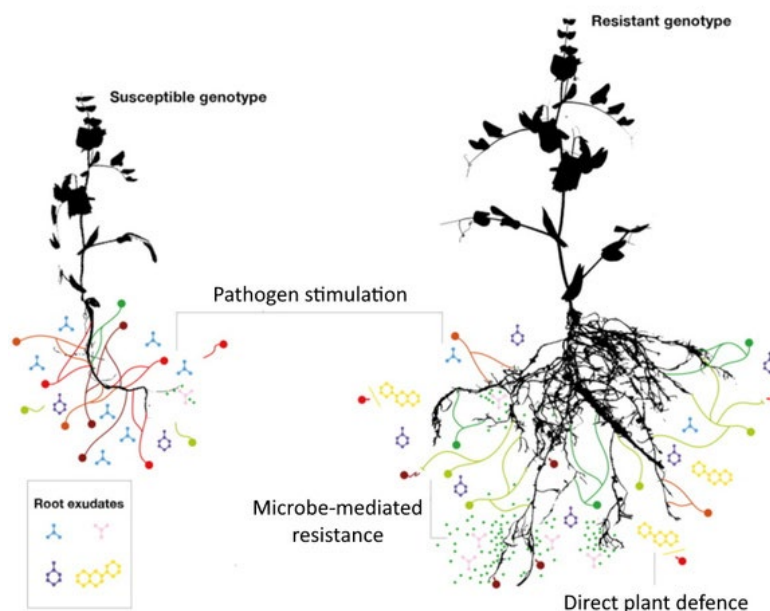


# 4<sup>th</sup> Workshop on Implementing Plant-Microbe Interactions in Plant Breeding

19 July 2024

Universitäts- und Forschungszentrum Tulln

Konrad Lorenz-Str. 24, 3430 Tulln an der Donau, Austria



modified from Wille et al. 2019, *Plant Cell Environ*

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

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## Description

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

Knowledge on plant genetic determinants for beneficial interactions with individual microbes (incl. biologicals) and entire communities is growing rapidly. However, the implementation of such knowledge in plant breeding is still in its infancy. Similarly, plant microbiome manipulation via the early introduction of biologicals (e.g. via seed treatments) offers great promise, but still suffers from variable outcomes.

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 its growing knowledge in plant breeding. Previous workshop outputs were synthesised in a perspective article on emerging research priorities towards microbe-assisted crop production ([Hohmann et al. 2020, FEMS Micr Ecol](#)).

This year's 4<sup>th</sup> EUCARPIA workshop will continue the discussion and foster the dialogue between the different research disciplines and industry to promote the development of holobiont-based breeding strategies for future resilient agroecosystems.

## Keynote Speaker

**Raffaella Balestrini**

(National Research Council, IT)

Title: Crop Breeding based on root traits and associated microbes

## On behalf of the Organizing Committee:

Pierre Hohmann<sup>1,2</sup>, Valentin Gfeller<sup>3</sup>, Friederike Trognitz<sup>4</sup>, Monika Messmer<sup>3</sup>

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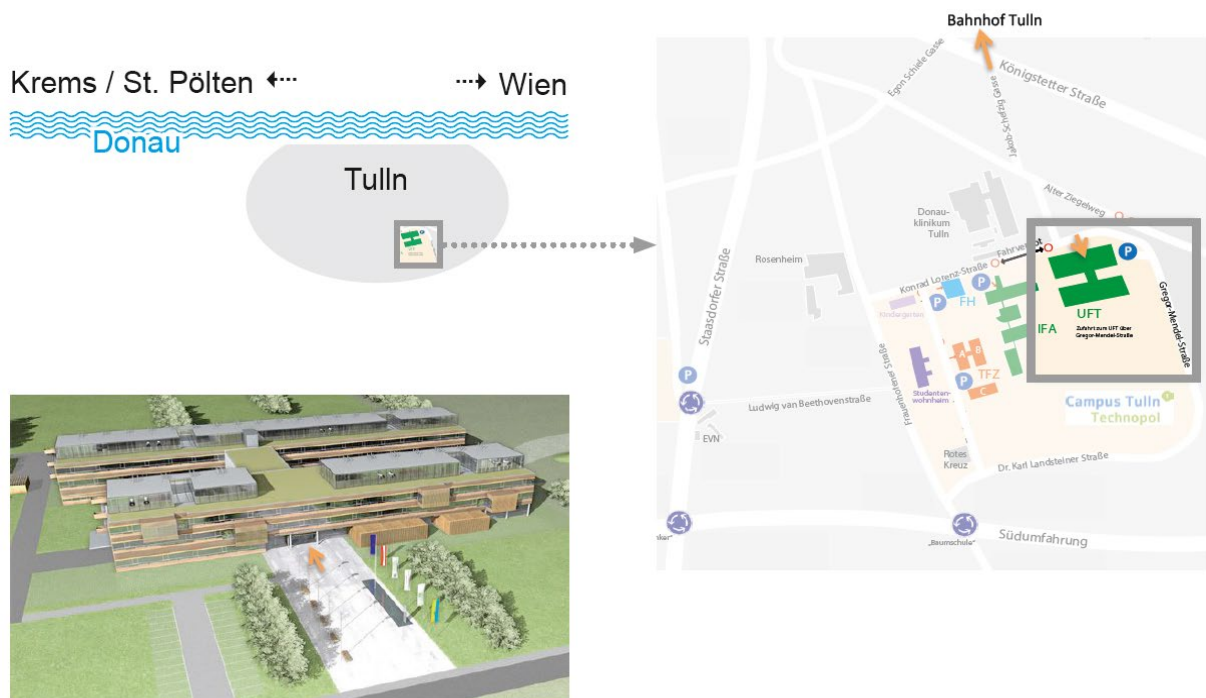
## Programme

9:00 - 9:30	Registration	
9:30 - 9:40	Welcome note	
<b>Session 1</b>		
9:40 - 10:05	<b>Keynote:</b> Crop Breeding based on root traits and associated microbes	<u>Raffaella Balestrini</u>
10:05 - 10:45	4-min flash talks and introduction round	
10:45 - 11:15	Coffee break	
11:15 - 11:30	Understanding the holobiontic nature of eggplant in bacterial wilt resistance	<u>Gaofei Jiang</u>
11:30 - 11:45	Synthetic bacterial community mimics the assembly pattern of a natural community on wheat roots grown in soil	<u>Frederik Bak, ... Mette Nicolaisen</u>
11:45 - 12:15	Summary of 3 <sup>rd</sup> workshop in 2022 and introduction to the group work session	
12:15 - 13:30	Lunch break	
<b>Session 2</b>		
13:30 - 15:00	Group work	
15:00 - 15:30	Coffee break	
15:30 - 16:15	Plenum discussion	

## Location

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

Travel option from Vienna Heiligenstadt: Take the REX<sub>4</sub> train departing at 8:09 or 8:38 to Krems an der Donau, arriving at Tulln at 8:31 or 8:59, respectively. From Tulln train station, it's a 10-minute walk to UFT Konrad Lorenz Str. 24 (see below). Safe travels!



### Universitäts- und Forschungszentrum Tulln (UFT)

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

## Abstracts - Oral presentations

### Keynote

#### **Crop Breeding based on root traits and associated microbes**

Raffaella Balestrini

National Research Council, Institute of Biosciences and Bioresources, Italy

In the context of climate change, crop and soil microbiota diversity are pivotal for addressing environmental threats, improving crop yields, and promoting soil health. Intercropping, i.e., the simultaneous cultivation of two or more crop species in the same field, enhances soil microbial diversity and decreases dependence on nitrogen fertilizers. This practice benefits from above- and below-ground plant interactions. In this context, a relevant role is played by the arbuscular mycorrhizal fungi (AMF), which are widely distributed fungi that can form a symbiosis with more than 80% of terrestrial plants. The application of AMF-based inocula currently represents a promising sustainable approach to improve plant nutrition and tolerance to stresses as well as soil quality. However, the effects of these cultivation practices (i.e., intercropping and use of AM fungal inoculum in the field) on soil microbes and crop traits are still poorly investigated as well as the mechanisms involved in responsiveness to AMF inoculation in diverse genotypes. Overall, the importance of considering the interactions between roots and soil microbes to improve plant nutrition and protection in the context of sustainable agriculture in the face of climate change will be discussed, providing data from pot and in field experiments. The importance of the phenotyping of roots, also in terms of the ability to interact with beneficial soil microbes, will be also highlighted.

## Understanding the holobiontic nature of eggplant in bacterial wilt resistance

Gaofei Jiang

Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, PR China; [gjiang@njau.edu.cn](mailto:gjiang@njau.edu.cn)

Breeding disease-resistant crops is essential to increase yield while cutting down the use of pesticides. Several diseases remain however off target for breeding due to the absence of classical resistance genes. Here we demonstrate that including the plant microbiome in disease resistance allows identifying new alleles that can be targeted to breed improved crops. We measured genome, rhizosphere microbiota and root exudates of 432 eggplant varieties, and reconstructed their relative contribution to the soil-borne bacterial pathogen *Ralstonia solanacearum*. Different cultivar broadly differed in disease tolerance. We ascribed high disease tolerance to a set of SNPs associated with root exudates, suggesting that these genes work by modulating the microbiome. In turn, these SNPs led to the recruitment of a set of bacterial species with putative beneficial properties. We further identified the combined effects of rhizosphere microbiota, root exudates, and host genetics on eggplant resistance, with host genetics contributing the most to phenotype, while the rhizosphere microbiota played a relatively smaller role. Our results confirm the critical roles of host genetics and rhizosphere microbiota in shaping resistance phenotypes. These insights offer valuable understanding into the underlying mechanisms of crop resistance and provide a essential theoretical foundations for future crop breeding work.

## **Synthetic bacterial community mimics the assembly pattern of a natural community on wheat roots grown in soil**

Frederik Bak<sup>1,2</sup>, Jakob K. Meier<sup>1</sup>, Kitzia Y.M. Zamudio<sup>1</sup>, Clara Tang<sup>3</sup>, Mette H. Nicolaisen<sup>1</sup>

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<sup>2</sup> Bioresources Unit, AIT Austrian Institute of Technology

<sup>3</sup> Plant and Microbial Biology, North Carolina State University

Root-associated bacteria provide important nutrients and can alleviate biotic and abiotic stresses in the plant. Originating from the soil and the seed, they form a structured bacterial community, and the intimate host-microbe interactions have led to the view of the plant and its microbiota as a metaorganism. However it is still not clear, how the bacterial community assemble on the roots, due the high complexity in the soil. Here, we designed a synthetic community (SynCom) comprising 13 genera isolated from wheat roots, to study the community assembly on roots. We inoculated the SynCom to gamma-irradiated soil, with a low bacterial abundance and diversity, prior to wheat seedling transplantation, and tracked the community development on the roots over a 4-week period using 16S rRNA amplicon sequencing. This was compared to seedlings inoculated with a richer and more diverse natural soil community. Despite the low diversity of the SynCom, we did not find any changes in root and shoot length inoculated with the SynCom, as compared to inoculation with the natural community. The temporal dynamics of most SynCom members mimicked those of corresponding genera in the natural soil community. We demonstrate that a reductionist approach can be used to study bacterial community assembly on plant roots in soil and provide a conceptual framework for future studies which can shed light on factors determining assembly processes on the root and interactions among root-associated bacteria.



## Abstracts - Flash talks

### Breeding for Mycorrhizal Symbiosis

Natalie Ferro Lozano<sup>1, 2,3</sup>, Liesje Mommer<sup>1</sup>, Guusje Bonnema<sup>1</sup>, Thom Kuyper<sup>1</sup>.

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One of the most common organic practices is to improve soil fertility and plant nutrient acquisition by using beneficial soil microorganisms. Arbuscular mycorrhizal fungi (AMF) are one of such beneficial soil microorganisms that improve plant nutrient acquisition while also provides the plant with a primed immune system (Cardoso & Kuyper, 2006; Jung et al., 2012; Kuyper et al., 2021). The question is how the beneficial impact of AMF can be harnessed to increase or protect cocoa production? Cocoa is required to be grown organically if cocoa farmers want to access premium prices due to the Fairtrade certifications. Still, due to the lack of adequate organic agricultural technology, around 30% of the yield is currently lost due to diseases. Thus far breeding has focused on above-ground traits that can provide plant vigour, quality and yield of harvests, neglecting belowground traits, including traits related to roots or mycorrhizal symbiosis. Considering mycorrhizal symbiosis as a likely polygenic trait, it could be a durable comprehensive improvement regarding nutrition and defences. Therefore, I propose to unravel the root traits and genetics that could facilitate mycorrhizal symbiosis in cacao. *T. cacao* is highly responsive to AMF, in some cases triplicating the total dry matter in presence of AMF (Chulan Hashim & Ragu, 1986). Still, plant responses to AMF depend on plant and mycorrhizal fungal species and genotype, the interaction between these and environmental conditions in which the symbiosis develops (Johnson et al., 2015). That opens the possibility to harness the genetic variation present to enhance plant benefit from AMF symbiosis. Identifying markers is an essential contribution to fundamental understanding that will benefit the breeding of crops with increased mycorrhizal benefit and hence sustainably increased productivity.

Cardoso, I. M. & Kuyper, T. W. Mycorrhizas and tropical soil fertility. *Agric Ecosyst Environ* **116**, 72–84 (2006).

Jung, S. C., Martinez-Medina, A., Lopez-Raez, J. A. & Pozo, M. J. Mycorrhiza-Induced Resistance and Priming of Plant Defenses. *J Chem Ecol* **38**, 651–664 (2012).

Kuyper, T. W., Wang, X.-X. & Muchane, M. N. Interplay Between Roots and Arbuscular Mycorrhizal Fungi influencing Water and Nutrient Acquisition and Use Efficiency. *The Root Systems in Sustainable Agricultural Intensification* (2021).

(Hashim), A. C. & Ragu, P. Growth response of *Theobroma cacao* L. seedlings to inoculation with vesicular-arbuscular mycorrhizal fungi. *Plant Soil* **96**, 279–285 (1986).

Johnson, N. C., Wilson, G. W. T., Wilson, J. A., Miller, R. M. & Bowker, M. A. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol* **205**, 1473–1484 (2015).

## Holobiont genetic basis of pea root rot resistance

Valentin Gfeller<sup>1</sup>, Michael Schneider<sup>1</sup>, Ignacio Romero Lozano<sup>1</sup>, Natacha Bodenhausen<sup>2</sup>, Matthew W. Horton<sup>1</sup>, Daniel Ariza Suarez<sup>3</sup>, Lukas Wille<sup>1</sup>, Klaus Oldach<sup>4</sup>, Nora Temme<sup>5</sup>, Sebastian Kussmann<sup>6</sup>, Bruno Studer<sup>3</sup>, Martin Hartmann<sup>7</sup>, Monika M. Messmer<sup>1</sup>, Pierre Hohmann<sup>1,8</sup>

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Pea (*Pisum sativum*) cultivation is an important source of sustainable plant proteins. The production is, however, highly compromised by various soil-borne pathogens that can synergistically attack the roots and trigger soil fatigue. While resistant cultivars to individual pathogens exist, the combination of pathogens found in the pea root rot complex (PRRC) can still provoke root infections. Microbiome-mediated disease resistance poses a possible mechanism to mitigate yield loss through PRRCs. So far, it is largely unknown how the PRRC interacts with other members of the root microbial community and whether the pea genotype affects these interactions. To shed light on this, we sequenced amplicons of the root microbiota (fungi and bacteria) of 252 diverse pea lines grown under controlled conditions in a naturally-infested soil, and plants from the same batch of seeds were sequenced by genotyping-by-sequencing. We found that pea lines grown under root rot stress harbor a genotype-specific root microbial community and that the composition of the microbial community is associated with disease resistance. Further analyses enabled us to identify heritable microbial (highly connected) hub taxa that are associated with root rot resistance. Subsequent genome-wide association studies revealed plant genomic regions correlated with the microbiota abundance and overall community composition. Genomic predictions showed that the holobiont (plant and microbial markers together) significantly determines the resistance of the plant. The prediction ability was improved compared to standard plant genomic predictions. Some of the microbial markers for disease resistance were validated in three additional soils. Building on these findings, the breeding company KWS developed a marker assay for putative microbiome-mediated and direct root rot resistance and screened their breeding material. This will be instrumental in selecting pea breeding material for field validation of microbiome-mediated resistance against PRRCs. Overall, this research highlights the potential of microbiome-assisted breeding to promote sustainable farming practices.

## Assemblage and function of rhizosphere microbiome under drought stress during heterosis manifestation in maize

Ling Gu<sup>1, 2</sup>, Xiaoming He<sup>1, 2</sup>, Frank Hochholdinger<sup>2</sup>, Peng Yu<sup>1,2</sup>

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<sup>2</sup>Crop Functional Genomics, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany.

Drought is a major abiotic stress that threatens crop performance. Hybrids outperform their inbred parental lines in productivity and stress tolerance. Root-associated beneficial microbes can enhance plant growth and resilience to biotic and abiotic stresses. However, it is largely unknown whether and how heterosis manifestation has shaped their rhizosphere microbiome under abiotic stress, and in turn, how these microbes modify and improve the maize hybrids and inbred lines response to stress. In this study, we investigated 303 inbred lines from different genetic backgrounds and their F1 hybrid offspring which had been crossed with the common female parent B73 under drought and well-watered soil conditions. In general, the shoot biomass of all the maize hybrids was significantly higher than inbred lines under different water conditions. We observed that a large divergence in drought response existed between inbred lines and hybrids among different genotypes, thus suggesting the importance of genetic background in manifestation of maize heterosis underlying drought tolerance. In particular, the mid-parent heterosis of shoot biomass from the non-stiff stalk/tropical genetic background was significantly higher than that in stiff stalk background. The following work will investigate the biological mechanism for such pattern of heterosis and drought tolerance via different morphological, anatomical, and molecular approaches. We will validate whether and how soil microbes can confer such divergence of inbred lines and hybrids in response to drought. Taken together, we will integrate microbiome features and host gene expression profiles and aim to understand the causal relationship between roots and microbes, further enhancing the strategy to breed stress-resilient cultivars for future crops.

## Genetic basis and nutritional regulation of root development and rhizosphere microbiome assemblage in maize

Xiaofang Huang<sup>1,2\*</sup>, Frank Hochholdinger<sup>1</sup>, Peng Yu<sup>1,2</sup>

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The host-associated microbiome plays an irreplaceable role in the performance and fitness of natural plants. Although the root microbiome is mainly shaped by environment factors and soil properties, small part of host-mediated changes in microbiome composition can have large effects on plant fitness. However, the causal interactions between host genetic variation, gene regulation and the impact of the microbiome on the host phenotype remain unclear.

Here we profiled 1001 rhizosphere microbiome samples and 979 root transcriptome samples from 247 maize lines. These lines originate from a Mexican landrace 8-way Multi-parent advanced generation inter-cross (MAGIC) population, which covers major ecological groups and biogeographic regions in Mexico and has abundant genetic diversity. Combining the genome data of this MAGIC population, we will integrate multi-omics analysis to identify the differentially expressed genes and significant genome-wide associations for highly heritable amplicon sequence variants (ASVs). In addition, we performed high-throughput bacterial cultivation and identification from maize root and captured 583 ASVs. Together with BonnMu, which is a population of Mutator (Mu) induced mutants in several inbred lines of maize, we expect to obtain a mechanistic understanding of the genetic basis and nutritional regulation of root development and rhizosphere microbiome assemblage.

## Enhancement of cold tolerance of tomato plants using endophytic bacteria isolated from cold-adapted plants

Irma Milanese<sup>1,2</sup>, Malek Marian<sup>1,2</sup>, Michele Perazzolli<sup>1,2</sup>

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Cold stress is one of the major abiotic stresses that negatively affect crop production. Climate change is causing warm and short winters, increasing the risks of plant exposure to late spring frosts, especially for crops that are not cold-adapted, such as tomato. Microbial communities associated with plants growing in cold environments can promote plant growth at low temperatures and improve plant tolerance to cold stress. Thus, the use of psychrotolerant endophytic bacteria could be a promising approach to protect crop plants from cold stress. This work aims to investigate the effect of bacterial endophytes from cold-adapted alpine plants on the tolerance of tomato plants to cold stress. A total of 41 psychrotolerant bacterial isolates collected from the roots of three cold-adapted alpine plants were tested for their growth-promoting ability on tomato plants. Inoculated and control plants were grown under controlled conditions at 25°C and ten plant growth-promoting bacteria were selected according to their ability to increase dry and fresh weight. These bacterial isolates were then tested for their ability to promote tomato growth at 10°C, and two of them improved tomato growth under cold-stressed conditions, reducing the content of H<sub>2</sub>O<sub>2</sub> and modulating the expression of cold-stress-related genes. RNA-Seq analysis will better clarify genes and processes stimulated by bacterial inoculation in tomato plants exposed to cold stress. In particular, co-expression gene network analysis will highlight functional connections among tomato processes related to cold tolerance and stimulated by beneficial plant-microbiome interactions.

## **Microbe-induced phenotypic diversity attenuates competition outcomes in neighbor naïve plants**

Waseem Raza<sup>1, 2</sup>, Gaofei Jiang<sup>1</sup>, Zhong Wei<sup>1</sup>, Qirong Shen<sup>1</sup>, Alexandre Jousset<sup>1</sup>, George A. Kowalchuk<sup>2\*</sup>

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Plant-plant competition caused by the presence of neighbors negatively affects their productivity, usually by reducing the availability of resources and niches. This plant-to-plant competition is thought to be more severe in the case of clonal plants while the increase in diversity of plant species increases ecosystem productivity. We found that microbes induce phenotypic diversity of clonal plants that not only shows a reduction in competition outcomes but also exhibits transgressive overyielding. Here we extend this knowledge by showing that microbe-induced plant-plant phenotypic complementarity is also extended to the naïve neighbors by alleviating their competition outcomes for both growth and yield. We found that naïve plants growing beside microbe-treated plants showed a relative interaction index even higher than that of microbe-treated plants. These effects were found more definite at higher plant densities and were positively correlated not only to the numbers and diversity of microbe-treated plants but also to the phenotypic distances of measured life-history traits of microbe-treated plants. These results confirm the significant role of biodiversity in ecosystem functioning with the essential difference that in our experiment, the diversity of soil microbial mutualists could generate these effects in monoclonal plant assemblages.

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