

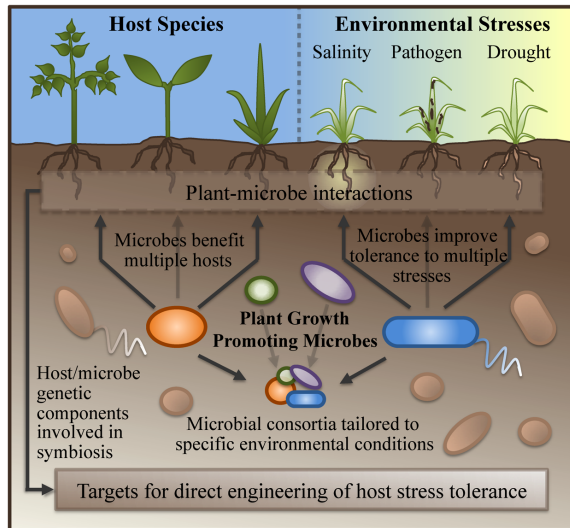
2nd EUCARPIA Workshop on Implementing Plant-Microbe Interactions in Plant Breeding

December 6th 2019

Universitäts- und Forschungszentrum Tulln

Konrad Lorenz-Str. 24

3430 Tulln an der Donau, Austria



Coleman-Derr & Tringe 2014. *Front. Microbiol.* 5:283

EUCARPIA Working Group Plant-Microbe Interactions
Section Organic and Low-Input Agriculture

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Description

The workshop aims to strengthen a network among plant breeders and scientist of different disciplines to explore the use of plant-microbe interactions in plant breeding.

In recent years, plant-associated microbes have received considerable attention in research for their ability to improve crop productivity and yield stability. Benefits include improved nutrient uptake and resistance against biotic and abiotic stresses. Influences of crop management, soil parameters and climatic effects are well documented. Knowledge on plant genetic determinants for beneficial interactions with individual microbes (incl. biologicals) and entire communities is growing rapidly. Several reports indicate that not only the host species but also the host genotype play a significant role in driving microbial community composition and activity, selecting for and against particular members of the microbial community. However, to what extent genetic factors are responsible for shaping beneficial plant microbiomes is still poorly understood. Similarly, seed or plant microbiome manipulation via the introduction of biologicals offers great promise, but still suffers from variable outcomes due to insufficient knowledge of the factors involved for a successful integration. In conclusion, there are still many uncertainties on how to implement this knowledge into plant breeding and seed multiplication programmes.

The Workshop is organised by the EUCARPIA Working Group on Plant-Microbe Interactions of the Section Organic and Low-Input Agriculture and will continue to discuss the potential and limitations of implementing the growing knowledge on plant-microbe interactions in plant breeding in order to improve stress resistance, plant nutrition, plant health and general adaptability, and links between upstream disciplines and breeding. This shall foster the dialogue between the different disciplines in order to develop advanced breeding strategies for the future.

Session I: Genetic determinants of plant-microbe interactions

Keynote: Dr. Davide Bulgarelli
(Royal Society of Edinburgh Research Fellow at Dundee University)

Session II: Integration of plant-microbe interactions into breeding

Keynote: Dr. Martin de Vos (Program Scientist Trait Biology at KeyGene N.V.)

On behalf of the Organizing Committee:

Pierre Hohmann¹, Friederike Trognitz², Monika Messmer¹

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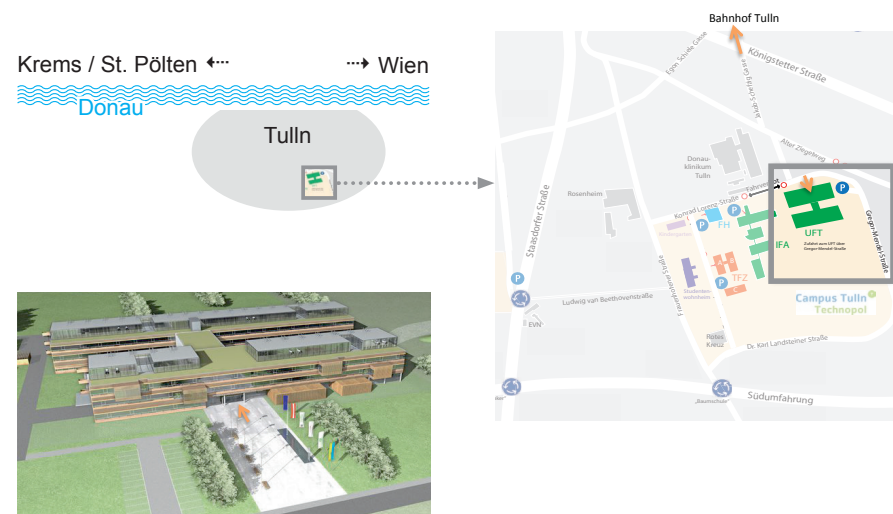
² AIT Austrian Institute of Technology GmbH, Tulln, Austria

Supported by:



Location

The workshop is taking place at **Universitäts- und Forschungszentrum Tulln, Konrad Lorenz-Str. 24, 3430 Tulln an der Donau, Austria.**



Universitäts- und Forschungszentrum Tulln (UFT)

Universität für Bodenkultur
Konrad Lorenz-Str. 24, 3430 Tulln an der Donau
(Zufahrt über Gregor-Mendel-Str.)

Programme

9:00 – 9:30 Registration & coffee

9:30 Welcome note

Session 1

- 9:40 – 10:10 **Keynote:** Defining the host genetic determinants of the barley rhizosphere microbiota Davide Bulgarelli (Dundee Univ.)
- 10:10 – 10:25 Effect of different Serendipita species on Fusarium wilt in a susceptible and tolerant tomato cultivar Negar Ghezel Sefloo, ..., [Karin Hage-Ahmed](#)
- 10:25 – 10:40 Influence of bacteria inoculation of the gene expression during soybean germination [Friederike Trognitz](#), ..., Angela Sessitsch
- 10:40 – 10:55 Genetically determined responsiveness to beneficial microbiomes in the rhizosphere can suppress phytonematodes [Ahmed Elhady](#), ..., Holger Heuer
- 10:55 – 11:10 Genetic differences in barley govern the receptiveness to priming agent [Abhishek Shrestha](#), ..., Adam Schikora

Session 2

- 11:10 – 11:40 **Keynote:** Strategies for molecular resistance breeding and directions for enabling beneficial plant-microbe interactions Martin de Vos (KeyGene N.V.)
- 11:40 – 11:55 Breeding for priming triggered leaf rust resistance in barley [Gwendolin Wehner](#), ..., Frank Ordon

11:55 – 12:40 Lunch break

- 12:45 – 13:00 Innovative biofertilizers improving yielding of horticultural crops and soil fertility [Sas Pasz L.](#), ..., [Krzysztof Weszczak](#)
- 13:00 – 13:15 Heritability of root rot resistance of pea assessed on naturally infested field soil [Lukas Wille](#), ..., Pierre Hohmann
- 13:15 – 13:30 Insights of a WUR workshop on Breeding for Diversity [Guusje Bonnema](#), ... [Liesje Mommer](#)
- 13:30 – 14:15 Flash talks (3 minutes each)
1. Caroline Schneider & Mirjam Seeliger, INOQ
 2. Mauricio Dimitrov, KeyGene
 3. Szilvia Bencze, ÖMKi
 4. Jaderson Armanhi & Bárbara Bort Biazotti, University of Campinas
 5. Ulf Feuerstein, DSV
 6. Céline Hamon, Vegenov
 7. Anurag Chaturvedi, Université de Lausanne
 8. Michelle Carkner, University of Manitoba
 9. Shree Pariyar, Forschungszentrum Jülich
 10. Abco de Buck, Louis Bolk Institute
- 14:15 – 14:45 Group work
- 14:45 – 15:10 Coffee break
- 15:15 – 15:30 Group work wrap-up
- 15:30 – 16:00 Plenum discussion
- 16:00 – 16:15 Closing of the workshop

Abstracts

Defining the host genetic determinants of the barley rhizosphere microbiota

Davide Bulgarelli

Plant Sciences, School of Life Sciences, University of Dundee at the James Hutton Institute.
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My group uses barley (*Hordeum vulgare*) as a model to gain novel insights into the functional significance of plant-microbiota interactions in the rhizosphere and devise novel strategies to enhance sustainable crop production.

We previously demonstrated that Elite varieties and wild barley ancestors host distinct microbiotas, possibly representing a footprint of plant domestication on the microbial communities inhabiting the rhizosphere. We recently extended these lines of investigation by characterising the microbiota of a bi-parental population between an elite variety and a wild barley ancestor. By combining 16S rRNA gene sequencing profiles, as ‘quantitative traits’, with thousands of SNPs in the barley genome we compiled a map of the plant loci shaping the rhizosphere microbiota. Strikingly, we did not observe a linear relationship between number of loci and bacteria putatively controlled by them. Rather, our data suggest that microbial community assembly in the barley rhizosphere is controlled by a few major alleles with a major effect. In particular, we identified a single locus on barley chromosome 3H significantly associated with the recruitment of several, phylogenetically unrelated, bacteria. We produced isogenic barley lines harbouring contrasting alleles at the locus of interest and we demonstrated that the introgression of wild barley genes into a modern variety on chromosome 3H is sufficient to shape, at least in part, the rhizosphere microbiota.

Here I will discuss the experiments that led us to these discoveries and their implications for basic science and translational agriculture.

Effect of different *Serendipita* species on *Fusarium* wilt in a susceptible and tolerant tomato cultivar

Negar Ghezel Sefloo, Constanze Hauser, Anna Marie Hallasgo, Krzysztof Wieczorek, Siegrid Steinkellner and [Karin Hage-Ahmed](#)

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Endophytic fungi from the family *Serendipitaceae* have recently gained increasing attention due to their growth-promoting and bioprotective properties. The majority of studies were conducted with the most prominent representatives, such as *Serendipita indica* and *S. vermifera*. However, due to their origin, they might be critical for application in Europe. To date, little is known about other members of this family, such as *S. williamsii* and *S. herbamans*, which are of European origin. To test their biocontrol and growth-promoting potential, a susceptible and tolerant tomato cultivar (Kremser Perle and Micro-Tom, respectively) was inoculated with *S. williamsii*, *S. herbamans*, *S. indica* or *S. vermifera* and challenged with the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) in greenhouse experiments. Furthermore, *in vitro* assays on the direct inhibitory effects of *Serendipita* spp. against *Fol* were performed. Negative effects of *Fol* on the phenological growth in the susceptible cultivar were alleviated by all four applied *Serendipita* spp. Apart from these similar effects on biometric parameters, disease incidence was only reduced by *S. herbamans* and *S. vermifera*, respectively. In the tolerant cultivar, disease parameters remained unaffected although shoot dry mass was negatively affected by *S. vermifera*. Direct effects of *Serendipita* spp. against *Fol* were not evident in the *in vitro* assays indicating an indirect effect via the host plant. Our results highlight the importance of identifying cultivar-specific effects in pathogen–endophyte–plant interactions to determine the most beneficial combinations.

Influence of bacteria inoculation of the gene expression during soybean germination

Friederike Trognitz, Oscar Gonzales Lopez, Angela Sessitsch

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Many plants are reproduced through seeds. Seeds are adapted to survive for a period under unfavorable conditions. Longevity depends on the plant species, storage conditions but is also linked to certain microbial activities. To test the effect of bacterial seed endophytes germination, we isolated endophytes from different soybean varieties with different germination abilities. Isolates were tested on soybean for germination improvement under cool conditions and biocontrol activity *in vitro*. Several strains had a positive effect on seedling growth at 12° C. Two strains were selected for further analysis on the transcriptome on soybean, and of the two strains showed more biocontrol activities against *Rhizoctonia solani* and *Fusarium oxysporum*, whereas the other one had plant growth-promoting abilities. For the transcriptome experiment, the seeds were inoculated with either of the strains and kept for 24 and 48 hours at 18° C. The change in the plant gene expression due to bacterial inoculation was analyzed. Both strains had a different effect on the plant. The biocontrol strain influenced plant gene regulation already after 24 hours at 18° C and differential regulated genes belong to the defense pathway like FLS2, WRKY33, CDPK and PR1. After 48 hours genes involved in germination were up-regulated like genes involved in phytohormone production of auxin and cytokinin, whereas genes for ethylene and abscisic acid production were down-regulated. Inoculation with the plant growth-promoting strain resulted after 48 hours in the up- or down-regulation of only 500 genes, whereas 2000 genes were differentially expressed when inoculated with the biocontrol strain.

Applying both strains in a field trial, we found higher seed weight per 100 seeds compared to the control treatment and a higher oil content in the seeds, but no change in the protein content. Future tests are needed on different varieties to prove the findings.

Genetically determined responsiveness to beneficial microbiomes in the rhizosphere can suppress phytonematodes

Ahmed Elhady, Shimaa Adss, Johannes Hallmann, Holger Heuer

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

By plant-soil feedback, successors or neighbours of a plant growing in the same soil may benefit from recruited mutualistic microorganisms. Under the temperate condition of Germany, the susceptibility of short-season soybean genotypes towards naturally occurring populations of root lesion nematodes (RLN) in the field were found to vary among soybean genotypes. Cultivars Abelina, Primus, and Taifun3 supported the highest infestation and multiplication of a mixed population of *P. neglectus* and *P. crenatus*, while Sultana and Solena were least susceptible. Currently, we are investigating whether the defence capacity of soybean cultivars against RLN induced by the microbiome is determined by the plant genotype, i.e. different soybean genotypes may respond differently to a suppressive microbiome. We believe that differences in the expression of defence genes and biochemical compounds can explain the impact of the suppressive microbiome to induce genes involved in defence responses on suppression of nematodes between high and low responsive genotypes. Crossing lines of two soybean genotypes differed in their defence responsiveness by the bacterial quorum-sensing molecule N-3-oxotetradecanoyl-l-homoserine lactone (oxo-C14-HSL) against root lesion nematodes (RLN). Genetically determined responsiveness to beneficial microbiomes could be the basis for a breeding strategy.

Genetic differences in barley govern the receptiveness to priming agent

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Priming crop plants for enhanced resistance using biocontrol agents is an efficient disease management strategy, since it results in robust resistance and higher yield. The beneficial effects of the bacterial quorum sensing (QS) molecules e.g. N-acyl homoserine lactones (AHL) on resistance and plant growth have been shown in different plant species. Here, we present the effects of the AHL, oxo-C14-HSL, on the priming capacity of barley. We demonstrated that barley primed with *Ensifer meliloti*, expresses enhanced resistance against *Blumeria graminis*. We also showed that the capacity to induce priming varies among different barley genotypes. Among a set of barley genotypes, we identified “primable” genotypes that had better ability to enhance resistance and “non-primable” genotypes that were non-responsive to oxo-C14-HSL and therefore, did not have any ability to enhance resistance. This suggests that appropriate genetic component is required for AHL-induced priming. We further showed that priming for enhanced resistance in barley involves stronger activation of the barley ortholog of the MPK6, regulation of defense-related *PR1* and *PR17b* genes and remodeling of the cell wall structure. Noticeable was the stronger accumulation of lignin upon priming after a chitin challenge. Interestingly, the global metabolomic changes in barley during priming are rather subtle and specific. Identification of these metabolites is important as it opens doors to study the mechanisms and understand the relation between the plant genomic background and the priming agent. This understanding would further increase the efficacy of priming approaches and lead to novel breeding strategies.

Heritability of root rot resistance of pea assessed on naturally infested field soil

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Root and foot diseases severely impede pea (*Pisum sativum*) cultivation worldwide, and therefore a valuable protein source and important crop in low-input farming systems. Breeding lines with resistance against individual pathogens exist, but these resistances are often overcome by the interaction of multiple pathogens in the field. Moreover, resistance depends on the interactions between the plant associated microbial community and the host, and there is increasing evidence for the existence of genetic variation in the regulation of plant-microbe interactions that can be exploited by plant breeders. In order to acknowledge the entire native soil microbiome as a key element of plant resistance we designed a resistance screening experiment on naturally infested field soil. In a first step, DNA from diseased roots and rhizosphere soil from infected plants was isolated. Sequencing of the fungal ITS region in the roots and rhizosphere showed the presence of several putative pea pathogens along putative beneficials. Subsequently, 261 pea lines were grown under controlled conditions and evaluated after three weeks. Along significant genotypic differences, moderate to high heritabilities could be revealed for root rot resistance and growth performance traits. Relating different resistance traits allowed to distinguish between highly susceptible, tolerant and resistant lines and between resistance at different time points in plant development (i.e. emergence and young plant stage). The evaluation of a subset of pea lines on two field sites with moderate and high root-rot potential, respectively, confirmed the resistance ranking obtained under controlled conditions. Furthermore, we used quantitative real-time PCR targeting selected fungal pathogen species to show that the pathogen DNA detected in the roots differs among resistant and susceptible pea genotypes, respectively. Our results indicate the replicability and usefulness of naturally infested field soil-based screening systems. Such systems will allow to pursue research on plant-microbiome interactions and on the role specific pathogen species or potential beneficial microbes play in plant disease resistance.

Innovative biofertilizers improving yielding of horticultural crops and soil fertility.

Sas Paszt L., Paweł Trzciniński, Anna Lisek, Beata Sumorok, Edyta Derkowska, Sławomir Głuszek, Krzysztof Górnik, Mateusz Frąc, Michał Przybył, Krzysztof Weszczak

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To obtain high yields in intensive horticultural and agricultural production, high levels of mineral fertilization combined with the application of chemical plant protection products are commonly used. This results in a loss of the biological potential and erosion of soils, which leads to deterioration in the quality and fertility of cultivated soils. An alternative to such production is the use of microbially-enriched biofertilizers, biostimulators and composts. The aim of the study was to evaluate the effects of the applied microbial bioproducts on the growth and yield and develop innovative technologies for improving the quality of soils of selected species of horticultural plants. We have developed innovative consortia of beneficial microorganisms on the basis of the resources collected in SYMBIO BANK of the Department of Microbiology, Research Institute of Horticulture in Skierniewice. The results of the experiments demonstrated a positive influence of the microbial method of cultivating fruit and vegetable crops with the use of beneficial microorganisms, a compost based on biochar and humic acids on the vegetative growth and yielding of plants and the occurrence of beneficial groups of microorganisms in the rhizosphere of those plants. Moreover, the application of beneficial microorganisms positively affected the germination of vegetable seeds and seedlings emergence. Due to vegetable seeds treatments with beneficial microorganisms, the dynamics of root and hypocotyl growth at the early stages of plant development was improved as well as further growth of plants was stimulated. The application of beneficial microorganisms also positively influenced photosynthetic activity monitored by chlorophyll a fluorescence in leaves. As a result of the application of beneficial microorganisms and biofertilizers in cultivation significantly higher yields of the tested vegetable and fruit species were achieved, with better storage and processing qualities compared to conventional production. The use of beneficial microorganisms in cultivation of horticultural plants will multiply their positive impact on the yield

potential of horticultural plants and improve the quality of soils. Widespread use of the innovative bioproducts in organic cultivation of vegetables and fruit will help improve the quality of soils and increase the profitability of horticultural farms by reducing production costs.

Acknowledgement:

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Breeding for priming triggered leaf rust resistance in barley

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Leaf rust (*Puccinia hordei*) is one of the major diseases of barley (*Hordeum vulgare* L.) leading to yield losses up to 60%. Resistance genes *Rph1-Rph26* are known but most of these have been overcome, meanwhile. In this respect, priming may offer an opportunity to enhance resistance to *P. hordei*. During quorum sensing in populations of many Gram-negative bacteria, single cells produce *N*-acyl homoserine-lactones (AHL). Those molecules are known to induce resistance in plants. The present study aims therefore at the detection of genotypic differences in the response of barley to AHL, followed by the identification of genomic regions involved in priming capacity of barley, which is one of the most important cereal crops, worldwide. A diverse set of 200 spring barley accessions was treated with bacteria, i.e. a repaired *Ensifer meliloti* natural mutant strain *expR+ch* producing substantial amount of the AHL oxo-C14-HSL and a transformed *E. meliloti* strain carrying the lactonase gene *attM* from *Agrobacterium tumefaciens* which inhibits AHL accumulation. After three bacterial inoculations, plants at the three-leaf stage were infected with *P. hordei* strain I-80. 12 days after infection scoring of the diseased leaf area and the infection type was conducted and the relative susceptibility was calculated thereof. Results revealed significant effects ($p < 0.001$) of the bacterial treatment indicating a positive effect of priming on resistance to *P. hordei*. Based on the observed phenotypic differences and 23,417 filtered SNPs derived from the Illumina 9k iSelect chip and genotyping by sequencing (GBS), 5 quantitative trait loci (QTL) associated to improved resistance to *P. hordei* after priming with *E. meliloti expR+*, were identified on the short arms of barley chromosomes 6H and 7H. QTL for the priming inducibility may be interesting for a pre-selection of primable accessions. Finally, KASP markers will be developed, facilitating marker assisted selection of priming efficient accessions in barley breeding. Moreover, genes in QTL regions might be interesting candidates for further research on the mechanisms of plant-microbe interactions.

The authors thank the German Federal Ministry of Education and Research (BMBF) for funding this project (FKZ 031B0196C).

Workshop “Breeding for diversity”

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On 30 Oct 2019, scientists and representatives from breeding companies and the Dutch government gathered to discuss the opportunities to do plant breeding for crop mixtures, during the workshop ‘Breeding for Diversity’ on the Wageningen UR campus,

www.pe-rc.nl/breeding-for-diversity.

Crop diversification is one of the cornerstones in building sustainable, circular agricultural systems. One of the most promising strategies is mixed cropping – growing different crops and/or genotypes together. Currently, several initiatives are exploring the agronomic and economic benefits at the farm level. However, we do not know what traits make different crop species/genotypes a ‘winning team’, as the genotypes that perform best in monocultures may not be suitable for mixtures. By understanding crop complementarity and identifying these traits, the Dutch breeding sector will be equipped with the tools needed to breed for circular agriculture and catalyse this radical change. We aim to provide a scientific basis for developing ‘winning teams’, based on both above and below-ground plant traits, including interactions with soil biota. We aim to build a 3D modelling tool that allows efficient testing of multiple genotype combinations that differ in key traits.

The challenges associated with breeding for such diverse systems were presented by WUR scientists and breeding companies, and subsequently discussed in groups. In a short presentation I will summarize the outcome of the workshop in terms of opportunities, challenges and priorities in the domain of breeding for diversity.

List of participants

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