop yield. The resistance gene will be genetically fixed in DH lines, after crossing the resistant parent with different breeding lines.

Molecular characterization of the resistance locus *Ren3* against powdery mildew (*Erysiphe necator*) from the grapevine cultivar 'Regent'

Pierre Schneider, Reinhard Töpfer and Eva Zyprian
Julius Kühn-Institut, Institute for Grapevine Breeding, Siebeldingen
Email of corresponding author: pierre.schneider@jki.bund.de

Powdery mildew is one of the most devastating diseases of grapevine (*Vitis vinifera* L). The disease is caused by *Erysiphe necator* Schw. (syn. *Uncinula necator* (Schw.) Burr, anamorph *Oidium tuckeri* Berk.), an ascomycete fungus, which was introduced from North America to Europe in 1845. The traditional European cultivars are highly susceptible to the fungus and still today huge amounts of fungicides are necessary to counteract the pathogen.

Many North American *Vitis* species developed resistance against *Erysiphe necator* due to co-evolution of host and pathogen. This process promoted the development of a genetic locus called *Ren3*, which was characterized by WELTER et al. (2007) in the cultivar 'Regent'. Later DUDENHÖFFER J. and ZYPRIAN E. (2012) showed, that several genes are located within this region, which show great similarity to genes known to mediate resistances in other plants.

In the recently started project the physical map of the *Ren3* locus will be completed via finishing partial sequences obtained from BAC clones in the *Ren3* locus by amplicon sequencing.

The obtained sequences will allow further searching for open reading frames, which contain functional domain structures already known from identified resistance genes. Some of these genes will be cloned into a binary expression vector and checked for functionality upon transformation of susceptible grapevine cultivars with *Agrobacterium tumefaciens*.

Furthermore microscopic studies will be performed to follow the pathogen attack and its cytological effects in resistant and susceptible grape cultivars. This will help to elucidate the mechanisms of resistance.

Tn5 mutagenesis as a method for determination of necessary genetic features of bacterial antagonists

Christine Hübert¹, Kristin Dietel², Helmut Junge², Annette Wensing¹ and Wilhelm Jelkmann¹

¹Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim

²ABiTEP GmbH, Berlin

Email of corresponding author: christine.huebert@jki.bund.de

Fire Blight is a bacterial disease which attacks plants of the Rosaceae family. It can lead to great economic losses, especially in the area of fruit-production. An effective control strategy is based on streptomycin, but application of antibiotics in plant production is highly discussed. An approach on biological level depends on control by application of bacterial antagonists. These organisms are able to fight the Fire Blight pathogen *Erwinia amylovora* for example by competition or by production of toxic compounds.

Possible antagonists against *E. amylovora* are the closely related epiphyte *Erwinia tasmaniensis* and representatives of the genus *Bacillus*. *Bacilli* are often characterized by their ability to produce a broad spectrum of secondary metabolites with toxic effects on Gram-negative bacteria. Nevertheless, there might be other factors responsible for such inhibitory abilities. Identification of features involved in the pathogen-antagonist interaction could be a useful criterion for antagonist selection.

A screening method for antagonists *in planta* has been introduced for inhibition of *Pseudomonas syringae* DC3000 by *Sphingomonas* sp. Strain Fr1 in a model system of *Arabidopsis thaliana*.

This setup seems promising and might be adapted to the Fire Blight pathosystem as well, using pear slices, apple flowers or *in vitro* plantlets for screening.

Tn5 transposon mutagenesis allows generation of random mutations on chromosomal level. The plasmid pRL27 carries a hyperactive transposase which leads to high frequency transposon transfer. Another advantage of this transposon is a conditional origin of replication which allows an easier way of re-isolation by rescue cloning for further investigations. Using a luminescent *E. amylovora* strain as an indicator enables screening for antagonist mutants which have lost their inhibitory effect on pathogen growth. These measurements are feasible in micro titer plates which allow a high-throughput screening.

Determination of genetic features which are important for antagonistic abilities enables comparison to other pathosystems and might be useful to identify suitable antagonists in different areas. It also could be used for pre-selection of antagonistic organisms before application in field trials which have only a limited capacity for testing.

Elucidation of a novel mode of resistance of codling moth against *Cydia pomonella granulovirus* by homogenization experiments

Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn and Johannes A. Jehle
Julius Kühn-Institut, Institute for Biological Control, Darmstadt
Email of corresponding author: annette.sauer@jki.bund.de

In nearly all growing regions of apple and pear worldwide, the codling moth (CM, *Cydia pomonella*) is the most devastating pest; CM has developed resistance to many chemical insecticides. An alternative to the application of chemical insecticides is the use of *Cydia pomonella granulovirus* (CpGV, Baculoviridae). CpGV products are applied in both organic and integrated production.

Since 2005, codling moth populations with a reduced susceptibility to CpGV, products have been reported from about 40 European orchards. The resistance could be traced back to a single, dominant, sex-linked gene. Currently, resistance management strategies are based on the application of improved CpGV products containing resistance-overcoming CpGV isolates.

Recently, two CM field populations (NRW-WE and SA-GO) with a reduced susceptibility to even these improved CpGV products were found. First single pair crossing experiments between individuals of these resistant field colonies and a susceptible laboratory CM strain (CpS) indicated that the inheritance of resistance of these populations did not follow the previously

described pattern of Z-linked, dominant resistance. In single-dose bioassays the susceptibility of neonates of the resistant CM colonies NRW-WE and SA-GO to different CpGV isolates (CpGV-M, -S, -V15 and -E2) was estimated.

The aim of the current study was the genetic homogenization of the genetically heterogeneous field populations NRW-WE and SA-GO by two different methods: (i) repeated single pair crossings followed by family selection and (ii) successive mass crossing experiments under virus pressure. The resulting homogenous strains of NRW-WE and SA-GO with fixed resistance were used for backcrossing experiments with CpS to elucidate the mode of inheritance of their resistance. Single or multiple resistance genes, completely dominant as well as intermediate inheritance patterns are possible. Furthermore, the two different homogenization methods as well as the two different resistant field colonies NRW-WE and SA-GO will be compared based on potential differences in their mode of resistance. The intended reciprocal backcrossing experiments followed by bioassays can also define sex-linkage of the resistance.

Session 5: Environmental Impact on Agriculture

Soil composition matters – Minerals and charcoal control bacterial response to plant litter and to the pollutant phenanthrene

Doreen Babin¹, Cordula Vogel², Sebastian Zühlke³, Michael Spiteller³, Ingrid Kögel-Knabner^{2,4} and Kornelia Smalla¹

¹Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

²Technische Universität München, Lehrstuhl für Bodenkunde, Freising-Weihenstephan

³Technische Universität Dortmund, Institute of Environmental Research, Dortmund

⁴Technische Universität München, Institute for Advanced Study, Garching

Email of corresponding author: doreen.babin@jki.bund.de

Soil bacteria are known to be involved in many essential environmental processes such as nutrient cycling, plant growth promotion, and biodegradation of organic pollutants. However, the complex soil structure formed by heterogeneous compounds and their interactions hinders a mechanistic understanding of these processes. Here we used artificial soils under laboratory conditions to reduce the soil complexity. A microbial inoculant obtained from a natural soil and sterile manure were added to four different soil compositions varying in type of clay minerals and presence of charcoal or ferrihydrite, respectively. After more than two years of incubation, phenanthrene (2 mg/g) used as model compound for polycyclic aromatic hydrocarbons was spiked to artificial soils with or without added plant litter (1 wt%) in order to study bacterial responses as a function of the soil composition. After 21 days of incubation, total community DNA was extracted and bacterial structure and abundance was assessed by 16S rRNA amplicon-based techniques (DGGE, pyrosequencing, quantitative PCR).

The type of clay mineral present (illite, montmorillonite) was found to be the main driver of bacterial communities in

the long term while ferrihydrite and charcoal selected for specific bacterial classes. By DGGE, several populations were shown to be enhanced or decreased in abundance in response to the phenanthrene spike. In all artificial soils, *Actinobacteria* were revealed as dominant responders to phenanthrene. Soils containing illite showed stronger changes in the bacterial community than soils containing montmorillonite and soil composition-dependent responses to phenanthrene were identified by pyrosequencing at the genus level. Interestingly, the addition of plant litter, which was assumed to foster horizontal gene transfer and adaptation to the pollutant, affected bacterial communities but decreased their response to phenanthrene. Soils containing both phenanthrene and litter spike exhibited the highest 16S rRNA gene copy numbers indicating a synergistic effect. The decrease in phenanthrene concentrations in the treatments with and without litter suggested in both cases a biodegradation of the pollutant.

In conclusion, the present study proposes the long-term driving role of the soil composition (minerals, charcoal) on the establishment and functionality of bacterial communities.

The effect of soil types on the abundance and diversity of potato plant associated bacteria with antagonistic activity towards *Ralstonia solanacearum*

Tarek Elsayed¹, Rita Grosch² and Kornelia Smalla¹

¹Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

²Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Department Plant Health, Großbeeren

Email of corresponding author: tarek.elsayed@jki.bund.de

Ralstonia solanacearum is an epidemic phytopathogenic bacterium responsible for bacterial wilt in potato and many other plant species. This pathogen can survive in the soil for several years and can spread very fast via water streams and latent infections in the tubers are particularly problematic. Endophytic biocontrol agents might be a solution as they occupy the same niches as colonized by *R. solanacearum*. The aim of this study was to assess the diversity of bacterial antagonists of *Ralstonia solanacearum* in different soil types and potato plant spheres, and to estimate the effect of soil type on the abundance and diversity of antagonistic bacteria.

The total bacterial communities from bulk soil, rhizosphere soil and from the endophytic compartments were compared by PCR-DGGE fingerprints of 16S rRNA genes amplified from total community DNA. Approximately 2000 bacterial isolates obtained from different spheres of potato plants grown in three different soils (Diluvial sand, Alluvial loam and Loess loam) were screened for in vitro antagonistic activity towards *R. solanacearum* and 200 isolates with antagonistic activity were further characterized.

Using BOX-PCR fingerprints, the 200 antagonistic bacteria were compared. The biological control mode of action and the plant growth promoting potentials were investigated as well. The BOX-PCR fingerprint as well as the 16S rRNA sequences showed that some endophytic antagonistic isolates identified as *Pseudomonas umsongensis* colonized all potato plant ecto- and endospheres. The total bacterial communities denaturing gradient gel electrophoresis (DGGE) profile revealed distinct bacterial community structure associated with each plant sphere, and within each plant sphere the microbial community composition was mainly shaped by the soil type. The effect of soil type decreased in the endophytic compartments, suggesting that this endophytic compartment harbored unique indigenous bacterial communities protected from surrounding environment even so some of the endophytic antagonistic bacteria isolated from different soil types shared the same BOX-PCR fingerprints. Currently we investigate under greenhouse conditions whether endophytic bacteria can more effectively antagonize *R. solanacearum* than rhizosphere colonizing bacteria.

Soil suppression against the cereal cyst nematode *Heterodera avenae*

Caroline Eberlein¹, Jan Moos², Hans Marten Paulsen² and Andreas Westphal¹

¹Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Braunschweig

²Thünen-Institute of Organic Farming, Westerau

Email of corresponding author: caroline.eberlein@jki.bund.de

Cereal cyst nematodes (CCN), especially *Heterodera avenae*, can constrain the yield of cereal crops, affecting important annual losses. In addition to rotation with non-host crops, management relies on the use of resistant cultivars. Their use can be jeopardized by the existence of different pathotypes of CCN. In the UK, examples of nematode suppressive soils have been reported where population densities of *H. avenae* declined under a cereal monoculture. Two endoparasitic fungi frequently infected females, and were primarily responsible for this nematode population density decline.

Objectives of this study were (a) to monitor the population dynamics of the CCN *H. avenae* or *H. filipjevi* under a cereal monoculture, and (b) to test how female populations of *H. avenae* develop in soil of different origin (location and crop history). Microplots infested with *H. avenae* and *H. filipjevi* were cropped to a cereal monoculture from 2009 to 2012. Yields and nematode population densities were determined at each planting and harvest. Cysts were extracted, the eggs counted and categorized into healthy and diseased. Population densities of the two nematodes tended to decline, while the proportion of diseased eggs increased throughout these years.

In winter 2012/13, soil samples originating from three cereal fields were placed in root boxes of 1-L capacity, with the respective transparent side

leaned in 45 degree downwards. Soil was either left untreated or heat-treated (134°C, 10 min) before placing in the boxes to test if biotic causes impacted the number of females. Root boxes with soil from a field with a history of limited cereal cropping served as control. Susceptible *Hordeum vulgare* 'Hanka' was planted to the boxes at 6 plants per box. Roots boxes were inoculated with 10,000 J2 of *H. avenae* per box. Beginning at first appearance, nematode females visible on the root surface were counted weekly. Maximal female numbers were observed at the second week of observation. Thereafter, the numbers remained on a similar level. At all monitoring times, the numbers of females in the untreated soil were less than those in heated soil. During the first two weeks of observation, female numbers in the three soils were significantly lower than the one control soil. In soil from a wheat field, female numbers remained about one third lower than those in the control during the entire monitoring period. The results of this study suggested that a biotic component negatively affected the development of *H. avenae* females on the roots of a susceptible cultivar. Further investigations will focus on what microbial agents may be involved in this suppressive effect. Such microorganisms could play a vital role in the life history of CCN, and may be helpful to mitigate infestations of this nematode pest.

Influence of weather periods on plant diseases: a case study for *Puccinia triticina* and *Blumeria graminis* f.sp. *tritici* on winter wheat in Saxony-Anhalt

Bastian Stößel^{1,2}, Bernd Freier² and Frank Wechsung¹

¹Potsdam Institute for Climate Impact Research e.V. (PIK), Research Domain II –Climate Impacts and Vulnerabilities, Potsdam

²Julius Kühn-Institut, Institute for Strategies and Technology Assessment in Plant Protection, Kleinmachnow

Email of corresponding author: bastian.stoessel@pik-potsdam.de

Leaf rust (*Puccinia triticina*) and powdery mildew (*Blumeria graminis* f.sp. *tritici*) are two of the most important plant diseases of winter wheat in Germany. It is already known that both pathogens are influenced by weather, but detailed studies under field conditions regarding the temporal dimension of those influences are missing. Hence, the aim of our study was to detect timeframes during the course of the epidemic year, where weather variables have significant influence on disease severity of leaf rust and powdery mildew of winter wheat.

Randomly sampled monitoring data of 34 years of more than 20 monitoring fields per year between anthesis and early ripening were available for both diseases. The data were collected by the federal plant protection service of Saxony-Anhalt. Weather data for 11 variables at 61 stations were provided by the German Weather Service and missing or inhomogeneous data on specific variables were substituted and corrected using interpolation by the Potsdam Institute for Climate Impact Research. Further climatic variables like freezing days and days with precipita-

tion were calculated by using the original weather variables.

To identify influential periods we used a “window pane” algorithm presented in recent literature. The algorithm calculated Kendall correlation coefficients for each time window between monitoring date and sowing of the crop with a minimum window length of 5 days. Thus approximately 90.000 correlations per weather variable and pathogen were calculated and analyzed. Correlograms as introduced by Goldwin were used to map those correlations.

Both pathogens showed strongly differing structures comparing the correlation maps for different weather variables. Regarding leaf rust severity temperature during the whole vegetation period seemed to be an important influence. Powdery mildew severity showed the highest correlation coefficients with mean values of sunshine duration during the last month before the monitoring date. The results were compared to the relevant literature and discussed with regard to estimating future disease potentials under climate change.

Poster Session A

Polymer fibres as a prophylactic closure for vines

Melanie Molnar and Michael Fischer

Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen

Email of corresponding author: melanie.molnar@jki.bund.de

The Esca disease, a complex of approximately three or more fungi, has become one of the main diseases in vineyards during the last decades causing high losses and low quality wines. The origins of those wood living fungi are in the Mediterranean area, but due to international trades with grafted wood Esca can be found now all over the world. The main entrance for the fungi is wounds in the bark. As vines are pruned once a year during the winter season the spores find an easy entrance.

As wound protections with different waxes or resins have not lead to an improvement so far; the aim of this project is to find a wound closure to protect the pruning wounds from fungi spores. Therefore a prophylactic closure shall be tested. The closure shall be applied during the pruning time,

providing a physical barrier for pathogens first and foremost the Esca fungi.

The new closure is made of electrospun fibers making the material not only physically stable but also air- and water-permeable. Those characteristics shall promote the healing process as the wound can dry and rotting processes are prevented. Moreover the polymers have to be biodegradable as no residues shall be found in the produced wines.

In this study different polymers are tested at present in the lab for their tightness against different Esca spores. Furthermore the biodegradability of the material is tested over time in soil. Additionally the handling of the polymers and the application on vines is tested.

Automated analysis of tagged amplicon sequences from Next Generation Sequencing

Thomas Berner¹, Holger Heuer² and Jens Keilwagen¹

¹Julius Kühn-Institut, Institute for Biosafety in Plant Biotechnology, Quedlinburg

²Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

Email of corresponding author: thomas.berner@jki.bund.de

Repeated tasks can be handled with workflow management systems that allow for defining, managing and running series of task to produce specific results. Using the open-source software Galaxy, we provide a workflow management system for the JKI.

Based on this system, we provide a workflow for analyzing tagged amplicon sequencing data to predict operational taxonomic units (OTU) and analyse their phylogenetic affiliation. This can be used to compare communities of bacteria, fungi or nematodes between experimental samples.

In a typical experiment, soil or rhizosphere was sampled with replicates from two or more treatments. Total DNA of each sample was extracted and used as target for amplifying small subunit (SSU) rRNA genes (bacteria, nematodes) or internal transcribed space (ITS) fragments (fungi) by PCR. For Next Generation Sequencing, a library was constructed with fusion primers containing sequencing adapters

and a sample-specific eight-base barcode.

We used the 454 Genome Sequencer FLX platform (Roche – 454 Life Sciences, Branford, CT) at Biocant (Cantanhede, Portugal) for library construction and sequencing. Typically 2000-10000 sequences were obtained for each sample.

The workflow for SSU rRNA genes compares each sequence to a ribosomal database by BLAST. In addition, the RDP naive Bayesian classifier is used for taxonomic classification. All sequences are clustered into OTU based on pairwise similarities. For each OTU, the workflow reports its taxonomic classification as well as the number of observed sequences for each sample.

This OTU report is the basis for further analysis to statistically compare communities and find taxa, which respond to the experimental treatment.

A framework for a good review practice of existing evidence of potential impacts of genetically modified plants

Christian Kohl, Ralf Wilhelm and Joachim Schiemann

Julius Kühn Institut, Institute for Biosafety in Plant Biotechnology, Quedlinburg

Email of corresponding author: christian.kohl@jki.bund.de

The European regulatory framework for the market authorization of genetically modified plants (GMP) and products derived thereof demands a comprehensive risk assessment (RA). Even though a lot of safety research has been conducted on GMPs, it is very difficult for stakeholders, risk assessors and the general public to fully overview existing outcomes which are hence not entirely considered in decision-making.

The EU-funded research project GRACE (GMO Risk Assessment and Communication of Evidence) identifies the need to impartially compile existing evidence of potential impacts, including risks and benefits, caused by the deliberate release of GMPs on human and animal health, the environment and socio-economy. Evidence synthesis will be based on the outcomes of national, EU and international research activities.

Systematic evidence synthesis approaches are already established in other research fields to support evidence-based decision-making. They represent powerful tools to collect,

evaluate and summarize accessible research results in order to address a specific scientific issue in a transparent, reproducible and unbiased manner. Thus, their adaption to and implementation in the impact assessment process for GMOs and products derived thereof aims to increase the transparency and support the updating of science-based decision-making processes.

GRACE aims to identify and to integrate the most appropriate evidence synthesis approaches in a unified framework for the impact assessment of GMPs by drawing on and adapting existing concepts and general guidelines.

A set of research questions referring to health, environmental and socio-economic impacts will be reviewed by GRACE and stakeholders will be actively involved. Review teams and stakeholders are supported in preparing and using reviews through an open-access database that mirrors the entire evidence synthesis process. The database will be permanently established beyond the lifetime of the GRACE project.

The effect of *Pseudomonas jessenii* RU47 and *Bacillus amyloliquefaciens* FZB42 on the rhizosphere microbial community and plant growth of tomato and maize

Namis Etlbany and Kornelia Smalla

Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

Email of corresponding author: namis.eltlbany@jki.bund.de

Bio-effectors (BEs) affect plant performance directly and indirectly based on biological mechanisms interfering with soil-plant-microbe interactions. One example for BEs are plant growth promoting rhizobacteria, mostly *Pseudomonas* and *Bacillus* spp., which are applied to a wide range of agricultural crops to enhance growth by promoting seedling emergence, plant biomass and disease control.

In this study, the effect of *Pseudomonas jessenii* RU47 (RU47) and *Bacillus amyloliquefaciens* FZB42 (FB01) on plant growth and on indigenous bacterial and fungal communities in the rhizosphere as well as bulk soil of tomato and maize plants was investigated, with an aim to screen for promising and effective BEs. This work is embedded in the framework of the EU-project "BIOFEKTOR", which aims at increasing the field efficiency of the most promising BEs, with the final goal to contribute to the ecological intensification of agriculture by developing a viable alternative to mineral fertilizers.

A greenhouse experiment with two different plant species (tomato and maize), three application modes (control, RU47 and FB01) and four replicates was performed. The plant growth pa-

rameters were assessed. The effect of inoculant strains RU47 and FB01 on the relative abundances of dominant bacterial, fungal and *Pseudomonas* populations in the rhizosphere and bulk soil of tomato and maize plants was investigated using denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene and ITS fragments amplified from total community-DNA.

RU47 increased the growth of tomato plants compared to the control while FB01 increased the growth of maize plants. The DGGE fingerprint revealed that the application of RU47 and FB01 clearly affected the bacterial community composition in rhizosphere and bulk soil of tomato while for maize effects were only observed in the bulk soil. Furthermore, significant effects of RU47 and FB01 application on fungal communities were observed in the bulk soil of tomato but not in the rhizospheres of tomato and maize. The communities of *Pseudomonas* in rhizosphere and bulk soil were affected only by RU47.

In conclusion, RU47 and FB01 promoted the growth of tomato and maize, which might be related to significant effects on the plant-associated microbial communities.

Pest monitoring in cabbage crops with different sensor systems

Nelli Rempe-Vespermann¹, Martin Hommes¹, Daniel Mentrup² and Arno Ruckelshausen³

¹Julius Kühn-Institut, Institute for Plant Protection in Horticulture and Forest, Braunschweig

²iotec GmbH, Osnabrück

³Hochschule Osnabrück- University of applied science, Faculty of Engineering and Computer Science, Osnabrück

Email of corresponding author: nelli.rempe@jki.bund.de

The application of insecticides is not always opportune because of residues as well as other possible adverse side effects. According to literature the use of insecticides can be reduced by up to 65 % due to economic thresholds. The probability of infestation, which is associated with thresholds values, can be determined with different techniques e.g. crop monitoring. The currently available estimation methods for pests in vegetable crops under field condition are either very time consuming or not area-specific and for this reason rarely applied in practice. Therefore, a key aim of this research is to workout automated area-specific and easy-to-handle monitoring methods. The methods will be tested on the example of cruciferous vegetables under field conditions.

In 2013 three systems have been highlighted particularly under field conditions; a TriangleCameraSystem, a technically modified funnel trap and TrapView. The TriangleCameraSystem as well as the technically modified funnel trap were developed in collaboration with the Hochschule Osnabrück - University of applied science.

The TriangleCameraSystem detects the cabbage root fly (*Delia radicum*) near the ground by three HD web cameras which are arranged around a stem of cabbage crops. The recorded pictures are implemented into an image processing program for analysis. The technically modified funnel trap with a motorized turning unit shall detect lepidopteran pests with an open source based smart camera (leanXcam). TrapView is a technically modified delta pheromone-trap for the automatic detection of codling moth (*Cydia pomonella*) and is commercially available since 2012. According to the distributor iMetos® the trap can also be used for other moths and butterflies. However, the evaluation must be done visually on the basis of transmitted photos of the sticky card.

The first results have revealed that the tested systems in spite of further optimizations are generally well suited for the detection of cabbage root fly (TriangleCameraSystem) or lepidopteran pests (modified funnel trap or TrapView). This project is part of the "WeGa- Kompetenznetz Gartenbau".

High-Throughput screening of drought stress related traits in a set of *Brassica napus* genotypes

Sebastian Albrecht, Hans-Ulrich Jürgens, Christiane Balko and Frank Ordon
Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg
Email of corresponding author: sebastian.albrecht@jki.bund.de

Rapeseed (*Brassica napus* L.) has become the most important oilseed crop in Europe concerning production. Since high yielding modern varieties are based only on a thoroughly selected subset of the available genetic diversity, the project of the Pre-Breed Yield consortium aims at estimating the genetic diversity present in oilseed rape on the phenotypic and genotypic level as the base for a directed improvement of yield and yield stability. In this respect drought as one of the most serious production constraints worldwide came into focus in rape seed breeding. Therefore, a diverse panel of cultivars is evaluated for drought stress tolerance in rain-out shelter trials and in parallel a high throughput screening is established.

Physiological parameters indicating diversity of drought stress tolerance are examined, e.g. the concentration of osmotically active molecules like free proline, total soluble sugars, and relevant traits relating to water balance of plant tissues like membrane stability as well as osmotic adjustment and relative water content. As an indicator for drought stress induced leaf senescence, the chlorophyll content was measured indirectly by Minolta SPAD readings. Results indicate significant effects ($P \geq 0,001$) of induced drought stress

relative to the control treatment for all traits analysed.

By screening the PBY-collection at BBCH16, genotypic differences under drought were identified for the traits; proline, total soluble sugars, membrane stability and chlorophyll content.

Characterization of physiological and agronomic traits in a two-year field trial at Groß Lüsewitz, Germany displayed a significant treatment effect for almost all traits between the irrigated control and drought stress, generated by a rain-out shelter set-up.

The evaluation of adult plants in field pointed out genotypic differences for yield and thousand kernel weight. Drought related variations in physiological response were detected confirming the examined traits as drought stress indicators.

Results of growth chamber experiments were correlated to agronomic traits examined in rain-out shelter trials. Correlations among all traits were calculated and ranged from 0.04 to 0.5 for the physiological traits to yield per plant and thousand seed weight. Based on this, two parameters, i.e. the accumulation of total soluble sugars and the membrane stability index were chosen to be tested on a population comprising 1064 genotypes in growth chamber experiments.

Poster Session B

Characterization and quantification of antibiotic resistance genes and mobile genetic elements on fresh produce

Eva Fornefeld, Sven Jechalke, Katja Reimann and Kornelia Smalla

Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

Email of corresponding author: eva.fornefeld@jki.bund.de

Enterobacteria, especially human pathogens consumed with raw fresh produce can cause human illnesses. As foodborne outbreaks associated with fruits and vegetables are a growing concern, there is a need to further investigate the dissemination of human pathogens to plants and understand the factors influencing their survival on the surface and inside of plants. Especially the aspect of internalization into plant tissues is not entirely understood. Putative factors influencing the colonization of plants by human pathogens may be the presence of resistance genes and mobile genetic elements like plasmids.

Fresh produce from supermarkets was screened for occurrence of enterobacteria, antibiotic resistance genes and mobile genetic elements. The bacterial communities of lettuce, corn salad, cilantro and fresh-cut salad were compared using culture-dependent and -independent methods.

Antibiotic resistant bacteria, mobile genetic elements and resistance genes were found to be widespread on fresh produce. The incidence of Enterobacteria and resistant bacteria differed between plant species with the highest numbers found on cilantro. *E. coli* were detected in a few samples and no *Salmonella*. Among the resistance genes detected were *sul1* and *sul2*. These

genes confer resistance to sulfonamides, which are widely used in veterinary medicine. Furthermore the *qacE* gene was detected, which encodes for resistance against quaternary ammonium compounds that are used as disinfectants in food producing and clinical environments. *QacE* genes are often found on integrons, clinically important genetic elements which are able to integrate and exchange gene cassettes. Among the mobile genetic elements on fresh produce class I integrons were found in high prevalence. Also plasmids of the IncP-1 incompatibility group were detected in some of the samples. These plasmids have a broad host spectrum and are able to transfer resistance genes among almost all Gram-negative bacteria including human pathogens. Mobile genetic elements often carry more than one resistance gene which can lead to co-selection and therefore spreading of different resistances.

In summary, resistance genes and mobile genetic elements were detected on fresh produce which might serve also as reservoir for human pathogens. The influence they might have on survival and spread of human pathogens needs to be investigated in further experiments to avoid produce-related illnesses.

Effect of two different insecticides on the reproduction of pollen beetles in field tests

Meike Brandes^{1,2}, Udo Heimbach¹, Gerrit Hogrefe² and Bernd Ulber²

¹Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Braunschweig

²Georg-August-University Göttingen, Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Section Agricultural Entomology, Göttingen

Email of corresponding author: meike.brandes@jki.bund.de

One of the most important pests of oilseed rape is the pollen beetle (*Meligethes aeneus* F.). Beetles emerging from overwintering sites in spring immigrate to oilseed rape crops and feed on the buds to get access to pollen, which results in bud abortion and high yield loss.

In the past 30 years control of pollen beetle in Germany was mainly based on the application of synthetic pyrethroids. The extensive and indiscriminate use of this insecticide class resulted in a high selection pressure on the beetles, ensuing in the formation of resistance, which has spread over many European countries. Replacement of pyrethroids by insecticides with other mode of action is limited as only few alternative active substances are available.

One of these alternative substances is the neonicotinoid Biscaya (active ingredient thiacloprid). To test the effect of Biscaya and of the pyrethroid Karate Zeon (active ingredient lambda-cyhalothrin) on the reproduction of pollen beetles a field trial was carried out near Braunschweig. The field trial was divided into control plots without insecticide application, and plots sprayed on different dates with Biscaya or Karate Zeon. Shortly before and after application the number of beetles was counted in the different plots. Additionally the number of eggs per bud and the number of larvae dropping to the ground for pupation was recorded. Furthermore samples of adult pollen beetles were analyzed for resistance to pyrethroids and sensitivity to thiacloprid by using the Adult-Vial-Test.

Saponins from *Sapindus mukorossi*: Extraction, purification and biological activity against apple scab

Franziska M. Porsche and Andreas Kollar

Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim

Email of corresponding author: franziska.porsche@jki.bund.de

Saponins from soap nut, *Sapindus mukorossi*, show high antifungal activity against the plant pathogen *Venturia inaequalis*. In seedling assays, a treatment with *S. mukorossi* extract (1%) reduced apple scab symptoms. Pathogen sporulation on infected leaves was reduced (99%) as compared to control.

New extraction and purification methods to analyze and characterize the antifungal saponins from *S. mukorossi* were developed. Fine-grinded pericarps of *Sapindus mukorossi* were extracted with a chloroform-methanol-water mixture. In a next step chloroform and water was added to achieve phase separation. The supernatant was removed and lyophilized. Further purification was achieved by hydrophobic interaction preparative column chromatography.

For the first time Phenyl Sepharose High Performance was used for saponin separation.

Detection of membranolytic/hemolytic fractions was performed with an agar diffusion assay amended with sheep erythrocytes. Fractions were further purified and analyzed with TLC. Separated bands were visualized, scraped off and extracted with 80% ethanol. These compounds were further characterized by HPLC analyses. Peaks were fractionated and subjected to bioassays. One fraction showed significant fungicidal effect on conidia germination and growth of *Venturia inaequalis*.

A general approach for discriminative de-novo motif discovery from high-throughput data

Jan Grau^{1*}, Stefan Posch¹, Ivo Grosse¹ and Jens Keilwagen^{2*}

¹Institute of Computer Science, Martin Luther University Halle-Wittenberg, Germany

²Julius Kühn-Institut, Institute for Biosafety in Plant Biotechnology, Germany

*Both authors contributed equally

Email of corresponding author: jens.keilwagen@jki.bund.de

De-novo motif discovery has been an important challenge of bioinformatics for the last two decades. Since the emergence of high-throughput techniques like ChIP-seq, ChIP-exo, and protein binding microarrays (PBMs), the focus of de-novo motif discovery has shifted to runtime and accuracy on large data sets. For this purpose, specialized algorithms have been designed for discovering motifs in ChIP-seq or PBM data. However, none of the existing approaches works perfectly for all three high-throughput techniques.

Here, we propose *Dimont*, a general approach for fast and accurate de-novo motif discovery from high-throughput data.

We demonstrate that *Dimont* yields a higher number of correct motifs from

ChIP-seq data than any of the specialized approaches, and achieves a higher accuracy for predicting PBM intensities from probe sequence than any of the approaches specifically designed for that purpose. *Dimont* also reports the expected motifs for several ChIP-exo data sets.

Investigating differences between *in-vitro* and *in-vivo* binding, we find that for most transcription factors, the motifs discovered by *Dimont* are in good accordance between techniques, but we also find notable exceptions. We also observe that modeling intra-motif dependencies may increase accuracy, which indicates that more complex motif models are a worthwhile field of research.

Comparison of suppressiveness of soil to *Heterodera schachtii* after different management inputs at different depths

Rasha Nuaima, Holger Heuer and Andreas Westphal

Julius Kühn-Institut, Institute for Epidemiology and Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

Email of corresponding author: rasha.haj-nuaima@jki.bund.de

The cyst nematode *Heterodera schachtii* Schmidt is one of the most dangerous sugar beet pests; it causes serious stand and yield losses in many sugar beet production areas. The adoption of wide crop rotation and the cultivation of resistant cruciferous plants as cover crops and production of tolerant sugar beet cultivars help to maintain economic yields in infested soils. This poster reports *H. schachtii* field population levels under a cruciferous crop rotation and fallow period at two depths (0-30 cm and 30-60 cm) before tolerant sugar beet cultivation. Soil suppression levels to *H. schachtii* were expected to differ in the two soil depths as indicated by an earlier study in California (Roberts et al.). The objectives of our study were to identify major factors (cruciferous crop rotation, fallow crop rotation, soil depth, organic substrates) influencing population levels of *H. schachtii* in support of rational management decisions. Population densities of *H. schachtii* were determined, followed by a bioassay to quantify the number of juveniles (J2) that enter the roots. In this bioassay, equivalents of 50 g soil dry weight were adjusted to 10-20% moisture, seeded with *Raphanus sativus* cv. Saxa 3, and incubated at a day-night (16:8 h) cycle of 28/23 °C. After 4 days of incubation, J2 in radish roots were enumerated. In parallel treatments, loess soil samples received

the equivalent cyst numbers or the equivalent cyst numbers plus organic matter as amendment. Cysts were extracted by density centrifugation using magnesium sulfate solution, were broken to enumerate eggs. Ratios of J2 to eggs were compared by non-parametric statistics to evaluate the effect of soil depth and organic soil material.

No significant difference in the number of J2 penetrating radish roots in the bioassay were observed between fallow period treatment and fodder radish treatment, or between cysts from 0-30 cm and 30-60 cm soil depth.

In the fallow period treatment, the organic substrates showed a significant effect on the number of penetrated J2 in radish root with cysts from 0-30 cm depth. But in the fodder radish treatment the organic substrates did not show this effect. A significant difference in the ratios of J2 / eggs was apparent between field soil treatments of fallow period and fodder radish at 0-30 cm depth. As the soil moisture in the bioassay was 11% for all samples and also other assay conditions were standardized, the reason of the observed effects may not be related to the soil potential water but rather to differences in microbial communities. Possibly, microbial colonization of the organic matter played a critical role.

GRACE - A new European research project on GM crop risk assessment

Christian Kohl, Ralf Wilhelm and Joachim Schiemann

Julius Kühn Institut, Institute for Biosafety in Plant Biotechnology, Quedlinburg

Email of corresponding author: christian.kohl@jki.bund.de

GRACE (GMO Risk Assessment and Communication of Evidence) is a research project under the 7th Framework Program of the European Commission, starting in June 2012 with duration of 3.5 years. The Consortium comprises 18 partners from Europe and South Africa.

It will - a) elaborate and implement systematic, transparent and inclusive reviews of existing evidence of potential health, environmental, and socio-economic impacts (risks and benefits) of genetically modified plants (GMPs) or food and feed derived thereof by following a harmonized framework, and - b) reconsider the design, execution and interpretation of results of animal feeding trials as well as *in vitro* studies for assessing the safety of GM food and feed.

The framework will adapt recently elaborated evidence synthesis approaches to impartially compile existing evidence of potential adverse effects

caused by the deliberate release of GMPs on human and animal health, the environment and socio-economy.

The activity addresses the need for a well documented, transparent and sustainable representation of the evidence synthesis process itself and the derived results. An open access database and a “central access point” for data and information will enable risk assessors, managers, scientists and the general public to reiterate and update their evaluations and conclusions on GMPs.

Animal feeding trials and *in vitro* studies are analyzed with regard to the added value and necessity of 90-day feeding trials with whole foods. Feeding trials are compared with advanced state-of-the-art analytical, *in vitro* and *in silico* tools.

The project will provide guidance for relevant, alternative *in vitro* cell-based approaches within the overall food and feed safety assessment.

Poster Session C

Detection of antibiotic resistance genes and mobile genetic elements in piggery manures and fermentation residues from biogas plants

Birgit Wolters¹, Susen Hartung², Arum Widyasari², Robert Kreuzig² and Kornelia Smalla¹

¹Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Department of Epidemiology and Pathogen Diagnostics, Braunschweig

²Technical University of Braunschweig, Institute of Environmental and Sustainable Chemistry, Braunschweig

Email of corresponding author: birgit.wolters@jki.bund.de

Antibiotics are used extensively in animal husbandry and treated animals often excrete them unchanged or as metabolites. These antibiotics lead to a fitness advantage of resistant bacteria present in the intestine of the animals and in agro-ecosystems to which manures and fermentation residues are applied as fertilizers. This might lead to an enrichment of resistant bacteria and corresponding antibiotic resistance genes (ARGs) in the microbial communities. If they are located on mobile genetic elements (MGEs) these genes may spread even among phylogenetically distant bacteria and new resistance phenotypes of pathogens might occur.

The aim of our project is to monitor antibiotics and ARGs in manures and fermentation residues in order to develop mitigation strategies and to minimize the risk of resistance transfer to pathogens.

To identify parameters influencing the degradation of antibiotics and reduction of ARGs and MGEs during fermentation in biogas plants or storage of manure we sampled 8 mesophilic biogas plants (fermenters were fed with

pig manure except one which was fed with bovine manure) at different processing steps and stored manures of 16 different pig producing facilities. The samples were analyzed for the abundance of ARGs and MGEs (via PCR, Southern blot and quantitative real time PCR) as well as for the presence and content of antibiotics (via LC/MS/MS).

By using DGGE of 16S rRNA gene fragments amplified from total community DNA we analyzed differences in the composition of bacterial communities in manure from different farms and during fermentation in biogas plants.

We detected plasmids of different incompatibility groups (IncN, IncQ, IncP-1, IncW, IncU, Low GC), integrase genes of class 1 and class 2 integrons, several ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *tetM*, *tetX*, *aadA1*) and the resistance gene *qacEΔ1* (against quaternary ammonium compounds) in many samples.

Our data indicate that piggery manures as well as fermentation residues from biogas plants might contribute to the spread of ARGs and MGEs in the agro-ecosystem when used as fertilizer.

Promising alternatives for pest control in *Brassica* crops

Malaika Herbst and Martin Hommes

Julius Kühn-Institut, Institute for Plant Protection in Horticulture and Forests, Braunschweig

Email of corresponding author: malaika.herbst@jki.bund.de

From January 2014 the implementation of Integrated Pest Management (IPM) into modern agriculture is claimed by the EU regulation (2009/128/EU). In the EU project 'PURE' (Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management) the development of different IPM approaches takes place aiming to reduce the application of chemical Plant Protection Products (PPPs) and thereby effects on human health and environment.

In Germany the most damaging insect pests on *Brassica* crops are cabbage root fly (*Delia radicum*), aphids (e.g. the cabbage aphid *Brevicoryne brassicae*) and caterpillars (e.g. cabbage butterfly - *Pieris rapae*, diamondback moth - *Plutella xylostella* as well as the cabbage moth - *Mamestra brassicae*). Thus, different PPPs are tested concerning efficacy, contribution to pesticide reduction and impact on the dif-

ferent pests. For combating cabbage root fly in cauliflower the compound SpinTor® (spinosad) and different alternative PPPs like entomopathogenic nematodes (*Steinernema feltiae*), PERLKA® (lime nitrogen) and the entomopathogenic fungi Naturalis® (*Beauveria bassiana*) are compared. Insecticides for controlling aphids and caterpillars in white cabbage were applied when action thresholds were exceeded.

The impact of SpinTor® (spinosad), broad spectrum insecticides (Karate Zeon® (lambda-cyhalothrin) and Perfekthion® (dimethoat)), selective insecticides (Steward® (indoxacarb) and Pirimor® (pirimicarb)) and biological products (Xentari® (*Bacillus thuringiensis* ssp. *aizawai*) and Micula® (rape oil) against insect pests named above and on their natural enemies is investigated.

Influence of nitrogen supply and drought stress on the water use efficiency in potato

Philipp Meise, Annegret Schum, Sylvia Seddig and Frank Ordon
Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Groß Lüsewitz
Email of corresponding author: philipp.meise@jki.bund.de

Potato (*Solanum tuberosum* L.) is an important crop worldwide for food and non-food uses. Potatoes are produced commercially under various climatic conditions, including arid areas. In order to compensate for inadequate amounts and distribution of rainfall, irrigation systems are used in many potato-producing areas to ensure profitable yields and satisfactory quality. In particular, the tuber initiation stage is most sensitive to drought stress. Limited water availability in that phase can influence the number of tubers and subsequently yield. Many studies have shown that a balanced nitrogen (N) status alleviates drought stress by preventing cell membrane damage and by enhancing osmoregulation. In addition, sufficient N nutrition resulted in higher water use efficiency (WUE), as most of the leaf N is used to synthesize components of the photosynthetic apparatus, in particular RuBisCo playing a major role in carbon assimilation. However, plants with high photosynthetic capacity can consume more available water for the production of biomass. In contrast, water utilization is generally restricted in plants with low N levels and a decrease of WUE has been observed. Both water- and N-deficiency induce many typical modifications in plants at the morphological and physiological level. The aim of the present study is to investigate such abiotic stress response in a number of potato cultivars and to

identify divergent genotypes for further analysis of stress tolerances.

For this purpose a first pot experiment with two N-treatments and two water regimes was conducted in a rain-out shelter in 2013. Plants were supplied with 1040 mg total N in the control and with 260 mg in the N deficiency treatment. Well watered plants were kept at 60 % water capacity of the soil during the entire season, while plants of the drought treatment did not receive any water for 13 days in the tuber initiation stage. The trial comprised 14 commercial starch and three table potato varieties, which were tested in a randomized split-split-plot design.

Here we present first results of agronomic traits such as yield, WUE and N-content as well as physiological parameters including cell membrane stability (CMS), relative water content (RWC) and chlorophyll content (CC). Genotype dependent differences in the response to stress were observed. WUE and CMS decreased under drought- and N-deficiency stress. Furthermore, drought stress initially resulted in an increase in CC, especially under high N supply. While the cultivars did not show differences in RWC in relation to N supply, a significant impact of the water regime was observed. The determined physiological parameters will be correlated with the agronomic performance of the cultivars.

Identification of genomic regions involved in drought stress tolerance in winter barley by association genetics

Thore Fettköther, Christiane Balko and Frank Ordon

Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

Email of corresponding author: thore.fettkoether@jki.bund.de

In the face of global warming drought stress tolerance is becoming an important goal in barley breeding. So far there have been no comprehensive studies on genotypic differences in drought stress tolerance in German winter barley cultivars. Therefore, the project aims at determining diversity on the phenotypic and genotypic level in order to identify genomic regions involved in drought stress tolerance by genome wide association studies (GWAS).

For this purpose 113 German winter barley cultivars and 15 accessions of the Spanish Barley Core Collection (SBCC) were grown in rain-out shelter trials under end terminated drought stress conditions initiated at BBCH 45 and well irrigated field conditions (control) for three growing seasons. Furthermore, a pot trial has been conducted in a growth chamber applying end terminated drought stress from BBCH 45 ongoing. In parallel the cultivars were genotyped using the 9k iSelect SNP-chip.

First results obtained in rain-out shelter trials revealed significant differences between the stressed and the control variant and between genotypes in response to drought for yield and yield components, the content of chlorophyll, free proline and soluble sugars as well as for osmotic adaptation, photosynthetic activity and quality parameters.

Similar results were obtained in the growth chamber experiment.

By analysing of 6073 polymorphic SNPs a large genetic variation was detected within the set of genotypes. Based on the phenotypic results and the genotypic data association genetics studies will be conducted. First results show SNPs significantly associated to drought stress tolerance, which will be converted into easy to handle CAPS or pyrosequencing marker. These will allow the effective marker-assisted selection for drought stress tolerance in barley.

Analysis of *Plasmopara viticola* resistance locus *Rpv10* and comparison to *Rpv3*

Jens Dudenhöffer, Tina Moser, Reinhard Töpfer and Eva Zyprian
Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen
Email of corresponding author: jens.dudenhoeffer@jki.bund.de

The oomycete *Plasmopara viticola* is one of the most important pathogens of grapevine (*Vitis vinifera* L.). Especially all traditional European cultivars used for wine production are highly susceptible. As a result a great quantity of fungicides is necessary to enable a rewarding cultivation of grapes. For that reason newly bred resistant varieties are a major contribution to reduce the fungicidal applications in sustainable viticulture.

Many North American *Vitis* species possess resistance genes due to co-evolution with *Plasmopara viticola*. Beyond that Asian *Vitis* species also exhibit such resistances although their evolutionary origin is not understood yet. Among others, the two loci *Rpv3* (American origin e. g. cv. 'Regent') and *Rpv10* (Asian origin e. g. cv. 'Solaris') have been identified.

In the present study we compare *Rpv3* and *Rpv10* carriers by performing leaf disc assays of *Plasmopara viticola* infections and comparative RNA

sequence analysis to identify differentially expressed genes.

After analyzing the genomic DNA sequence of 'Solaris' at the *Rpv10* locus several candidate genes were found which might be responsible for the expression of resistance against *Plasmopara viticola*. Among these candidates there are five genes with protein domains similar to resistance genes from other plant species. First gene expression studies demonstrated that one major candidate gene was upregulated after infection with the pathogen.

Among several other partners the Julius Kühn-Institut at Siebeldingen is involved in a cooperative project called "Bacchus" funded by the inter-regional program "Interreg IV Upper Rhine" of the objective "European territorial cooperation". The grant is provided by the "European Regional Development Fund" (ERDF) allocated by the European Union (EU).

Molecular characterization of *Barley yellow dwarf virus* (BYDV) resistance gene *Ryd4^{Hb}* introgressed from *Hordeum bulbosum* into barley

Janine Wojacki¹, Brigitte Ruge-Wehling¹, Kristin Fischer¹, Margret Scholz¹, Antje Habekuß², Peter Winter³, Björn Rotter³ and Peter Wehling¹

¹Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz

²Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

³GenXPro GmbH, Frankfurt/M.

Email of corresponding author: janine.wojacki@jki.bund.de

BYDV is a widespread and economically important virus disease of cereals. The disease is caused by different viruses of the family *Luteoviridae* and results in high yield losses. As a consequence of global warming, aphids which may act as virus vectors are expected to be active for a longer time during the year, thereby increasingly threatening the growing of winter barley. The primary gene pool, which is represented by *Hordeum vulgare* and its subspecies *H. vulgare* subsp. *spontaneum*, contains genes for tolerance, rather than resistance, against BYDV. In contrast, the secondary gene pool, with the wild species *H. bulbosum* (Hb), was shown to comprise genes which confer complete resistance. In order to supply breeders with new sources of resistance, interspecific crosses between *H. vulgare* cv. 'Igri' and *H. bulbosum* were performed to generate a segregating mapping population.

Previous studies indicate that the resistance against BYDV was introgressed to barley chromosome 3HL and is governed by a dominant gene, *Ryd4^{Hb}*.

In the present study we develop molecular markers specific for the introgressed *Hb* segment. Three strategies are applied: (I) use of anchor markers from the barley genome, (II) make use of the orthology of parts of the *Hordeum* genome and the model genome of *Oryza sativa*, and (III) perform Massive Analysis of cDNA Ends (MACE) and RNASeq.

The approaches provide approximately 200 markers for the 3HL introgression. So far, we mapped about 40 markers on chromosome 3HL. Three markers are located in the vicinity of *Ryd4^{Hb}* and are of potential use for marker-assisted breeding programs.

„Berichte aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft“ erscheinen seit 1995 in zwangloser Folge

Seit 2008 werden sie unter neuem Namen weitergeführt:
„Berichte aus dem Julius Kühn-Institut“

- Heft 146, 2009: Chronik zum 75jährigen Jubiläum des Instituts für Pflanzenschutz in Ackerbau und Grünland. Bärbel Schöber-Butin, 47 S.
- Heft 147, 2009: NEPTUN 2007 – Obstbau. Dietmar Roßberg, 71 S.
- Heft 148, 2009: 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. July 5 – 10, 2009, Neustadt, Germany, 92 S.
- Heft 149, 2009: Netz Vergleichsbetriebe Pflanzenschutz – Jahresbericht 2008. Bearbeitet von: Bernd Freier, Bernhard Pallutt, Marga Jahn, Jörg Sellmann, Volkmar Gutsche, Wolfgang Zornbach, Eckard Moll, 64 S.
- Heft 150, 2009: NEPTUN 2008 – Hopfen. Dietmar Roßberg, 17 S.
- Heft 151, 2010: NEPTUN 2009 – Weinbau. Dietmar Roßberg, 19 S.
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