iMMM 2019

4th international Molecular Mycorrhiza Meeting

Torino (Italy), 6-8 February 2019

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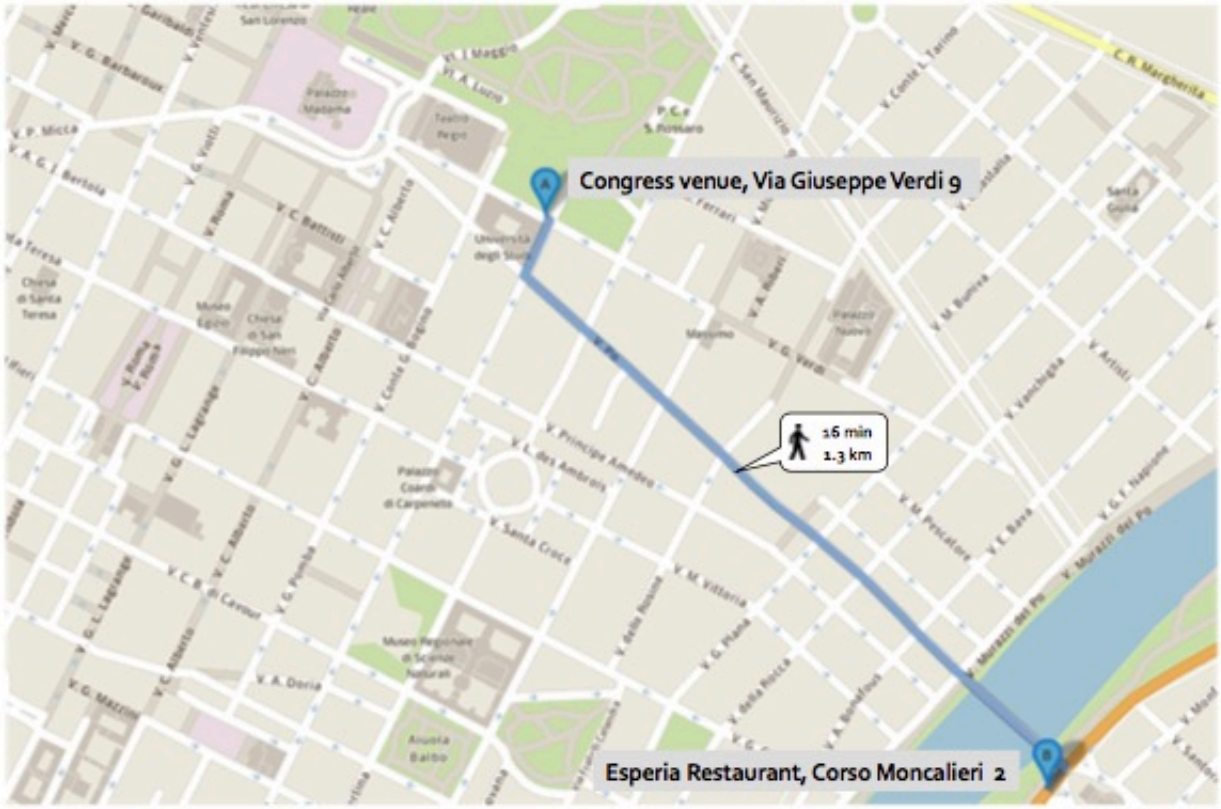

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Program

Wednesday February 6th, 2019

17,00 - 21,00 Registration and Poster mounting

Thursday February 7th, 2019

08,30 Registration and Poster mounting

09,00 - 09,30 Salutations and opening

09,30 - 10,00 **OPENING LECTURE**

Chair: Paola Bonfante

Alga Zuccaro: *Commonalities and differences of root associated fungi*

09,30 - 12,30 **Genomics and Natural variation**

Chair: Maria Harrison

10,00 - 10,30 **Nicolas Corradi:** *The genetics of arbuscular mycorrhizal fungi*

10,30 - 11,00 Coffee break

11,00 - 11,15 **Marisol Sanchez-Garcia:** *Understanding nuclear diversity within a single spore of arbuscular mycorrhizal fungi*

11,15 - 11,30 **Ian Sanders:** *Genetic variation in a mycorrhizal fungus drives the molecular regulation of the currency of exchange in symbiosis with the food security crop cassava*

11,30 - 12,00 **Claude Murat:** *Pezizomycete genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle*

12,00 - 12,30 **Poster flash talks**

12,30 - 14,00 Lunch and poster session

14,30 - 16,00 **Signaling in mycorrhizal symbioses**

Chair: Giles Oldroyd

14,00 - 14,30 **Natalia Requena:** *Getting to the root of the AM symbiosis*

14,30 - 14,45 **Chenglei Wang:** *Revealing the genetic mechanisms involved in systemic regulation of mycorrhizal colonization*

14,45 - 15,00 **Hector Montero-Sommerfeld:** *ARK1 mediated signalling in the post-arbuscule development stage*

15,00 - 15,15 **Johanna Wong:** *Small RNA regulation and crosstalk in symbiosis between Eucalyptus grandis roots and the mutualistic ectomycorrhizal fungus Pisolithus microcarpus*

15,15 - 15,30 **Tian Zeng:** *A LysM effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis*

15,30 - 16,00 **Johnatan Plett:** *Within Hailing Distance: The critical role of pre-symbiotic fungal signals to the colonization success of the ectomycorrhizal fungi*

16,00 - 16,30 Coffee break

16,30 - 18,00 **Cellular and molecular aspects of mycorrhizal interactions** ***New Phytologist Trust session***

Chair: Uta Paskowsky

16,30 - 17,00 **Caroline Gutjahr:** *Hormone signaling in arbuscular mycorrhiza development of Lotus japonicus*

17,00 - 17,15 **Erik Limpens:** *A Medicago truncatula SWEET transporter required for arbuscule maintenance*

17,15 - 17,30 **Maria Harrison:** *Genetic dissection of AM symbiosis in Brachypodium distachyon*

17,30 - 17,45 **Sebastian Schornack:** *Spatially distinct sugar signatures along the plant-fungus interface*

17,45 - 18,00 **Veronica Basso:** *Ectomycorrhizal symbiosis development: from fungal effectors to plant hormones*

18,00 - 20,00 Poster session with beer tasting
Sponsored by Birrificio Baladin

20,00 Apericena

Friday February 8th, 2019

09,00 - 12,00 **Symbiosis functioning**

Chair: Andrea Genre

09,00 - 09,30 **Ertao Wang:** *Signalling and nutrient exchange in mycorrhizal symbiosis*

09,30 - 09,45 **Krista Plett:** *Avoiding exploitation: Host tree controls over the ectomycorrhizal colonization process*

09,45 - 10,00 **Jeongmin Choi:** *Rice arbuscular mycorrhizal symbiosis establishment depends on the removal of the suppressor SMAX1*

10,00 - 10,15 **Juan Antonio Lopez-Raez:** *Deciphering the role of flavonoids and strigolactones in the AM symbiosis*

10,15 - 10,30 **Judith Felten:** *Decrypting the molecular mechanisms behind fungal root invasion during ectomycorrhiza formation with focus on pectin remodelling and fungal auxin*

10,30 - 11,00 Coffee break

11,00 - 11,30 **Pierre-Marc Delaux:** *Evolution of the arbuscular mycorrhizal symbiosis*

11,30 - 11,45	**Clémence Bonnot:** *Investigation of the regulation of ectomycorrhizal symbiosis by nutrient signalling in Poplar*
11,45 - 12,00	**Thomas Irving:** *A kinase functions downstream of RAM1 to maintain the arbuscular mycorrhizal symbiosis in Medicago truncatula*
12,00 - 14,30	Lunch and poster session

14,30 - 17,30 Multiple interactions

Chair: Guillaume Bécard

14,30 - 15,00	**Ainhoa Martinez-Medina:** *Root Fungal Symbionts: Allies of Plants in a Multitrophic Context*
15,00 - 15,15	**Gregor Langen:** *Fungal competition reduces the virulence potential of the root rot pathogen Bipolaris sorokiniana*
15,15 - 15,30	**Ivan Fernandez Lopez:** *Defence mechanisms triggered by herbivory in ectomycorrhizal oak trees over successive growth flushes*
15,30 - 16,00	**Aurélie Deveau:** *Fungal - bacterial interactions in the mycorhizosphere: friends and foes*
16,00 - 16,30	Coffee break
16,30 - 16,45	Best poster prize *Sponsored by Frontiers in Plant Science*
16,45 - 17,15	**CLOSING LECTURE**

Chair: Paola Bonfante

Davide Bulgarelli: *Defining the host genetic control of the rhizosphere bacterial microbiota*

17,30 - 17,45	Meeting closure
17,45 - 19,00	Poster session
20,30	Gala dinner at Società Canottieri Esperia

Speaker abstracts

OPENING LECTURE

L1 Commonalities and differences of root associated fungi

Alga Zuccaro

Universität zu Köln, CEPLAS, Botanisches Institut

Plants associate with a wide range of beneficial fungi in their roots which facilitate plant mineral nutrient uptake in exchange for carbohydrates and other organic metabolites. These associations play a key role in shaping terrestrial ecosystems and are widely believed to have promoted the evolution of land plants. To establish compatibility with their host, root-associated fungi have evolved diverse colonization strategies with distinct morphological, functional and genomic specializations as well as different degrees of interdependence. They include obligate biotrophic arbuscular mycorrhizal (AM), facultative biotrophic ectomycorrhizal (ECM) interactions and root endophytic associations, which due to their inconspicuous nature have been often overlooked. Recent research into the biology and genomics of root associations revealed fascinating insight into the phenotypic and trophic plasticity of these fungi and underlined genomic traits associated with biotrophy and saprotrophy. In this talk we will consider the commonalities and differences of AM and ECM associations and contrast them with root endophytes. Additionally we will present data on the mechanisms driving multipartite interactions and the different colonization strategies of pathogens and mutualistic root associated fungi by addressing how the interaction between the beneficial root endophyte *Serendipita vermifera* and the pathogen *Bipolaris sorokiniana* affects fungal behavior and determines host responses using a gnotobiotic natural soil-based split-root system for phenotypic and transcriptional analyses.

Genomics and Natural variation

I1.1 The genetics of arbuscular mycorrhizal fungi

Nicola Corradi

University of Ottawa, Department of Biology, Ottawa, Canada.

The genetics of arbuscular mycorrhizal fungi (AMF) have always been notoriously difficult to study. In particular, their perpetual multinucleated state, with thousands of nuclei floating in the same cytoplasm, and their obligate biotrophy, has made it difficult to isolate good quality DNA and single nuclei to better understand their nuclear complexity and overall biology. My presentation will discuss how the latest advances in the field of genomics and single cell/nuclei isolation have been playing a major role in advancing our understanding of the AMF life-cycle, shifting long held paradigms regarding the genetics and (para)sexual potential of these organisms.

S1.1 Understanding nuclear diversity within a single spore of arbuscular mycorrhizal fungi

Marisol Sánchez-García, Mercè Montoliu-Nerin, Anna Rosling

Department of Ecology and Genetics, Uppsala University

Arbuscular mycorrhizal (AM) fungi form vital symbiotic relationships with the roots of most plants, and they have been considered key components in the transition from aquatic to terrestrial habitats. Despite the importance of these organisms in natural and agricultural ecosystems, we know very little about their genome organization. A long-standing question has been whether these coenocytic fungi are homokarytic or heterokaryotic. Some evidence supports the hypothesis of genetically identical nuclei, while other lines of evidence support the hypothesis of the presence of many divergent nuclei that co-exist within a common cytoplasm. For this study, we used a single nucleus sequencing method to sequence nuclei from a single spore of four different species of AM fungi in order to detect whether the nuclei are genetically identical or not. We analyzed the distribution of k-mer frequency and GC content across all the nuclear genomes within the same species (spore) and we estimated the completeness of individual assemblies and annotations in order to find regions that were sequenced across all nuclei and compared their genetic content. These results will provide further evidence to understand the genetic structure and organization of a multinucleate spore of AM fungi.

S1.2 Genetic variation in a mycorrhizal fungus drives the molecular regulation of the currency of exchange in symbiosis with the food security crop cassava

Romain Savary[a], Cindy Dupuis[a], Frederic G. Masclaux[a,b], Ivan D. Mateus[a], Edward C. Rojas[a,c] and Ian R. Sanders[a]

[a]Department of Ecology and Evolution; University of Lausanne; [b]Vital-IT Group, Swiss Institute of Bioinformatics; University of Lausanne; [C]Department of Plant and Environmental Sciences; University of Copenhagen.

Arbuscular mycorrhizal fungi (AMF) form symbioses with most land plants, increasing plant growth and plant diversity. For decades, it was assumed that AMF trade phosphate for carbohydrates from their hosts. But recent studies show that plant-derived lipids represent an essential currency of exchange. The role AMF genetic variation plays in the regulation of this currency has been completely ignored. We used an ultra-high resolution phylogeny of one AMF species to investigate whether patterns of fungal genetic variation drive the regulation of the plant fatty acid pathway. Here we show that in cassava the complete fatty acid pathway from induction to transport was commonly switched on by all fungi. Unexpectedly, however, the regulation of this pathway was explained by clear patterns of fungal genome-wide variation representing the precise fungal evolutionary history. This represents the first demonstrated link between the genetics of AMF and reprogramming of an essential plant pathway regulating the currency of exchange in the symbiosis. Our study opens the door to discovering characteristics of AMF genomes responsible for interactions between AMF and cassava that will lead to optimal cassava growth. Given that cassava feeds one billion people daily, in regions of extreme poverty, this represents a pursuit of the highest priority.

I1.2 Pezizomycete genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle

Murat C[a], A. Kohler[a], E. Morin[a], F. Todesco[a], P. Wincker[b], A. Kuo[c], I. Grigoriev[c], Mycorrhizal Genome Initiative consortium, and F. Martin[a]

[a]INRA, UMR1136, 54280 Champenoux, France; [b]Genoscope, 91057 Evry Cedex, France; [c]US DOE-JGI, Walnut Creek, CA 94598, USA

Tuberaceae is one of the most diverse lineages of symbiotic truffle-forming fungi. To understand the molecular underpinning of the ectomycorrhizal truffle lifestyle, we compared the genomes of Piedmont White Truffle (*Tuber magnatum*), Périgord Black Truffle (*T. melanosporum*), Burgundy Truffle (*Tuber aestivum*), Pig Truffle (*Choiromyces venosus*) and Desert truffle (*Terfezia boudieri*) to saprotrophic Pezizomycetes, Black Morel (*Morchella importuna*), *Ascobolus immersus*, and *Pyronema confluens* [1]. Genomic features in *Tuber* species appear to be very similar with high transposon content, few genes coding lignocellulose-degrading enzymes, a substantial set of lineage-specific fruiting body-upregulated genes and high expression of genes involved in volatile organic compounds (VOCs) metabolism. Developmental and metabolic pathways expressed in ectomycorrhizae and fruiting bodies of *T. magnatum* and *T. melanosporum* are unexpectedly very similar owing to the fact that they diverged ~100 Mya. VOCs from pungent truffle odors are not the products of *Tuber*-specific gene innovations, but rely on the differential expression of an existing gene repertoire. Thanks to a successful collaboration between academic researchers and truffle grower federations, truffles represent a unique model for the quick transfer of genomic resources to the field to address fundamental questions in the ecology and biology of these fungi. For example, we developed several qPCR protocols using these genomic resources to investigate the monthly dynamic variation of truffle DNA in the soil of orchards and its link with climatic factors.

[1] Murat et al. (2018) *Nature Ecology and Evolution*, **2**, 1956–1965

Signaling in mycorrhizal symbioses

I2.1 Getting to the root of the AM symbiosis

Meike Hartmann, Ruben Betz, Sven Heidt, Carolin Heck, Victor Gourain, Natalia Requena

Molecular Phytopathology, Botanical Institute, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 4, D-76131 Karlsruhe, Germany.

Arbuscular mycorrhizal (AM) symbiosis is one of the most widespread plant adaptations to low soil phosphate. AM fungi colonize the root cortex and transfer phosphate from the soil to the plant at arbuscules where dedicated plant phosphate transporters load it into cortical cells. From there, and by mechanisms not yet fully understood, phosphate is transported towards the xylem towards the shoot which, ultimately, controls the phosphate status of the whole plant. However, high phosphate inhibits AM fungal colonization and arbuscule development, and thus, it is unclear how the elevated concentration of phosphate downloaded at arbuscules is compatible with symbiosis progression. Surprisingly, the phosphate starvation response (PSR) is high in arbuscule-containing cells despite a systemic reduction of the PSR in mycorrhizal roots. Here we show that *Rhizophagus irregularis* employs at least three AM conserved effector proteins that redundantly alter the plant PSR. Ectopic expression in *Medicago truncatula* roots of any of these effectors triggers systemic PSR with accumulation of microRNAs (pri-miR399s) in leaves and down-regulation of the ubiquitin E2 conjugase PHO2 in roots. Silencing their expression *in planta* impairs phosphate allocation to the shoots and triggers arbuscule degeneration, suggesting that the effectors work locally to rewire the PSR, preventing symbiosis arrest. Interaction, localization and deregulation assays indicate that this effector family modulate the PSR by intersecting with the plant mRNA processing machinery. These results highlight the finesse of symbiotic arbuscular mycorrhizal fungi in manipulating the plant cell to ensure their permanence in the root while contributing to plant health and nutrition.

S2.1 Revealing the genetic mechanisms involved in systemic regulation of mycorrhizal colonization

Chenglei Wang[a], James B. Reid[a], Eloise Foo[a]

[a]School of Natural Sciences, University of Tasmania, Hobart, TAS, Australia

Some plants can form mutualistic symbiotic relationships with arbuscular mycorrhizal fungi (AMF) and/or rhizobia (nodulation) to improve nutrient supply to the plant partner. However, the formation of these symbioses is energetically costly for the plant. In order to balance the energy cost with the benefit gained, plants employ systemic negative feedback loops to control the formation of these symbioses, systems known as autoregulation of nodulation (AON) and autoregulation of mycorrhizae (AOM). The molecular elements involved in AON are relatively well understood while the genetic basis of AOM is largely unknown. Elegant physiological studies in legumes have indicated that there is at least some overlap in the genes and signals that regulate these two symbioses, but there are major gaps in our understanding. We have tested the function of putative orthologues of AON genes in mycorrhizal associations in the non-legume tomato using a mutant based approach. Our results show that a tomato *clv1* mutant and a CRISPR-Cas9 knock out line that targets the *CLV2* gene are more heavily colonized by mycorrhizal fungi than wild type plants, consistent with a role for these genes in suppressing mycorrhizal development. We also reveal an important role for these genes in controlling root development. We also explore whether these genes act systemically in AOM, monitor the expression of genes encoding CLE peptides (well-known systemic elements in AON) and explore the crossover with nutrient signalling. Our results provide genetic evidence about the AOM pathway in a non-legume and add to our understanding of the evolutionary origin of AOM.

S2.2 ARK1 mediated signalling in the post-arbuscule development stage

Hector Montero, Jennifer McGaley, Jonathan Schnabel, Mieke Jürgens, Ronelle Roth, Roxanne Sicat, Susannah Parkhill, Uta Paszkowski

Department of Plant Sciences, University of Cambridge, UK

Arbuscular mycorrhizal fungi and plants coexist in its most extremely coalesced form in arbusculated cells. Here, reciprocal exchange has been demonstrated to occur for an array of plant and fungal derived nutrients. Mutual perception and surveillance of cellular events conceivably takes place in divergent ways as compared to non-colonized root cells. However, little is known about the signalling landscape in arbusculated cells beyond broad transcriptional surveys in this cell type. Recently, Arbuscular Receptor-Like Kinase 1 (ARK1) was described to function in the rice periarbuscular membrane (PAM) with a role in supporting fungal fitness. Quantification of wheat germ agglutinin (WGA) stained roots and live cell imaging employing the secretory carrier membrane protein SCAMP show arbuscules of ark1 mutant to develop normally placing its function in the post-arbuscule development stage. As rice ARK1 lacks structural competence for perception in the periarbuscular space, approaches to the identification of ARK1 interactors will be presented and potential candidates discussed. In addition, RNA seq, pull down assays, kinase assays and lipid imaging are implemented to unveil mechanistic aspects of ARK1 function and those of its close homolog, ARK2 whose protein domain architecture parallels ARK1 throughout evolution.

S2.3 Small RNA regulation and crosstalk in symbiosis between *Eucalyptus grandis* roots and the mutualistic ectomycorrhizal fungus *Pisolithus microcarpus*

Johanna WH Wong[a], Vivian Ng[b], Igor V Grigoriev[b], Francis Martin[c], Ian C Anderson[a], Jonathan M Plett[a]

[a]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, 2753, NSW, Australia; [b]U.S. Department of Energy, Joint Genome Institute, Walnut Creek, California, USA; [c]UMR1136 Interactions Arbres-Microorganismes, Laboratoire d'Excellence ARBRE, INRA, Université de Lorraine, Champenoux, France

Small RNAs (smRNAs), particularly microRNAs (miRNAs), are known to regulate plant-microbe interactions. Plant miRNAs such as miR171 and miR393 are crucial in establishment of legume-rhizobia interaction and plant-arbuscular mycorrhizal (AM) fungal interaction [1]. In other systems emerging evidence would also suggest that smRNAs can be translocated between microbes and plants to facilitate symbiosis [2]. The roles of smRNAs in plant-ectomycorrhizal (ECM) fungal interactions, however, have yet to be explored. In this study, we investigate the role of smRNAs during the mutualistic interaction between *Eucalyptus grandis* and the ECM fungus *Pisolithus microcarpus.* Transgenic suppression of smRNA-synthetic pathway in *E. grandis* reduced the root colonization rate significantly, suggesting that smRNAs are involved in crucial steps initiating ECM symbiosis with host plants. We then sequenced the smRNA transcriptomic profiles of both the plant roots and fungal partners at different timepoints along the formation of mycorrhizal root tips between *E. grandis* and *P. microcarpus*. Using these data, we have identified novel plant/fungal miRNA regulators that are differentially expressed during ECM establishment. Intriguingly, amongst these differentially expressed miRNAs, a fungal miRNA was found in root tissues after pre-symbiotic interaction with *P. microcarpus*. Preliminary data suggested this fungal miRNA is a plausible cross-kingdom signalling smRNA that facilitate colonization of plant roots by targeting a defense-related R gene in *E. grandis.* Further molecular experiments will be reported concerning the function of this effector-like fungal small RNA in ECM symbiosis.

[1] Lelandais-Brière et al. (2016) Mol Plant Microbe Interact, **29**, 170-180

[2] Weiberg et al. (2015) Curr Opin Biotechnol, **32**, 207-215

S2.4 A LysM effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis

Tian Zeng[a], Luis Rodriguez-Moreno[b], Artem Mansurkhodzaev [a], Peng Wang[a], Jieyu Liu[a], Olga Kulikova[a], Willy van den Berg[c], Virginie Gasciolli[d], Sylvain Cottaz[e], Sébastien Fort[e], Bart P.H.J. Thomma[b], Jean-Jacques Bono[d], Ton Bisseling[a], Erik Limpens[a]

[a]Laboratory of Molecular Biology, Wageningen University & Research, 6708 PB Wageningen, the Netherlands; [b]Department of Plant Sciences, Laboratory of Phytopathology, Wageningen University & Research, 6708 PB Wageningen, the Netherlands; [c]Laboratory of Biochemistry, Wageningen University & Research, 6708 WE Wageningen, The Netherlands; [d]INRA, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, 31326 Castanet-Tolosan, France; [e]Univ. Grenoble Alpes, CNRS, CERMAV, 38000 Grenoble, France

A striking ability of mutualistic arbuscular mycorrhizal fungi is their ability to intracellularly colonize root cells of the vast majority of land plants, despite the fact that they have common MAMPs such as chitin in their cell wall, which trigger immune responses in the plant. Pathogenic fungi have evolved strategies to evade or down-regulated chitin triggered immune responses, especially by secreting LysM effectors. However, it is unknown how AM fungi subvert chitin triggered immune responses. Here we study whether AM fungi use similar effectors as pathogenic fungi to suppress this response during symbiosis.We identified and characterized a LysM domain containing effector, called RiLysM, from *Rhizophagus irregularis*. We show that a homolog of this LysM effector, consisting of a single LysM domain, is conserved in a wide-range of AM fungal species, showing signs of diversifying selection. RiLysM is one of the most highly expressed effectors in the interaction with an evolutionary diverse range of AM host plants and is especially highly expressed in the intraradical hyphae, but not in arbuscules. We show that RiLysM can bind chitin, protect fungal hyphae from plant chitinases, and that it efficiently interferes with chitin-triggered immune responses. Host-induced gene silencing of *RiLysM* greatly reduces fungal infection and arbuscule abundance in the host. Taken together, our results indicate that, in analogy to pathogenic fungi, AM fungi use LysM effectors to suppress chitin-triggered immunity to mediate a successful symbiosis.

I2.2 Within Hailing Distance: The critical role of pre-symbiotic fungal signals to the colonization success of the ectomycorrhizal fungi

Jonathan M. Plett

Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia

In forest ecosystems, tree roots are typically colonized by a range mutualistic ectomycorrhizal fungi. Despite the prevalence of these important fungi, little is understood concerning the inter-kingdom communication that occurs between host and fungal cells to favor the formation of these symbioses. This is especially true with regards to our knowledge of how signals secreted by the ectomycorrhizal fungus during pre-symbiosis, defined here as the period of time just prior to physical contact between the fungus and the host plant, might affect later stages of fungal colonization of plant tissues. The objective of our research is to 'listen in' on this molecular dialogue at the very early stages of pre-symbiosis and to characterize the signals used by both mutualistic fungi and their host plants to negotiate symbiosis. Using examples from our high- and medium-throughput screening (e.g. sequencing, meabolomics, protein activity analysis), I will discuss the steps forward we have made in recent years in understanding the evolution and role of both protein- and metabolomic-based dialogue between mutualistic fungi and their hosts. Using the model system of *Pisolithus microcarpus* and its host *Eucalyptus grandis*, I will dissect how a very small contingent of effector-like proteins induced by the presence of the host are able to target key plant pathways to foster symbiosis. Further, I will present some of our recent work using untargeted metabolomics to demonstrate how pre-symbiotic signals from *Pisolithus* can alter critical molecular patterns to support the later fungal aggregation and penetration into the host root. I will conclude by reflecting upon some of the bigger-picture ramifications of these findings and highlight some avenues of research that may be attractive to pursue in the future.

Cellular and molecular aspects of mycorrhizal interactions

I3.1 Hormone signaling in arbuscular mycorrhiza development of *Lotus japonicus*

Caroline Gutjahr

Plant Genetics, School of Life Science Weihenstephan, Technical University of Munich (TUM), Emil Ramann Str. 4, 85354 Freising, Germany

Arbuscular mycorrhiza (AM) symbioses are ancient and widespread in the plant kingdom and are based on the exchange of nutrients. The fungus provides minerals (most prominently phosphate) to the plant and receives organic carbon in return. Symbiosis establishment is initiated with an interchange of diffusible signaling molecules ensuring reciprocal recognition and promoting symbiosis-facilitating responses of both partners. It was shown that the rice alpha-beta hydrolase D14L participates in early recognition of AM fungi: a rice *d14l* mutant is not colonized and is transcriptionally unresponsive to exudates from germinating fungal spores. D14L forms a receptor complex with the F-box protein MAX2 for the smoke compound karrikin, which induces germination of fire following plants. Furthermore, this complex regulates seedling morphogenesis in Arabidopsis. We use the legume *Lotus japonicus*, which is permissive to rapid hairy root transformation, to study the molecular function and signaling downstream of the D14L-MAX2 complex that is relevant to symbiosis establishment. Surprisingly, the phenotype of *Lotus japonicus* karrikin receptor mutants is much weaker than in rice and the mutant roots are colonized at about 50% of the wild-type level; suggesting differences in molecular wiring of the signaling pathway or partial redundancy with other molecular functions in *Lotus japonicus.* I will report on our progress in understanding the role of the karrikin receptor complex in AM symbiosis of *Lotus japonicus*.

S3.1 A *Medicago truncatula* SWEET transporter required for arbuscule maintenance

Jianyong An[a], Ji Chuanya[a], Tian Zeng[b], Sanne de Graaf[b], Zijun Zheng[a], Shunyuan Xiao[a,c], Xiuxin Deng[a], Ton Bisseling[a], Zhiyong Pan[b] & Erik Limpens[a]

[a]Key Laboratory of Horticultural Plant Biolog, College Horticulture and Forestry Sciences, Huazhong Agricultural University; [b]Laboratory of Molecular Biology, Wageningen University; [c] Institute for Bioscience and Biotechnology Research & Department of Plant Sciences and Landscape Architecture, University of Maryland

Arbuscular mycorrhizal fungi rely on their plant hosts to obtain carbon. It has become clear that fatty acids are transported from the host to the fungus, likely serving as a major nutritive carbon source. In addition it was shown that the host also provides sugars as carbon source. However, the importance of and mechanism by which sugars are transported to the fungus has remained unclear. In plants sugar export requires the action of SWEET (Sugar Will be Eventually Exported Transporter) transporters. Therefore, we searched for SWEET members that are active in the arbuscule-containing cells where reciprocal nutrient exchange is thought to occur. By cell-specific transcriptome analyses in *Medicago truncatula*, we identified one SWEET member, MtSWEET1, that is specifically and dominantly expressed in arbuscule-containing cells. It localizes to the peri-arbuscular membrane across which nutrient exchange takes place. Heterologous expression of MtSWEET1 in a yeast hexose deficiency mutant showed that it transports glucose. Overexpression of MtSWEET1 promoted AM colonization levels. Retrotransposon insertion lines, causing truncated MtSWEET1 protein variants impaired in glucose transport ability, did not lead to defects in AM symbiosis. However, arbuscule-specific overexpression of two MtSWEET1 (Y57A and G58D) mutant versions that act in a dominant-negative manner significantly impaired the maintenance of arbuscules, causing the early collapse of arbuscules. Our data suggest that glucose transport by MtSWEET1 plays an important role in the maintenance of the arbuscules.

S3.2 Genetic dissection of AM symbiosis in *Brachypodium distachyon*

Armando Bravo[a*], Lidia Campos-Soriano[a*], Liudmila Mainzer[b*], Stephanie Watts-Williams[a], Yi Ding[a], Lena Müller[a], Veronique Levesque-Tremblay[a], Matthew E. Hudson[b,] and Maria J. Harrison[a]

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The grass species, *Brachypodium distachyon* has been adopted as a biofeedstock model species and a range of genomics and genetics resources are being developed to enable the dissection of traits of significance for biofeedstock production, including AM symbiosis. Initial analysis of *Brachypodium distachyon* symbioses with 3 diverse arbuscular mycorrhizal (AM) fungi, *Glomus versiforme*, *Rhizophagus irregularis* and *Gigaspora gigantea* reveals that each of these fungal symbionts has an almost exclusive intracellular growth habit in the Brachypodium root cortex that appears to be a hybrid of the *Arum*-type and *Paris*-type AM symbioses[1]. All three fungal symbionts promote plant growth with variation in the growth phenotype. RNA-seq analysis reveals that the Brachypodium transcriptional responses during interaction with these three fungal symbionts are highly conserved. Additionally, a common transcriptional response is apparent in the three fungi, including strong expression of lipid metabolism-related genes and a variety of transport proteins, including phosphate transporters. To enable functional analysis of Brachypodium genes, we used CRISPR/Cas9 gene editing technology to generate predicted loss-of-function mutants. Editing known symbiosis genes enabled us to establish a baseline for evaluation of mutant phenotypes in this system. Simultaneous editing of multiple genes is particularly efficient in Brachypodium and the analysis of higher order transport mutants is in progress.

1. Hong, J. J. et al. Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. Planta 236, 851-865.

S3.3 Spatially distinct sugar signatures along the plant-fungus interface

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Arbuscules are highly differentiated fungal structures within plant cells. While their architecture differs depending on the host species, ranging from Arum- to Paris-types, they are always surrounded by a specialised periarbuscular membrane. However, a cellulosic plant cell wall is missing, while many cell wall components are still secreted into the periarbuscular matrix, the space between both organisms. Branch-domain-specific plant proteins have helped defining functionally different arbuscule branch and trunk domains. Their localisation relies on a repolarisation of secretory processes coupled with their expression. Given this temporal/spatial relationship of arbuscule secretion we hypothesized that the periarbuscular matrix of branch and trunk domains will markedly differ in its composition of secreted cell wall components. Using monoclonal antibodies towards cell wall components we identified specific glycan-modifications that have spatially distinct or equal distribution along the arbuscule. Comparing these signatures in arbuscules formed in *Nicotiana benthamiana* and *Medicago truncatula* allowed us to identify host- and colonisation-type associated differences. Extending this work into intracellular haustoria of pathogenic filamentous microbes will define common and specific signatures of matrix carbohydrates at the interface between plants and microbes and will therefore further our understanding on how similar pathogenic and symbiotic interfaces are.

S3.4 Ectomycorrhizal symbiosis development: from fungal effectors to plant hormones

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Ectomycorrhiza (ECM) are mutualistic interactions occurring between the roots of about 6000 tree species and hyphae of soil-borne fungi of the *Basidiomycota* and *Ascomycota* phyla. ECM are pivotal in forest ecosystems but not much is known about the molecular and physiological mechanisms underpinning their establishment. Previously we showed that the Mycorrhiza-Induced Small Secreted Protein 7 (MiSSP7) of the ectomycorrhizal fungus *Laccaria bicolor* is secreted upon ECM establishment, penetrates the nuclei of cortical root cells of the host tree *Populus trichocarpa* and here stabilizes the repressor of jasmonic acid (JA) signaling JAZ6, dampening plant responses to JA and promoting mutualism [1]. Here, through protein-protein interaction studies, we show how MiSSP7 impacts the structure of the *Populus* JA perception complex and, using poplar transgenic lines, we investigate the function of JA-responsive genes regulating ECM symbiosis. Further, through physiologic and transcriptomic analysis, we demonstrate that dampening of JA-signaling is only part of a complex rearrangement of phytohormone biosynthesis and perception in poplar roots during ECM development. Therefore, we propose to consider ECM establishment not simply the output of effector-driven fungal evasion of plant immunity, but a multilayered process integrating fungal and plant-derived signals, to reprogram root and mycelial physiology for a successful mutualistic interaction.

[1] Plett et al. (2014) PNAS, **111**(22): 8299-8304.

Symbiosis functioning

I4.1 Signalling and nutrient exchange in mycorrhizal symbiosis

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Most land plant species enter a mutualistic symbiosis with arbuscular mycorrhizal (AM) fungi to improve nutrient acquisition from the soil. In return, up to 20% of host plant photosynthate is transferred to the mycorrhizal fungus in the form of lipids and sugar. Nutrient exchange must be regulated by both partners in order to maintain a stable symbiotic relationship. However, the mechanisms that regulate lipid transfer from plant to AM fungus are elusive. Here, we show that MtAP2-domain transcription factors are master regulators of AM symbiosis by controlling lipid transfer and periarbuscular membrane formation. For instance, MtWRI5 binds to AW-box *cis*-regulatory elements in promoters of the lipid transporter *STR* and phosphate transporter *MtPT4* in *M. truncatula*. *MtWRI5* mutants displayed impaired arbuscule formation, whereas overexpression of MtWRI5 activates *FatM*, *STR* and *MtPT4*, suggesting that *MtWRI5* controls bidirectional nutrient exchange in AM symbiosis. Finally, we showed that *MtWRI5* and *RAM1* regulate each other at the transcriptional level, forming a positive loop to orchestrate bidirectional nutrient transfer between two symbionts. Our data suggest a role for *MtWRI5* in controlling lipid transfer and periarbuscular membrane formation via regulation of fatty acid biosynthesis and transporter genes in arbuscule-containing cells. I will also discuss our new finding on recognition of mycorrhizal fungus.

S4.1 Avoiding exploitation: Host tree controls over the ectomycorrhizal colonization process

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Ectomycorrhizal (ECM) symbiosis is generally considered to be a process that is beneficial to both symbiotic partners. However, emerging information is questioning where the balance of power in the relationship actually lies. Using stable isotope tracing and transcriptomic analyses, I will consider mechanisms used by the plant to protect its own interests from unhelpful symbionts, and how these mechanisms affect the exchange of nutrients between the two symbiotic partners. Particularly, I will consider the effects of nutrient availability and competition on the interaction. Overall, these results demonstrate that the plant is able to restrict access to its roots in situations where the ECM fungus is less beneficial, however, the plant is unable to influence the amount of nitrogen that it receives from its partner. I further characterize potential sugar transporters used by the plant to feed the ECM fungus and demonstrate through transgenic modification that their differential expression affects not only the amount of carbon acquired by the fungus, but also its ability to colonize the plant tissues. These results demonstrate that the plant does exert a strong level of control over the ECM symbiosis to protect its own interests.

S4.2 Rice arbuscular mycorrhizal symbiosis establishment depends on the removal of the suppressor SMAX1

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Under nutrient deficient condition, more than 80% terrestrial plants are engaged with arbuscular mycorrhizal (AM) fungi to obtain minerals such as phosphate and nitrogen. AM symbiosis begins with a bidirectional chemical dialogue where both symbiotic partners perceive diffusible signaling molecules to recognize each other prior to physical contact [*1*]. Sensing of AM fungi by rice requires the alpha-beta fold hydrolase Dwarf14-like (D14L) and the F-box protein Dwarf3 (D3) [*2*]. The same receptor complex has previously been described for the perception of the smoke derived-compound karrikin, triggering seed germination and seedling growth post wildfire. I investigated the symbiosis signaling pathway downstream of D14L/D3 and identified *Suppressor of MAX2 -1 (SMAX1)* as an essential component. Mutation of *SMAX1* led to a higher level of colonization than wild-type functions suggesting SMAX1 functions as a negative regulator of AM symbiosis. Indeed, absence of fungal colonization in the rice receptor mutants *d14* or *d3* was suppressed in *d14l/smax1* and *d3/smax1* double mutants. *SMAX1* operates therefore epistatically downstream of the two known signaling components. The subcellular localization of *SMAX1* in the nucleus, suggested that SMAX1 suppresses transcription of AM symbiosis genes. RNAseq analysis of non-colonized *smax*1 mutant indeed uncovered a suite of AM symbiosis-induced genes. In summary, we found that successful AM colonization depends on the removal of the SMAX1 suppressor for transcriptional reprogramming rice roots for symbiosis.

[1] Choi et al (2018) *Annu Rev Phytopathol*, (2018). **56**, 135-160.
[2] Gutjahr et al. (2015) Science, **350**, 1521-1524.

S4.3 Deciphering the role of flavonoids and strigolactones in the AM symbiosis

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The use and abuse of chemical fertilizers and pesticides is becoming increasingly controversial due to their negative impact on the environment, contaminating soil and groundwater, on the safety of farmers and on consumers' health. A sustainable alternative to reduce their use in agriculture are the beneficial microorganisms present in the rhizosphere, including the arbuscular mycorrhizal (AM) fungi. The establishment and correct functioning of AM symbiosis requires a high degree of communication and coordination among the two partners [1, 2]. The molecular dialogue takes place during the pre-symbiotic phase, and begins with the production and exudation into the rhizosphere of signalling molecules by the host plant, including strigolactones (SLs) [3]. Other important cues in the rhizosphere are the flavonoids, a class of secondary metabolites involved in plant biology and physiology [4]. Flavonoids are essential in another beneficial symbiosis, the legume-Rhizobium symbiosis [5], where SLs seems to play also a role [6, 7]. Surprisingly, very little is known about the role of flavonoids in AM symbiosis. In the present work, using the flavonoid-deficient tomato mutant anthocyanin free (af), we aim to dephicer the potential role of these compounds in AM symbiosis, as well as their interaction with SLs. The colonization levels by R. irregularis in af and the corresponding wild-type (cv. Red Cherry) were analysed over time. Transcriptomic and metabolic analyses were also performed in the two genotypes. Results suggest a contrasting role of flavonoids during the early stages of the plant-AM fungus interaction, depending of the type of inoculum used, and a cross-talk with SLs and other phytohormones.

[1] Bonfante and Genre (2015) Trends Plant Sci., 20, 150-154.
[2] Pozo et al. (2015) New Phytol., 205, 1431-1436.
[3] López-Ráez et al. (2017) Trends Plant Sci., 22, 527-537.
[4] Weston and Mathesius (2013) J. Chem. Ecol., 39, 283-297.
[5] Oldroyd (2013) Nat. Rev. Microbiol., 11, 252-263.
[6] McAdam et al. (2017) Plant Physiol., 175, 529-542.
[7] Peláez-Vico et al. (2016) Plant Sci., 245, 119-127.

S4.4 Decrypting the molecular mechanisms behind fungal root invasion during ectomycorrhiza formation with focus on pectin remodelling and fungal auxin

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Mycorrhiza development requires cell wall modification at the contact site of the interacting partners to achieve flull symbiotic functionality. The Hartig Net (HN) in ectomycorrhizal symbiosis is a prerequisite for the nutrient exchange between plant and fungus and is a prime example for cell wall alterations. During HN formation, adjacent root cells loosen from each other and fungal hyphae invade the apoplastic space. This requires a release of the pectin rich middle lamella between adjacent root cells. Using immunolocalization with antibodies against epitopes of pectin in different methylesterification states on material from a time course of ectomycorrhiza development between the fungus *Laccaria bicolor* and *Populus* roots, we show that pectin demethylesterification increases during Hartig Net formation, suggesting pectin loosening. This is accompagnied by an increase in fungal *pectin methylesterase* (*PME*) expression as well as in PME activity. Transgenic *L. bicolor* lines with altered levels of *LbPMEs* have been generated to identify the role of LbPMEs for Hartig Net formation. We furthermore investigate the contribution of fungal auxin for HN formation, as this phytohormone can facilitate cell wall loosening. We have characterized the genes and metabolites of the auxin biosynthesis pathway in *L. bicolor.* Transgenic alteration of *Aldehyde Dehydrogenase* family gene expression within the *L. bicolor* auxin biosynthesis pathway alters auxin production and auxin metabolite levels in *L. bicolor* lines. These lines are used to reveal the effect of altered auxin production on Hartig Net formation as well as on pectin remodelling in *Populus* and Norway spruce roots.

I4.2 Evolution of the arbuscular mycorrhizal symbiosis

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The plant lineage faced two major transitions over the last 450 million years: the colonization of lands and the transition from a gametophyte- to a sporophyte-dominant lifestyle, resulting in the divergence of vascular plants. These two events required the evolution of new mechanisms and the recruitment of existing pathways in new developmental contexts. The fossil record and its broad host range suggest that the Arbuscular Mycorrhizal (AM) symbiosis evolved in first land plants and was one of the critical innovations that allowed plants to successfully colonize lands. Using phylogenomic approaches we have identified genes specifically conserved in land plants that maintain the AM symbiosis. Comparative genomics in the Marchantia genus and analysis of 90 wild populations of *Marchantia polymorpha* ssp. *ruderalis* confirmed this finding, leading to the identification of a core-set of genes that have been committed to AM symbiosis for the last 450 Million years. In parallel, genetic analyses in the liverwort *Marchantia paleacea*, combined with inter-species complementation assays and biochemistry supports that the AM symbiosis evolved in first land plants by the rewiring of ancient eukaryotic gene networks. A model for the origin of the AM symbiosis will be discussed.

S4.5 Investigation of the regulation of ectomycorrhizal symbiosis by nutrient signalling in Poplar

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To improve their mineral nutrition, trees form symbiotic associations with mutualistic microorganisms [1]. Symbiotic associations are mutually beneficial interactions in which the microorganism provides the plant with mineral nutrients in exchange for organic carbon. These associations have an energetic cost for the plant. Therefore, to maintain an optimal growth, plants have to integrate environmental and metabolic nutritional cues to regulate adequately their symbiotic associations [2]. In Legumes, Small-Secreted Peptides (SSP) mediate local and systemic signals regulating nutrient stress responses including symbiotic interactions with nitrogen fixing bacteria [3,4]. Occurring between 6000 trees species and several fungi of the Basidiomycota and Ascomycota phyla, ectomycorrhizal (ECM) symbioses are pivotal for nutrient cycling in forests [1]. However, little is known regarding the molecular and physiological mechanisms controlling ECM-associations [5]. Members of several SSP families were found in the model tree Populus [6,7]. To investigate whether and how trees nutrient signals and SSPs control the formation and maintenance of ECM associations, we propose to (i) characterize the effects of nutrient stresses on ECM symbiosis in Populus canescens associated to the ECM fungus Laccaria bicolor, (ii) to identify the members of SSPs families in Populus by genome scan and phylogenetic analysis and (iii) to assess their role in the transduction of nutrient signals regulating Populus-Laccaria ECM by transcriptomics and secretomics.

[1] Martin et al. (2016) Nat Rev Microbiol 14.
[2] Nehls et al. (2016) Mol. Mycorrhizal Symbiosis 161–178
[3] de Bang et al. (2017) Curr. Opin. Plant Biol. 39, 31–39
[4] Bisseling et al. (2014) Science 346, 300–301
[5] Garcia et al. (2015) New Phytol. 208, 79–87
[6] Ghorbani et al. (2015) J. Exp. Bot. 66, 5257–5269
[7] Goad et al. (2017) New Phytol. 216, 605–616

S4.6 A kinase functions downstream of RAM1 to maintain the arbuscular mycorrhizal symbiosis in *Medicago truncatula*

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Drawing from a forward genetic screen for genes of the model legume *Medicago truncatula* that were important for the arbuscular mycorrhizal symbiosis, but not in the common symbiosis pathway shared with the root nodule symbiosis, we identified a transmembrane kinase, called KIN3 by Bravo et al (2016, Nature Plants). We used our independent *kin3* knockout mutant to show that this kinase is essential for the maintenance of a productive symbiosis, and demonstrate the expression and localisation of the protein. We used RNAseq to validate our previously presented model of KIN3 function in arbuscule containing cells, and explore the function of the putative 'receptor' domain of KIN3 that is not present in several plant linages. This work demonstrates the utility of the Noble Foundation's collection of tnt1-mutagenised *Medicago truncatula* in the discovery of new parts of the mycorrhizal symbiosis machinery, and that whole genome sequencing offers a cost-effective way to overcome the large number of transposons present in this collection.

Multiple interactions

I5.1 Root Fungal Symbionts: Allies of Plants in a Multitrophic Context

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Plants nurture a vast community of symbiotic microbes, that in analogy to microbial communities in the human gut, provides their host with essential functions related to nutrient acquisition and protection against infections. Among them arbuscular mycorrhizal (AM) and Trichoderma fungi are widespread soil inhabitants that establish symbiosis with the vast majority of terrestrial plants, conferring positive effects on growth and fitness. Root microbial symbionts represent thus a promising contribution to sustainable pest management strategies. However, the mechanisms underlying the impact of root symbionts on plant-insect interactions remain largely unknown. Moreover, besides improving plant growth and inducing defenses against herbivorous insects, root-colonizing microbes may affect plant-insect performance via interactions at higher trophic levels. Still, the effects of symbiotic mutualistic fungi on the recruitment of natural enemies of herbivores (indirect defenses) are largely unknown. In my research, I take an 'integrative approach', combining transcriptomics and metabolomics, with performance and behavioral studies to uncover key traits driving the impact of AM and Trichoderma fungi on the interaction of tomato plants with herbivores and herbivores natural enemies.

S5.1 Fungal competition reduces the virulence potential of the root rot pathogen *Bipolaris sorokiniana*

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In nature, beneficial and pathogenic fungi often simultaneously colonize plants. To address how multipartite plant-fungal interactions shape local and systemic host responses, we established a reductionist approach taking advantage of a gnotobiotic natural soil-based split root system. Phenotyping, cytological and transcriptional analyses showed that barley roots respond remarkably different to infection with the pathogen *Bipolaris sorokiniana* and to colonization with the beneficial root endophyte *Serendipita vermifera*. Endophyte colonization only marginally affected plant gene expression, whereas 2741 (1643 with log2FC≥|1|) host genes were deregulated during pathogen infection. In the presence of *S. vermifera*, pathogen infection and disease symptoms were significantly reduced despite the lack of marked alterations of the plant transcriptional response. During direct fungal confrontation in soil, highly up-regulated genes in *B. sorokiniana* are involved in fungal stress responses while in *S. vermifera* genes encoding hydrolytic enzymes were strongly induced. Interestingly, competition with the endophyte resulted in a significant down-regulation of genes encoding secreted proteins in *B. sorokiniana* possibly limiting the pathogen's ability to colonize its plant host. Moreover, expression of the identified fungal antagonistic genes was not induced in planta, indicating that fungal competition occurs outside the plant host.

S5.2 Defence mechanisms triggered by herbivory in ectomycorrhizal oak trees over successive growth flushes.

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The pedunculate oaks (*Quercus robur*) as long-lived, immobile and large organisms have to adapt to environmental changes and biotic interactions via growth regulation and defence molecule production. A particular trait of oaks is their endogenous rhythmic growth (ERG) with alternating aboveground and belowground growth flushes. Alternating shoot flushes (SF) and root flushes (RF) are paralleled by fluctuations in resource allocation. This alternating SF and RF impacts plant defence responses against detrimental interactors and the level of protection by beneficial partners. However the genetic mechanisms are barely understood and were never related to metabolite profiles. Here we investigate defence mechanisms triggered by herbivory in ectomycorrhizal oak trees over successive growth flushes. The *Q. robur* clone L. DF159 was propagated and cross-inoculation experiments were performed at different stages of the ERG. Leaf herbivory was inflicted by *Lymantria dispar* and root ectomycorrhizal fungus inoculation (EMF) by *Piloderma croceum*. Using this multitrophic system, we aimed to: 1) Study how leaf herbivory in a SF influences the plant defence response to a second herbivory event in the next SF. 2) Analyse how EMF inoculation affects the plant defence response triggered by herbivory. 3) Investigate how two different growth stages (RF and SF) affect the plant defence response triggered by above-belowground interactions. Our preliminary results show how leaf herbivory in SF modulates the plant defence responses to leaf herbivore in the next SF, whereby both the EMF and the plant stage (RF or SF) have a strong influence on the nature and strength of the response.

I5.2 Fungal-bacterial interactions in the mycorrhizosphere: friends and foes.

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Mycorrhizal fungi interact with complex communities of bacteria all along their life cycles ; spores, mycorrhizae, extramatrical mycelium and fruiting bodies provide habitats to a wide range of bacteria that can thrive on the surface of the hyphae or inside the fungal cells. The output of the interactions ranges from beneficial to detrimental and their effects can extend to the host plant of the mycorrhizal fungi. Although bacteria tend to be classified according to a specific phenotype (*e.g*. mycorrhiza helper bacteria, mycophagous bacteria, plant pathogen…) many bacteria have versatile behaviours and adapt the way they interact with mycorrhizal fungi depending on abiotic and biotic conditions. However, little is known on the exact parameters that drive behaviour shifts. Conversely, mycorrhizal fungi may be able to recognize bacteria and to adapt their response accordingly. We have explored these different aspects of the interactions by combining genome mining, mutagenesis, transcriptomic and microscopy analyses.

CLOSING LECTURE

L2 Defining the host genetic control of the rhizosphere bacterial microbiota

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My group uses barley (*Hordeum vulgare*) as a model to gain novel insights into the genetic basis of plant-microbiota interactions in the rhizosphere. 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 this line 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 nine, phylogenetically unrelated, bacteria. Here I will discuss the experiments that led us to these discoveries and their implications for basic and applied science.

Poster abstracts

Genomics and Natural variation

P01 MAZE - Accessing arbuscular mycorrhiza-mediated drought stress resistance in a maize diversity panel

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Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between more than 80% of land plants and fungi of the phylum Glomeromycotina. The fungi provide plants with mineral nutrients in exchange for carbohydrates and lipids. AM associations with plants have been shown to enhance maize performance under low nutrient and under drought stress conditions. The positive effect of AM symbiosis on plant performance depends on the maize genotype, suggesting that the so called 'AM -responsiveness' is subject to genetic variation. We examined the impact of AM symbiosis with the model fungus *Rhizophagus irregularis* on the growth performance of a panel of one American and 12 European maize inbred lines of the Dent pool under drought stress and well-watered conditions. We determined significant genotype-dependent variation in AM-responsiveness for plant height, stem diameter, green leaf area, shoot and root dry weight. These data provide a promising basis for mapping genetic polymorphisms underlying variation in AM-responsiveness of maize and their future use for breeding AM-optimized crops.

P02 Arbuscular mycorrhizal community associated to *Vitis vinifera* cv. Pinot Noir in a Piedmont vineyard treated with integrated pest management

Patrizia Cesaro[a], Lara Boatti[b], Elisa Bona[a], Nadia Massa[a], Giorgia Novello[a], Valeria Todeschini[a], Flavio Mignone[a,b], Elisa Gamalero[a], Guido Lingua[a], Graziella Berta[a]

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Vitis vinifera (L.) is an economically important crop whose value largely depends on fruit quality, a feature that can be influenced by soil microorganisms, including arbuscular mycorrhizal fungi (AMF). AMF, able to establish symbiotic associations with vine roots, have beneficial effects on grapevine performance, including water use efficiency and replant success. Most grapevine varieties are susceptible to diseases, whose control can be performed by different approaches, including integrate pest practice (IPM). Previous reports suggested a specificity in the symbiosis between grapevine and AMF and the importance of soil characteristics on this association.

In the present study, we examined the AMF communities in the rhizospheric and bulk soil of *V. vinifera* cv Pinot Noir, subjected to IPM, by using 454 Roche sequencing technology.

The bulk and the rhizospheric soil of the grapevines were sampled before and after grape production. Genomic DNA was amplified, after extraction, according to the methods for pyrosequencing, by nested PCR using AMF specific primers of the large ribosomal subunit (LSU rDNA). Sequences were compared with both NCBI and an AMF LSU rDNA reference databases.

Our data showed different AMF communities in the rhizospheric and bulk soil of *V. vinifera* and the importance of the sampling time in regulating AMF biodiversity.

P03* Single nucleus sequencing reveals evidence of inter-nucleus recombination in arbuscular mycorrhizal fungi

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Eukaryotes thought to have evolved clonally for millions of years are referred to as ancient asexuals. The oldest group among these are the arbuscular mycorrhizal fungi (AMF), which are plant symbionts harboring hundreds of nuclei within one continuous cytoplasm. Some AMF strains harbor two co-existing nucleotypes (dikaryons) but there is no evidence that such nuclei recombine, as is expected for sexual fungi. Here, we show that AMF nuclei with distinct genotypes can undergo recombination. Inter-nuclear genetic exchange varies in frequency among strains, and despite recombination all nuclear genomes have an average similarity of at least 99.8%. The present study demonstrates that AMF can generate genetic diversity via (para)sexual processes in the absence of observable mating. The AMF dikaryotic life-stage is a primary source of nuclear variability in these organisms, highlighting its potential for strain enhancement of these symbionts.

* *Selected flash talk presentations*

P04 The genetics behind AM symbiosis: the case of Tomato wild relatives

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Arbuscular mycorrhiza (AM) is the most widespread mutualistic symbiosis established between land plants and Glomeromycotina, a group of soil fungi [1]. Among mycorrhizal crops, tomato has been extensively investigated for AM interactions being a valuable plant model. However, genetics behind AM symbiosis responsiveness in tomato has been mostly faced using functional genomics approaches (RNAseq and microarray). However, precious genetic resources are available to afford this topic, such as wild relatives, introgression lines and mutants. In particular, tomato wild relatives, offer an effective genetic reservoir for cultivated tomato [2,3], but have rarely been investigated for their susceptibility and responsiveness to the AM symbiosis at root and systemic level. We analyzed the responses of two species, *Solanum pennellii* and *Solanum neorickii* and a cultivated tomato (M82), to the colonization by *Funneliformis mosseae*. Plants were grown for 60 days in an alveolar tray with a substrate containing or not the AM fungal inoculum and watered with low phosphate fertilizer. Plant growth traits, SPAD and mycorrhizal status were assessed. Arbuscule morphology was obtained using Wheat Germ Agglutinin, while symbiosis functionality was tested by evaluating AM-marker gene's expression. Results indicate that, although in all genotypes arbuscule morphology was maintained, in *S. pennellii* mycorrhization was reduced while in *S. neorickii* was similar to M82. Interestingly, under these growth conditions, in both wild relatives a negative growth response was observed.
In conclusion, tomato wild relatives are a powerful tool to understand AM genetics suggesting that mycorrhiza responsiveness is a crucial trait to be considered in breeding programs.

[1] P. Bonfante, A. Genre 2010 Nature Communications, 1:48
[2] A. Bolger *et al*. 2014 Nature Genetics, 46, 1034-1038
[3] N.P. Castañeda-Álvarez et al. 2016 Nature Plants, 2:6022

P05 Let's upgrade our molecular tool box to trace and identify Glomeromycotina and no longer overlook accompanying Mucoro-, Mortierello- and Olpidiomycotina

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Analyses on fungal community assembly have been hampared by mismatches between available phylotaxonomic markers and deep sequencing technologies. The new *Sequel platform* of Pacific Biosciences together with sample tagging with *Barcoded Universal Primers* to multiplex samples for sequencing eliminates this bottleneck and allows convenient and cost-efficient generation of hundreds of long and high quality nucleotide sequences from hundreds of community samples. We will present an analytical procedure that was already used for two sets of approximately 300 samples of roots, colonised by natural fungal communities. The procedure relies on one pair of consensus and wobble PCR primers derived from the outer pair of the primer sets introduced by Krüger et al. [1], tailed with *Universal Sequences*, and potassium and ammonium ion-containing PCR chemistry, which increases the specificity and efficiency of amplification. Need for just one round of amplification reduces formation of chimeric amplicons. Use of large amounts of template DNA gurantees that rare taxa are detected and that the abundance ranking of the community members is retained. Using the concatenated 270 bp-18S, 5.8S and 930 bp-28S rRNA gene sequences, we show that occurrence and relative abundance of Glomero-, Mucoro-, Mortierrelo-, and Olpidiomycotina in roots of matgrass (*Nardus stricta*) is driven by soil humidity and that the communities of symbionts of the legume shrub rooibos (*Aspalathus linearis*) shift from being dominated by Gigasporaceae to being dominated by *Rhizophagus* in response to fertilisation. High polymorphism in the ITS1 and ITS2 sequences allows analyses on possible strain-strain interactions within abundant fungal lineages for which polymorphisms within and among strains can be separated across replicate samples.

[1] Krüger et al. (2009) New Phytol., **183**, 212-223.

P06 A Genome Wide Association Study to disentangle legume-specific root responses to phosphate

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Phosphate is an essential plant macronutrient and its low soil availability leads farmers to massive application of phosphate-based fertilizer. Being also a key regulator of Arbuscular Mycorrhizal symbiosis, a better understanding of plant genetic and molecular bases of phosphate absorption and utilization is needed. Much progress in that area has been made over the past years, but most approaches focused on Arabidopsis thaliana, a plant that lost its capacity to form arbuscular mycorrhizal symbiosis. However, it is unclear which of the discovered mechanisms are conserved and which ones are specific to it. Here, we approach this problem by using a combination of high throughput root phenotyping and phosphate quantification of natural populations of three different plant species, Arabidopsis thaliana and the legumes Lotus japonicus and Medicago truncatula. Genome Wide Association allowed us to identify multiple candidate genes regulating phosphate metabolism and root growth responses to varying phosphate levels. The parallel root phenotyping of multiple natural populations constitutes therefore a powerful tool for detection of genes involved in such complex traits such as root growth and could represents an important tool for further characterize multiple connection linking root system architecture, nutrient homeostasis and mycorrhizal symbiosis.

P07* Missing metabolic pathways concerning symbiosis-based auxotrophy revealed by genome sequencing of an arbuscular mycorrhizal fungus *Rhizophagus clarus* HR-1

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Arbuscular mycorrhizae are the most widespread mycorrhizae originated from more than 400 million years ago. However, moleculer and genomic studies of arbuscular mycorrhizal (AM) fungi were not so advanced than other fungi because of difficulty in culture and genome sequencing. Until last year, available genomic sequences of AM fungi were limited to only one model species, *Rhizophagus irregularis* [1][2]. We sequenced the genome of a non-model AM fungi *R. clarus* (strain HR-1) and compared its character with *R. irregularis* and other fungi. Missing of cytosolic fatty acid synthase (FAS), as reported for the genome of *R. irregularis* [3] and the transcriptome of *Gigaspora* spp. [4], was confirmed to be general feature of genomes of two AM fungi, whereas all mitochondrial FAS components were present. Common absence of several metabolic pathways including thiamine biosynthesis and conversion of vitamin B6 derivatives were also identified. Most of glucose-producing polysaccharide hydrolases except for trehalase and glycogen phosphorylase also appear to be absent in AM fungi, whereas ectomycorrhizal or pathogenic fungi maintain most of those enzymes. These absence of primary metabolic pathways may indicate that AM fungi depend their metabolites on host plants and may have evolved during long symbiotic history. Our results [5] may also useful to clarify auxotrophy and improve the culture efficiency of AM fungi.

[1] Tisserant et al. (2013) P. Natl. Acad. Sci. USA, **110**, 20117-20122.
[2] Lin et al. (2014) PLoS Genetics, **10**, e1004078.
[3] Wewer et al. (2014) Plant J., **79**, 398-412.
[4] Tang et al. (2016) Front. Microbiol., **7**, 233.
[5] Kobayashi et al. (2018) BMC Genomics, **19**, 465.

* *Selected flash talk presentations*

P08 Improved genome sequence gives evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*

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Rhizophagus irregularis DAOM-181602 is a widely used model species for the study of arbuscular mycorrhizal fungus (AMF). We constructed much improved reference genome assembly of the model AMF (total contigs = 210, N50 = 2.3 Mb, Total length of contigs = 150 Mb, NSDC# = BDIQ01000000) [1]. Improved genome revealed that common concepts of eukaryotic rDNA are not applicable to AMF. Its rDNA copies are highly heterogeneous, reduced in number, and lack TRS. *R. irregularis* has only ten or 11 copies of complete 45S rDNAs[1], whereas the general eukaryotic genome has tens to thousands of rDNA copies[2]. Moreover, *R. irregularis* rDNAs are highly heterogeneous and lack a tandem repeat structure[1]. The rDNA marker which frequently used in the ecological and phylogenetic field should be adopted cautiously for AMF. The sequence diversity and reduced copy number of rDNA may result from the collapse of the concerted evolution system due to the lack of TRS. Although the adaptive significance of the TRS lacking in AMF remains to be determined, future investigations on the functional differences among the heterogenous rRNAs may reveal mechanisms that facilitate adaptation to various environmental conditions in AMF. RNA-Seq analysis confirmed that all rDNA variants are actively transcribed[1]. Observed rDNA/rRNA polymorphisms may modulate translation by using different ribosomes depending on biotic and abiotic interactions.

[1] Maeda et al. (2018) Communications Biology, **1**, 87.
[2] Eickbush et al. (2010) Genetics **175**, 477–485

P09* Dual RNA-seq reveals large-scale non-conserved genotype x genotype specific genetic reprograming and molecular crosstalk in the mycorrhizal symbiosis

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Arbuscular mycorrhizal fungi (AMF) impact plant growth and are a major driver of plant diversity and productivity. We quantified the contribution of intra-specific genetic variability in cassava (*Manihot esculenta*) and to gene reprogramming in symbioses using dual RNA-sequencing. A large number of cassava genes exhibited altered transcriptional responses to the fungus but transcription of most of these plant genes (72%) responded in a different direction or magnitude depending on the plant genotype. Two AMF isolates displayed large differences in their transcription, but the direction and magnitude of the transcriptional responses for a large number of these genes was also strongly influenced by the genotype of the plant host. This indicates that unlike the highly conserved plant genes necessary for the symbiosis establishment, most of the plant and fungal gene transcriptional responses are not conserved and are greatly influenced by plant and fungal genetic differences, even at the within-species level. The transcriptional variability detected allowed us to identify an extensive gene network showing the interplay in plant-fungal reprogramming in the symbiosis. Key genes illustrated that the two organisms jointly program their cytoskeleton organisation during growth of the fungus inside roots. Our study reveals that plant and fungal genetic variation plays a strong role in shaping the genetic reprograming in response to symbiosis, indicating considerable genotype x genotype interactions in the mycorrhizal symbiosis. Such variation needs to be considered in order to understand the molecular mechanisms between AMF and their plant hosts in natural communities

* *Selected flash talk presentations*

P10 The fungal root endophyte *Serendipita indica* modifies extracellular nucleotides to modulate plant signaling and fungal accommodation.

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Extracellular adenosine 5′-triphosphate (eATP) is an essential signaling molecule that mediates different cellular processes through its interaction with membrane-associated receptor proteins in animals and plants. eATP regulates plant growth, development and responses to biotic and abiotic stresses. Its accumulation in the apoplast induces ROS production and cytoplasmic calcium increase mediating a defense response to invading microbes. We demonstrate that perception of extracellular nucleotides, such as eATP, is important in plant-fungus interactions and that during colonization by the beneficial root endophyte *Serendipita indica* eATP accumulates in the apoplast at early symbiotic stages. Using liquid chromatography-tandem mass spectrometry, cytological and functional analysis we show that *S. indica* secrets *Si*E5′NT, an enzymatically active ecto-5′-nucleotidase capable of hydrolyzing nucleotides in the apoplast. *A. thaliana* lines producing extracellular *Si*E5′NT are significantly better colonized and have reduced eATP levels and nucleotides signaling, indicating that *Si*E5′NT functions as a compatibility factor. Our data show that extracellular bioactive nucleotides and their perception play an important role in fungus-root interactions and that fungi can modify plant-derived metabolites in the apoplast to modulate host immunity.

P11* Multigene vs rDNA based species delimitation of Glomeromycotina

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Availability of DNA sequencing approaches to identify fungi in samples from nature revolutionized the research on fungi and are now standard. DNA sequencing of fungal specimens and cultures, and natural soil and root samples has considerably changed the understanding of the diversity of Glomeromycotina, fungi forming arbuscular mycorrhiza (AM). It is now possible to identify AM fungal taxa by DNA sequencing irrespective of the presence of microscopically identifiable structures. Further developments of species proxies (Virtual Taxa, VT; Species Hypotheses, SH) and concurrent systematic organization of information about their occurrences in databases provides tools and data for broad research community to target these fungi in studies from taxonomy to physiology and genomics or to ecosystem sciences and beyond. However, it remains unsolved, how to appropriately delimit DNA sequence based species proxies. Using *Rhizophagus intraradices-irregularis* species group as a model, I'll present the current knowledge of the phylogenetic diversity within the group in the context of recent genotyping data, the global geographical and habitat-wise taxon distribution in the group, and the share of data in culture collections vs DNA sequences from various samples. I will conclude with future prospects in the direction of unravelling the AM fungal diversity and its patterns in the nature.

* *Selected flash talk presentations*

P12* The role of host plant in symbiosis stability of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi, form symbiosis with more than 80% of terrestrial plants. Under the assumption of AM fungi are heterokaryotic, the multi-nucleus spores formed may have different genetic composition. In this study, we test the hypothesis that genetically distinct nuclei segregate within mycelial network in response to different hosts.
In a pilot study, leak, sorghum, petunia, plantago were grown with *Gigaspora rosea*, *Claroideoglomus claroideum* and *Cl. candidum*, *Scutellospora calospora* to identify the best host-fungus combinations. Sorghum (C4), leak (C3), *G. rosea* and *Cl. claroideum* are chosen.
In a follow-up experiment, three host combinations (i.e. sorghum:sorghum, leak:eak, sorghum:leak) are inoculated with spores of either *G. rosea* or *Cl. Claroideum* single spore cultures. Two T-shape PVC sewers are attached to form a dual-compartment pot, separated in the middle with a mesh (50 µm pore size) [1]. An amount of 40 g of inoculum (soil plus spores) is evenly distributed across the mesh before filling the pot with soil and tera-green and planting the seedlings. For the control, seedlings are inoculated with the autoclaved inoculum. After two months, the colonized roots are subjected to RNA-seq analysis. In addition, fitness of the host (biomass, height) and fungus (spore count) will be evaluated. Host preference and fitness effects on AM fungi will be discussed.

[1] Koyama et al., (2017) Mycorrhiza, 27, 553-563

* *Selected flash talk presentations*

P13* Connecting phylogenomics and fossil data

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For a long time it has been hypothesized that symbiotic associations between plants and fungi - the endomycorrhizas - were essential to the establishment of plant life on land, however much remains to be understood about how these interactions originated. Calibrated phylogenetic trees provide insights into the origins of plant and fungal groups, and genomics furthers our understanding of how the complex set of symbiotic traits evolved. The fossil record offers an additional and complementary source of insights. While the known geological record of these associations is generally sparse, certain exceptional geological sites provide direct evidence of early plant-fungal associations. Among these are the 407 Ma Rhynie chert (Scotland) and Late Carboniferous (ca 310 Ma) wetland soils (e.g., Grand'Croix, France). Organism associations in the Rhynie chert include several forms of mycorrhizal symbiosis. This is consistent with molecular studies that demonstrate that there is a greater diversity of mycorrhizal fungi in living bryophytes and pteridophytes than previously recognized. The earliest direct fossil evidence of endomycorrhizas in roots comes from trees of the Carboniferous Period. In these ecosystems there is evidence of arbuscular mycorrhizas (AM) in clubmosses (endodermis absent) and early relatives of the conifers (endodermis present). Since roots in these two groups of plants are known to have evolved independently the fossil record indicates at least two separate origins for root colonization by AM fungi in plants with significant differences in root anatomy.
Our aim is to highlight the potential of a cross disciplinary approach integrating phylogenetic, genomic and palaeontological sources.

* *Selected flash talk presentations*

P14 Effects of nuclei selection on symbiotic efficiency of *Rhizophagus irregularis*

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Arbuscular mycorrhizal fungi (AMF) can colonise the majority of all land plants. Symbiosis with these fungi offer the host plant many advantages, including an increased uptake of nutrients and protection against both biotic and abiotic stresses. The efficiency of this symbiosis varies between fungal isolates and even among offspring of a single AMF individual. The molecular basis of these differences is still unclear.

Previous studies have suggested that AMF can maintain their genetic composition by supporting a population of genetically differing nuclei. By forming spores filled with a subset of this population, the fungus can create progeny that differ from their parent colony. It is unknown to what extent this variation contributes to the adaptability of AMF.

We attempt to find how individual nuclei differ from each other and how this may affect symbiotic efficiency of AMF by assessing their ability to colonise new hosts, changing from carrot to tomato. AMF lines were derived from a single colony (*Rhizophagus irregularis* isolate C3) and adapted by introducing genetic bottlenecks or "training" them on tomato root cultures before host switch. Using these selection methods we aim to alter the genetic composition of the fungus. These selected lines will be scored on colonisation rate, effect on plant growth, and nutrient exchange efficiency. The colonies will also be compared by RNA sequencing of colonised plant roots and DNA sequencing on nucleus, spore, and metagenome level of the fungus. Through this approach we aim to link natural (epi)genetic variation in AMF to their symbiotic efficiency.

Signaling in mycorrhizal symbioses

P15* Exploring the tomato plant – *Rhizophagus irregularis* interactome

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Arbuscular mycorrhiza is the most widespread beneficial plant symbiosis. In order to establish this symbiosis, a fine molecular cross-talking has to take place between the plant and this obligatory biotrophic fungus. The initial signal exchange between both symbionts have been well established. However, the molecular communication needed at later steps of the interaction is far from be elucidated.

Analysis of the available *Rhizophagus irregularis* genomic sequence revealed that AM fungus potentially secrete an array of protein effectors to manipulate the host physiology [1]. Proteinaceous effectors derived from microbes often interact with host proteins to suppress immunity or to create the ideal conditions for niche occupation. So far, just two effector proteins have been studied in more detail being SP7 that interact with the plant ERF19 protein involved in ethylene response [2] and SIS1 activated by strigolactones treatment [3]. Elucidating the host protein network to which the effectors bind will reveal the molecular network that is essential for the establishment of the AM symbiosis. In addition, a diverse range of effectors might target only a small set of host proteins, called hub proteins, that are expected to be highly important during AM.

Here we use yeast two hybrid coupled to Illumina sequencing as a high through-put method to establish the tomato root proteome interacting with AMF effectors. Additionally, complemented with GFP trapping in order to study the interaction between the AMF effectors and tomato proteins to reveal the tomato protein network that plays an important role in the symbiosis establishment.

[1] Zeng et al. (2018) The plant journal, **94**, 411-425.
[2] Kloppholz et al. (2011) Current Biology, **21 (14)**, 1204-1209.
[3] Tsuzuki et al. (2016) Mol Plant Microbe Interact, **29 (4)**, 277-286.

* *Selected flash talk presentations*

P16 Independent signalling cues underpin arbuscular mycorrhizal symbiosis and large lateral root induction in rice

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Perception of arbuscular mycorrhizal fungi (AMF) triggers distinct plant signalling responses to enable the parallel establishment of symbiosis and induction of lateral root formation. Rice receptor kinase CHITIN ELICITOR RECEPTOR KINASE 1 (OsCERK1) and α/β-fold hydrolase DWARF14-LIKE (OsD14L) are involved in pre-symbiotic fungal perception. After 6 wk post-inoculation with *Rhizophagus irregularis*, root developmental responses, fungal colonization and transcriptional responses were monitored in two independent *cerk1* null mutants; a deletion mutant lacking *D14L*, and with *D14L* complemented as well as their respective wild-type cultivars (cv Nipponbare and Nihonmasari). We show that although essential for symbiosis, D14L is dispensable for AMF-induced root architectural modulation, which conversely relies on CERK1. These results demonstrate uncoupling of symbiosis and the symbiotic root developmental signalling during pre-symbiosis, with CERK1 required for AMF-induced root architectural changes. Meanwhile, ongoing efforts aim to identify the signalling mechanisms of *OsCERK1* during symbiosis. Symbiotic defects in *oscerk1* failed to be complemented by a common mycorrhizal network supported by neighbouring wild-type plants; suggesting that the compromised signal exchange downstream of CERK1 cannot be provided in *trans* unlike other symbiotic mutants.

P17* *OmSSP1*, a hydrophobin-like small secreted protein, is a putative effector in ericoid mycorrhiza

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Plant-colonizing fungi secrete "effector" molecules that can facilitate fungal invasion of plant tissues by manipulating the host cell metabolism. Symbiosis-induced small secreted proteins (SSPs) identified as fungal effectors have been characterized in ectomycorrhizal and arbuscular mycorrhizal fungi, whereas no information is available for ericoid mycorrhizal (ERM) fungi. ERM fungi are soil-born fungi, mostly belonging to Leotiomycetes (Ascomycetes), which form endomycorrhiza with plants in the family Ericaceae. The sequenced genome of the ERM model fungus *Oidiodendron maius* contains 445 *SSPs* genes. Aim of our work was to identify, among them, SSPs potentially involved in the molecular dialogue governing the ERM symbiosis.
RNA-Seq data revealed that 20% of *O. maius SSPs* are up-regulated during ERM symbiosis. The most highly symbiosis-induced SSPs, named OmSSP1, was functionally characterized through: a) gene expression validation; b) molecular characterization *in silico*; c) secretion assays; d) null-mutants generation; e) *in vitro* mycorrhizal synthesis with null-mutants.
OmSSP1 was confirmed to be strongly up-regulated during ERM symbiosis (FC>100). The protein shares some features with class I hydrophobins (i.e. cysteines pattern, hydrophobicity and length) but lacks the hydrophobin PFAM domain. OmSSP1 features a signal peptide and was experimentally confirmed to be secreted. The absence of a Nuclear Localization Signal suggests that OmSSP1 may interact with different plant cell compartments. A significant reduction in the degree of root mycorrhization was observed for three independent OmSSP1 null-mutants, suggesting that this protein is involved in the mycorrhization process.

* *Selected flash talk presentations*

P18* Nutrient status regulates plant recognition of signals from beneficial microbes for promotion of symbiosis and suppression of immunity

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Plants associate with myriads of microorganisms in the rhizosphere, that can have detrimental or beneficial effects on plant survival. Recognition of these micro-organisms occurs through receptors located at the cell surface, which can activate immunity signalling to restrict or symbiosis signalling to facilitate microbial invasion. Immunity signalling is induced following recognition of chitooligosaccharides (COs) present in fungal cell walls and peptidoglycan (PGN) on the surface of bacteria, but such recognition does not discriminate a pathogen from a symbiont. Consistently, we have found that root cells of *Medicago truncatula* activate symbiosis and immunity signalling following recognition of COs and PGN. This CO perception contributes to successful colonisation by arbuscular mycorrhizal fungi, in concert with the perception of lipochitooligosaccharides (LCOs). LCOs act synergistically with COs during the induction of symbiosis signalling, but antagonistically for induction of immunity signalling. We further demonstrated that nutrient status of the plant is also important for defining the degree of symbiosis or immunity signalling. Our work reveals how plants adapt their reaction to the microbial community, to be more welcoming or more restrictive according to their need for microbial nutrient services.

* *Selected flash talk presentations*

P19* Exploration of novel arbuscular mycorrhizal symbiosis signals transduced by *D14L/KAI2* pathway

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DWARF14 LIKE / KARRIKIN INSENSITIVE2 (D14L/KAI2) is a paralog of DWARF14, a strigolactone receptor. D14L/KAI2, however, perceives not strigolactones but karrikins, smoke-derived compounds which induce seed germination and photomorphogenesis. In addition, D14L/KAI2 is thought to perceive unidentified plant hormones other than karrikins, because rice *d14l* mutant and Arabidopsis *kai2* mutant exhibit disturbed seed germination and photomorphogenesis phenotypes without karrikin treatment. Furthermore, rice *d14l* mutant lacks the responses to arbuscular mycorrhizal fungi (AMF), indicating that AMF-derived signaling compounds induce symbiotic responses to AMF in rice via *D14L/KAI2* pathway. We aim to identify these unidentified plant hormones and AMF-derived signaling compounds.

We revealed that *DWARF14 LIKE2a* (*D14L2a*) and *DWARF14 LIKE2b* (*D14L2b*), paralogs of *D14* and *D14L*, are induced during AM symbiosis. They were not induced in *d14l* mutant, indicating that this induction is *D14L* dependent. Therefore, we established an assay system to detect the compounds which activate *D14L/KAI2* pathway based on *D14L2b* expression. We found that the extract of AMF induces *D14L2b* expression and are trying to isolate the compounds.

* *Selected flash talk presentations*

P20 On the rocks: Investigating the role of plant immunity in arbuscular mycorrhizal symbiosis

Radoslaw Kowalczyk

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Small secreted proteins known as effectors have recently emerged as integral regulators of molecular plant microbe interactions. Despite the identification of a few candidates in the last decade, the existence of a full effector repertoire in arbuscular mycorrhiza, as well as potential roles, host targets and mechanisms have thus far remained elusive. A bioinformatic approach was used to identify candidate effectors in the genome of AM fungus *Rhizophagus irregularis*. Candidate effectors were investigated for homology with known effectors and for effects on symbiosis using a combination of microscopic and gene expression analysis. Results showed a family of 15 effector candidates called R5 sharing sequence similarity to the secreted in xylem 6 (Six6) effector from the fungal pathogen *Fusarium oxysporum*. Transient co-expression assays revealed that R5 family members mimic Six6 activity by suppressing cell death triggered by the recognition of *Fo* effector Avr2 by the host R-gene I-2. These findings led to the hypothesis that *R. irregularis* may deploy R5 effectors to promote AM symbiosis by modulating host immunity using a pathogen-like effector strategy. To test this, I will use a combination of microscopic and gene expression analysis to investigate the impact of *in planta* effector expression on AM symbiosis under optimal and antagonism-inducing experimental conditions, with a particular focus on modulation of plant immunity.

P21 Role of fungal secreted peptides in arbuscular mycorrhizal symbiosis

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The Arbuscular Mycorrhizal (AM) symbiosis is a beneficial association established between land plants and the members of a subphylum of fungi, the Glomeromycotina. How the two symbiotic partners regulate their association is still enigmatic and secreted fungal peptides are candidates for regulating this interaction. We recently observed that in *Rhizophagus irregularis*, an unexpected high number of secreted proteins display the canonical features of ascomycete alpha-pheromones. These proteins present repetitions of identical motifs, separated by putative endoproteolytic cleavage sites, targeted by the KEX2 protease. We expect that these proteins are processed within the fungal cell and delivered as peptides in the extracellular space.
We developed a bioinformatic pipeline to identify KEX2-processed repeat proteins (KEPs) in publicly available fungal protein catalogs. Our survey of 250 fungal species revealed that nearly all fungi contain KEPs. Interestingly, in addition to glomeromycetes, some basidiomycetes also display protein with striking similarities with ascomycete alpha-pheromones. We characterized in detail one KEP in *R. irregularis*.
In *Gigasora rosea*, another AM fungus, one KEP present repetitions of a peptide almost identical to plant CLE peptides. In plants, CLE peptides are described as peptide hormones and regulate numerous developmental processes. Other AM species including *R. irregularis* also contain a CLE-containing protein, with a unique CLE domain at their C-terminus. Interestingly, besides plants and some plant-parasitic nematodes, only AM fungi seem to encode these proteins.
We will present a selection of data on *R. irregularis* secreted peptides. Based on physiological and molecular approaches, we will highlight their role during AM symbiosis.

P22 The symbiotic role of ARK2

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ARK2 is a receptor-like kinase transcriptionally induced in arbusculated cells that together with ARK1 belong to an undescribed class of receptor-like kinases. Their strict presence in plant lineages that engage in the arbuscular mycorrhizal symbiosis and absence in plant lineages that do not, points towards their dedicated, and probably concerted, symbiotic role. The symbiotic role of ARK2 in the symbiosis has not been reported. Most ARK1-ARK2 orthologues have an ectodomain that was likely lost early in the evolution of the monocot lineage. Interestingly, this ectodomain is present in a few other receptor-like kinases but that are unrelated based on kinase domain homology. Strategies to unveil the function of this ectodomain will be presented together with details on the comparative phenotypic characterization of ark1 and ark2 mutants lines in rice. In general, in relation to wild type plants, ark2 reduced colonization mutant phenotype is less severe than that of ark1. This correlates with its gene induction levels which are less pronounced than that of ARK1. Their cooperation as a signalling module operating in the arbusculated cell is thus a likely scenario.

P23 AM for quality - use of chito-oligosaccharides to enhance plant mycorrization and forage quality

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Population increase and ecosystem welfare urge XXI century farmers to send an SOS message to soil micro-organisms such as arbuscular mycorrhizal (AM) fungi, ancient symbionts providing mineral nutrients and water to most crop plants. Experimental evidence showed that plant treatment with AM fungal signaling molecules (short chain chito-oligosaccharides, or Myc-COs) enhanced AM establishment under controlled conditions. In view of Myc-CO application in biosustainable agricultural practices, we analyzed four conditions in an experimental meadow: untreated, Myc-COs, a commercial AM inoculum (Mycosat F), and Mycosat F + Myc-COs. The meadows were composed by a mixture of forage species: *Festuca arundinacea*, *Dactylis glomerata*, *Festulolium*, *Trifolium pratense*, *Medicago sativa*, *Onobrychis viciifolia*, *Poa pratensis*. We present here the results from the studies of AM colonization intensity and biomass yield carried out in 2017 and 2018. Myc-CO treatment significantly increased the intensity of AM colonization and biomass in mycorrhizal fields compared to control, fully supporting our laboratory results. Moreover, Myc-CO treatment improved biodiversity, by generating a better balance among forage species. We are currently monitoring the experimental meadows for the third growing season. Furthermore, the recording of seasonal patterns in AM development suggests that root colonization is anticipated in Myc-CO-treated compared to non-treated fields. Concerning the nutritional and organoleptic properties of forage, we will relate the symbiotic status of the forage plants with their content in crude protein, neutral detergent fibre (NDF) and acid detergent fibre (ADF).

P24 Small but powerful - complex regulation of microRNAs after arbuscular mycorrhizal root colonization

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A few specific miRNAs were shown to regulate plant-arbuscular mycorrhizal interactions, but many regulatory elements remain to be discovered. To understand how plant miRNAs are reconfigured during plant-arbuscular mycorrhizal fungal (AMF) interactions we performed a large-scale miRNA analysis. *Nicotiana attenuata* empty vector (EV) plants and an isogenic line silenced in *CCaMK* expression (ir*CCaMK*) impaired in AMF-interactions were grown under competitive conditions with and without AMF inoculum. Next generation sequencing of miRNAs in roots of these plants revealed 67 conserved and 82 novel unique miRNAs. We found major changes in the miRNA profiles due to mycorrhizal colonization, with clearly distinct patterns for a fully functional AMF interaction (EV plants) compared to inoculated plants impaired in the interaction (ir*CCaMK*) and non-inoculated plants. Five conserved miRNAs (miR6153, miR403a-3p, miR7122a, miR167-5p and miR482d) were present in all conditions and showed the highest accumulation in AMF inoculated EV plants compared to inoculated ir*CCaMK* plants. Furthermore, one sequence variant of miR473-5p specifically accumulated in AMF-inoculated plants. Also abundances of miR403a-3p, miR171a-3p and one of the sequence variants of miR172a-3p increased in mycorrhizal EV compared to inoculated ir*CCaMK* plants and to non-inoculated EV plants, while miR399a-3p was most strongly enriched in inoculated ir*CCaMK* plants grown in competition with EV. The analysis of putative targets of selected miRNAs revealed an involvement in P starvation, phytohormone signaling and defense. Further investigations are ongoing to determine the molecular mechanism of action and to elucidate the molecular players of the smRNA pathways, such as AGOs, involved in AMF-mediated regulation.

* *Selected flash talk presentations*

P25* Hormones in endomycorrhizal symbiosis: Study of the production and transfer of (phyto)hormones by endomycorrhizal fungi

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The molecular dialogue between the Arbuscular Mycorrhizal Fungi (AMF) and their host regulates symbiosis establishment. It involves strigolactone, a phythormone released by host roots, and Myc-factors released by fungal spores. Other phytohormones are involved in the development of this symbiosis as well, but they are always examined from the plant point of view. Recently, AMF cytokinins and ethylene receptors were identified *in silico*. It is therefore tempting to speculate that AMF perceive cytokinines or ethylene produced by plants as well. Furthermore, many ectomycorrhizal fungi are reported to produce phytohormones, but there is no strong evidence for AMF. We thus wonder whether AMF may perceive phytohormones imported from root-fungus exchange, or metabolized by AMF themselves. We therefore developed biochemical strategies to detect phytohormones in AMF. A global extraction and enrichment protocol for soluble phytohormones was adapted to different matrixes, like Germination Spores Exudates (GSE) and double compartment culture. In parallel, their detection and identification by liquid chromatography coupled to mass spectrometry was optimized. Ethylene production by fungal spore was investigated by gas chromatography, to decipher its biosynthesis pathways. We have been able to detect for the first time several phytohormones released by spores and extra-radical mycelium. Their metabolic origins, as well as their effect on AMF development still remain unknown and will be further investigated through this project.

* *Selected flash talk presentations*

P26 The role of MIGs (Mycorrhiza induced GRAS) in root development during symbiosis

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Arbuscule development is governed by a complex molecular regulation largely controlled by proteins of the GRAS transcription factor family which modulate different processes from nutrient transport to root development. In *M. truncatula,* MtMIG1 (Mycorrhiza induced GRAS1) controls the radial expansion of arbuscule-containing cells and its downregulation impairs arbuscule development. *MtMIG1* has two close paralogues also induced during symbiosis. To better understand their function, promoter-reporter assays and overexpression analyses were carried out. The results showed that *MtMIG2* and *MtMIG3* are expressed in all cortical cells under non-mycorrhizal conditions but are induced in arbuscules during symbiosis. Overexpression *MtMIG2* caused in, similar to *MtMIG1*, root thickening due to an increase in the number of cortical cell layers and a radial expansion of cortical cells. In contrast, overexpression of *MtMIG3* also increases the number of cortical cell layers, but it counteracted the root thickening phenotypes caused by *MtMIG1* and *MtMIG2.* This indicates that MtMIG3 might function as a negative regulator of the cortical radial cell expansion process. Previous results showed that MtMIG1 interacts with other GRAS-proteins such as MtDELLA1 and MtNSP1. Furthermore, the defect in arbuscule development caused by the downregulation of *MtMIG1* was counteracted in a constitutive DELLA background. However, the relationship with MtNSP1 was not clear. Here we show by expressing *MtMIG1* in a *Δnsp1* genetic background that the effect of MtMIG1 in root thickening is independent of MtNSP1. Future analyses will be towards identifying the targets of MtMIG1 and its paralogues that are responsible for the expansion of arbuscule cells during symbiosis.

P27 *In silico* evidences of fungal small RNAs functional significance in the interplay between the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and its host plant

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Small RNAs (sRNAs) are short non-coding RNA molecules (20-30 nt) that regulate gene expression at transcriptional or post-transcriptional levels in many eukaryotic organisms, through a mechanism known as RNA interference (RNAi). Recent studies have highlighted that they are also involved in cross-kingdom communication: sRNAs can move across the contact surfaces from "donor" to "receiver" organisms and, once in the host cells of the receiver, they can target specific mRNAs, leading to a modulation of host metabolic pathways and defense responses. Very little is known about RNAi mechanism and sRNAs occurrence in Arbuscular Mycorrhizal Fungi (AMF), an important component of the plant root microbiota that provide several benefits to host plants, such as improved mineral uptake and tolerance to biotic and abiotic stress.

Taking advantage of the available genomic resources for the AMF *Rhizophagus irregularis* we described its putative RNAi machinery, which is characterized by a single Dicer-like (DCL) gene and an unusual expansion of Argonaute-like (AGO-like) and RNA-dependent RNA polymerase (RdRp) gene families. *In silico* investigations and experimental assays provided evidence of gene expression for most of the identified sequences. Focusing on the symbiosis between *R. irregularis* and the model plant *Medicago truncatula*, we characterized the fungal sRNAs population, highlighting the occurrence of an active sRNA-generating pathway and the presence of microRNA-like sequences. Moreover, the *in silico* analysis, supported by degradome data, predicted 237 plant transcripts as putative targets of specific fungal sRNAs providing initial evidence of cross-kingdom post-transcriptional gene silencing during AMF colonization.

P28* The *Medicago truncatula LDP1/LDP2* proteins restrict epidermal entry of symbiotic microbes

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The ability to simultaneously undergo two types of symbiotic interactions namely with rhizobia and arbuscular mycorrhiza fungi (AMF), provided legumes with the ability to efficiently explore or survive on nitrogen and phosphate deprived soils. However, due to the use of a genetically identical signalling pathway (the 'common sym pathway', CSP) by both symbioses legumes require additional regulatory pathways to keep tight control over these colonisations. Here, we identified LDP1 and LDP2, two novel and paralogous membrane-resident legume- type (L-type) lectin-domain containing proteins, which exclusively evolved in the Fabaceae clade. LDP1 is strongly induced upon formation of AMF hyphopodia as well as during initial steps of rhizobial inoculation in a Nod-Factor-dependent but CSP-independent manner. Simultaneous silencing of LDP1 and LDP2 resulted in significantly decrease both AMF and rhizobial infections, clearly indicating that LDP1/LDP2 are novel but redundant CSP proteins that localize to the primary host cell entry sites. We will present additional data, which provide evidence that LDP1 functions in conjunction with symbiotic receptors and serves as a key player when the epidermal penetrated by symbiotic microbes.

* *Selected flash talk presentations*

P29 Recognition of chitooctamers and lipochitooligosaccharides activate symbiosis signaling in plants

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AM fungi signal to the plant via diffusible lipochitooligosaccharides (LCOs) or chitooligosaccharides (COs) signaling molecules that can activate the common symbiosis signaling pathway with induction of calcium oscillations. Recently, we have studied how COs and LCOs produced by AM fungi, *Sinorhizobium meliloti* and *Mezorhizobium loti* can activate calcium spiking, a marker of symbiosis signalling, in barley, wheat and maize. We have found that LCOs can be perceived by cereals, but that this recogntion lacks the stringency of perception present in legumes. Whereas legumes show LCO perception early in development, cereals only percieve LCOs when they are showing signs of nutrient starvation. Both nitrogen and phosphate status contribute to the regulation of LCO perception in cereals.

P30 Chito-oligosaccharide treatment anticipates and enhances arbuscular mycorrhizal colonization

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During the establishment of arbuscular mycorrhizal (AM) symbiosis a molecular dialogue occurs between the AM fungus and the host plant, through an exchange of chemical signals that is crucial to the recognition of both partners. In particular, AM fungi release short-chain chitin oligomers (Myc-COs) that trigger symbiotic responses in the host plant. Here we investigated the impact of exogenous Myc-CO application on pot-grown *Medicago truncatula* inoculated with the AM fungus *Funneliformis mosseae*. We firstly evaluated the biomass production and leaf photosynthetic surface in treated and untreated mycorrhizal plants, where we observed an increase of both parameters respect to the control plants. In addition, we investigated the colonization level by morphological and molecular approaches over 4 weeks. The Myc-CO treatment enhanced AM colonization with an extensive development of arbuscules in several layers of root cortical cells, compared to untreated mycorrhizal plants. Gene expression analyses during the course of AM development recorded an increase in the expression of AM marker genes - such as *MtPT4* (a symbiosis-specific phosphate transporter) and *MtBCP* (a blue copper binding protein) - for Myc-CO treated plants at early time points. By contrast, the transcript levels for both genes were lower in Myc-CO treated plants at later time points. Overall, these results suggest that the Myc-CO treatment anticipated the whole process of AM development, encouraging the use of Myc-COs as a promising tool to promote AM establishment in sustainable agricultural practices.

Cellular and molecular aspects of mycorrhizal interactions

P31* Local endoreduplication is associated with the accommodation of an arbuscular mycorrhizal fungus in *Medicago truncatula*

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An increase in nuclear DNA content has been described in several plant interactions with symbiotic, pathogenic and parasitic microbes [1]. In arbuscular mycorrhizas (AM) the high metabolic levels has also been related to a global increase in nuclear DNA content in the root system, suggesting that the degree of ploidy is influenced by AM colonization [2,3]. Nevertheless, the precise location of such endoreduplication events has anyway never been defined. The study of pre-penetration responses to AM fungi revealed that root cells undergo nuclear enlargement prior to fungal penetration [4]. On this basis we hypothesized that endoreduplication events can be part of the pre-penetration response. To test this hypothesis, we investigated ploidy changes in wild-type *Medicago truncatula* roots upon inoculation with the AM fungus *Gigaspora margarita*. Furthermore, we have extended our studies to *M. truncatula* dmi2-2 and dmi3-1 mutants, where pre-penetration responses are absent. Our experimental approaches were based on the targeted analysis of endoreduplication in the colonized area of the root. Cell ploidy was analyzed by combining cytofluorimetry and confocal microscopy imaging. Parallel gene expression and cellular localization analyses were used to highlight the localized activity of endocycle initiation markers. Our results clearly indicate that endocycle activation occurs in the wild-type colonized area of the root and is limited to those cells that host fungal structures and a few of the surrounding cells, suggesting that ploidy increase is indeed related to pre-penetration responses. In fact, no such changes were observed in the controls or *dmi2-2* and *dmi3-1* mutants.

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[2] Berta et al. (2001). Plant and Soil, **226**, 37-44
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* *Selected flash talk presentations*

P32 Transcriptomic analysis of plant and fungal Cd-regulated genes in the ericoid mycorrhizal symbiosis

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The success of Ericaceae in stressful habitats enriched in heavy metals has been ascribed to the unique abilities of their mycorrhizal fungal partners to withstand environmental stress and to enhance metal tolerance in their host plants. Heavy metal tolerance has been investigated in ericoid mycorrhizal (ERM) fungi, but the molecular and cellular mechanisms that confer metal tolerance to the host plant are currently unknown.

Here, we show that mycorrhizal roots of *Vaccinium myrtillus* colonised by the metal tolerant fungus *O. maius* Zn have a reduced Cd content when they are exposed to Cd *in vitro*, as compared with non-mycorrhizal roots. To better understand this phenotype, we applied next-generation sequencing technologies to analyse gene expression in *O. maius* Zn and its host plant *V. myrtillus* grown under normal and Cd stressed conditions, in the free living and in the mycorrhizal status.

The results clearly showed that, under the conditions tested, Cd had a stronger impact on plant gene expression than symbiosis, whereas regulation of fungal gene expression was mainly driven by symbiosis. The higher Cd content in non-mycorrhizal roots likely explains the higher abundance of transcripts coding for stress related proteins, such as HSPs. Regulated plant metal transporters have been identified, that may play a role in reducing the Cd content in mycorrhizal plants exposed to this metal.

P33 The arbuscular mycorrhizal transportome, what next!

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Understanding how arbuscular mycorrhizal (AM) symbioses establish and function is one of the most important current challenges in microbial ecology. Despite the fact that the AM symbiosis requires some complex and fine molecular tuning among symbionts in order to take place, both partners benefit from each other in a number of ways. For instance, the availability, uptake and exchange of nutrients in this biotrophic interaction are key factors driving plant growth and modulating biomass allocation. This underground trade is regulated by both plant and fungal transport components [1], as for instance the long distance transport of photosynthates from leaves towards colonized roots and the subsequent reciprocal transfer of nutrients from the soil to the plant through the fungal partner. More than a trophic role, it appears that nutrients themselves, acting as signals, function as major regulators of mycorrhizal associations [2]. The establishment and functioning of plant-fungal trophic interface as well as the local adaptation of fungal mycelium rely on complex and highly coordinated plant and fungal transcriptome regulations that are not well understood. Here, we focus in particular on key players involved in the nutritional exchanges between the mycorrhizal plant and the associated AM fungi. A better understanding of the underground trade will be useful for developing future engineering of new agro-ecological systems.

[1] Casieri et al. (2013) Mycorhiza, **23**, 597-625.
[2] Garcia et al. (2017) TIPS, **21**, 937-950.

P34 Zaxinone, a natural apocarotenoid, is involved in the establishment of the arbuscular mycorrhizal symbiosis

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Carotenoids are precursors of several hormones and signaling molecules in plants which are involved in the establishment and the maintenance of arbuscular mycorrhizal (AM) symbiosis. Recently, we have identified zaxinone, a product of the rice carotenoid cleavage dioxygenase (*Oszas*), as a novel natural metabolite and carotenoid-derived signaling molecule that exerts different developmental activities in plant, affects arbuscular mycorrhizal symbiosis, and suppress strigolactone (SL) biosynthesis (Wang et al. Nature Communications, *in press*). A loss-of function mutant (*zas*) contained less zaxinone, shows decreased root content of zaxinone, decreased root biomass, higher SL release and decreased mycorrhization level. To gain further insights on the role of zaxinone in mycorrhization, we generated *Oszas* over-expressing lines under the control of *35S*-promoter. These lines exhibited an increased mycorrhizal colonization level compared to WT. In order to understand whether zaxinone has a direct impact on the fungus during its pre-symbiotic phase, we tested the effect of this compound on spore germination of *Gigaspora margarita*, in comparison with the synthetic SL analog, GR24. To unravel the role of zaxinone during the colonization process, zaxinone content and the cross-talk with SLs has been evaluated in *oszas* e WT plants during a time course experiment. In their whole the results suggest that, zaxinone, a novel carotenoid compound, plays a crucial role in plant development and in the establishment of the AM symbiosis in rice plant.

P35 Characterization of copper transporters of the CTR family in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*

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Copper (Cu) is an indispensable and structural cofactor that drives a wide array of important biochemical processes essential for life; however, at elevated levels it becomes toxic. Due to this duality, Cu homeostasis is tightly controlled in all organisms. In eukaryotes, the entrance of Cu into the cells takes place through transporters of the CTR family [1]. This family has been widely studied in *Saccharomyces cerevisiae*; however, the mechanisms of Cu uptake in arbuscular mycorrhizal fungi (AMF) are unknown. The aim of this work was to get some insights into the mechanisms of Cu uptake in the AMF *R. irregularis* through the characterization of three CTR genes, *RiCTR1*, *RiCTR2* and *RiCTR3*, previously identified in its genome [2]. Interestingly, *RiCTR3* produces two types of transcripts as a consequence of an alternative splicing event. Functional analyses in yeast revealed that *RiCTR1* and *RiCTR2* encode functional plasma membrane and vacuolar membrane Cu transporters, respectively. However, none of the splicing variants of *RiCTR3* restored the mutant phenotypes of the yeast CTR deletion strains. Gene expression patterns suggest that RiCTR1 is the main transporter for Cu acquisition by the extraradical mycelium while CTR2 is involved in the mobilization of the vacuolar Cu stores. RiCTR3 was the most puzzling member of the RiCTR family, as it is up-regulated under Cu toxicity and reverts metal sensitivity of the *Δyap1* yeast strain. Our results also highlight the importance of Cu for arbuscule formation. More detailed results and the role of each family member will be presented and discussed.

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P36 Role(s) of fungal proteins involved in plant potassium nutrition during ectomycorrhizal symbiosis

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A major role of ectomycorrhizal symbiosis is the improvement of plant nutrition and water, due to a better exploration of the soil and due to an efficient absorption of poorly available nutrients in forest ecosystems. Physiological studies, recent genome sequencing projects and transcriptome analyses have allowed progress towards the identification and characterization of the fungal symbiotic transportome [1]. Potassium (K^+) is the most abundant cation in plant cells and is involved in various processes. We have shown improvement of K^+ nutrition by ectomycorrhizal symbiosis [2] under K^+ shortage conditions using the model couple *Pinus pinaster* and *Hebeloma cylindrosporum*. Questions are raised to identify the fungal transport systems involved in the uptake of nutrients from the soil and in their transfer towards the plant at the symbiotic fungus-plant interface, called Hartig net. On the fungal side, two types of K^+ transporters, Trk and HAK, are candidates to absorb K^+ from the soil, and two types of K^+ channels, Shaker-like and TOK, may release K^+ in the Hartig net [3]. In my PhD, I focus my research on the three TOK (Two-pore Outward K^+) channels of *H. cylindrosporum*, a family specific for fungi. These TOK channels belong to two subfamilies, which may imply different roles in potassium nutrition and in symbiosis. My aim is to complete the results obtained previously on their characterization and localization [4,5]. Thus, we can have a better understanding on the specific roles of these K^+ channels within the fungus and within the symbiosis.

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[2] Garcia et al. (2014) New Phytol., **201**, 951-960.
[3] Garcia et al. (2014) Front. Plant Sci., **5**, 337.
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[5] Guerrero-Galán et al. (2018) Plant Sign. Behav., **13**, e1480845.

P37* Glycerol-3 phosphate dehydrogenase *GPDH3* is involved in arbuscule branching in *Lotus japonicus*

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Arbuscular mycorrhizal (AM) fungi receive lipids from their host. Mutations in fatty acid synthesis-related genes, including the β-ketoacyl-acyl carrier protein synthase *DIS*, acyl carrier protein-thioesterase *FatM* and glycerol-3-phosphate acyltransferase *RAM2*, lead to the formation of stunted arbuscules [1–5]. *WRI5a–c*, homologous genes of the *Arabidopsis* AP2/EREBP transcription factors required for the regulation of fatty acid biosynthesis, are also involved in arbuscule formation [6]. In the present study, we analyzed the function of glycerol-3-phosphate dehydrogenases in AM symbiosis using a *lore1*-insertion line of *Lotus japonicus*, which has five glycerol-3 phosphate dehydrogenase (*GPDH*) genes in its genome. *GPDH3* was strongly induced by approximately 30-fold in AM roots compared with in non-mycorrhizal roots. The expression was exclusively observed in arbuscule-containing cortical cells and markedly suppressed in AM roots of the *WRI5* RNAi plants. When *GPDH3-GFP* fusion genes were agroinfiltrated into *Nicotiana benthamiana* leaves and expressed, fluorescent signals were observed in the cytoplasm. In the *gpdh3-1* mutant, the arbuscule density was significantly lower than in wild type, and stunted arbuscules were frequently observed. GPDH3 has several conserved amino acids that are required for GPDH activity, indicating that GPDH3 catalyzes the conversion of dihydroxyacetone phosphate into glycerol-3-phosphate using NADH. Thus, GPDH3 appears to be involved in arbuscule formation, possibly owing to the production of glycerol-3-phosphate, a substrate of RAM2, during AM fungal colonization.

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[2] Jiang et al. (2017) Science, **356**, 1172-1175.
[3] Keymer et al. (2017) eLife, e29107.
[4] Luginbuehl et al. (2017) Science, **356**, 1175-1178.
[5] Brands et al. (2018) Plant J., **95**, 219-232.
[6] Jiang et al. (2018) Mol. Plant, **11**, 1344-1359.

* *Selected flash talk presentations*

P38 pReNuK, a nuclear fluorescence protein reporter for promoter studies in ectomycorrhizal basidiomycetes

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At the moment, there are no fluorescence protein (FP) reporter vectors available for promoter studies in ectomycorrhizal basidiomycetes. With this mean we have developed pReNuK (= Red Nucleus with consensus Kozak), a vector for easy cloning of promoter sequences of interest, whose activity can be studied via nuclear localizing mCherry-*Laccaria bicolor* histone H2B fusion protein *in vivo*. pReNuK contains a flexible multiple cloning site for promoter introduction and a FP-H2B ORF with optimized Kozak sequence for efficient marker translation. pReNuK was designed to be compatible with hygromycin B resistance cassette bearing *Agrobacterium* binary vector pHg [1] and therefore promoter-FP-H2B constructs can be introduced via ATMT, or alternatively via other plasmid DNA based transformation methods functional in the fungus under study. The pReNuK was initially tested using the constitutive *gpd* gene promoter of *L. bicolor* in the dikaryotic strain S238N of *Laccaria*. The functionality of the FP-marker vector was further confirmed with *Laccaria* nitrate reductase (Nr) promoter sequence, which is transcriptionally regulated by nitrogen sources [2]. As expected, growth under no-inducing conditions resulted in low level signals in the pReNuK/NrP transformants, while under inducing conditions very potent nuclear fluorescence signals could be observed. The stability of the FP-reporter was demonstrated by replicating a set of pReNuK /gpdP transformants without selection antibiotic pressure for two months. This cultivation did not result in loss of fluorescence. The pHg/pReNuK vector system presented here offers a novel molecular tool for *Laccaria* studies and we expect it to be functional in other basidiomycete species as well.

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[2] Kemppainen et al. (2010) Environmental Microbiology Reports 2(4), 541–553.

P39 Potassium nutrition in rice under salt stress: role of arbuscular mycorrhizal symbiosis?

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Arbuscular mycorrhizal fungi (AMF) establish a symbiotic association with the roots of 80% of terrestrial plants and form complex tree-shaped feeding structures called arbuscules in colonized root cells. This association with AMF not only provides more efficient uptake of nutrients for the plant, but also confers protection against pathogens and increased tolerance to environmental stress such as salt stress [1]. Rice (*Oryza sativa*), the most salt-sensitive crop species amongst cereals, has a productivity strongly reduced around the world due to soil salinity/salinization and increased sea level (in deltas). High Na^+ concentrations (salt stress conditions) impairs K^+ uptake and inhibits many K^+-activated enzymes. Rice exhibits molecular mechanisms to alleviate salt stress such as maintaining a high cellular K^+/Na^+ ratio, e.g. by a more efficient K^+ uptake, which was recently shown to occur upon root/AMF interaction [2]. Knowledge on the role of root/AMF interaction on K^+/Na^+ transport upon salt stress is sparse [2, 3]. The aim of the research is to understand the mechanisms by which AMF improve plant K^+ nutrition upon salt stress, taking *Rhizophagus irregularis* (model AMF)-rice interaction as a model. We will investigate the mechanisms by which AMF mediate K^+ uptake and translocation towards root cells. Rice loss-of-function mutants for each of the three major K^+ uptake systems of the plant, OsAKT1, OsHAK5 and OsHAK1, were inoculated with *R. irregularis* and submitted to salt stress, and their K^+ contents and K^+/Na^+ ratios were monitored to identify key player(s) for AMF-mediated improved rice K^+ nutrition and salt tolerance.

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P40 Independent of arbuscular mycorrhizal symbiosis: Positional cloning and characterisation of a novel arbuscular mycorrhizal mutant in *Zea mays*

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Arbuscular mycorrhizal fungi from the Glomeromycota phylum form a beneficial symbiotic relationship with the root systems of a majority of plant species, enabling plants to profit from increased phosphate uptake efficiency. Further understanding of this interaction may prove vital in meeting the challenge of an increasing demand for phosphate supply in agriculture. A Zea mays mutant identified in a forward genetics screen, independent of arbuscular mycorrhizal symbiosis (ina) displays an inability to provide a signal to the arbuscular mycorrhizal fungus, Rhizophagus irregularis, that is required for fungal colonisation of plant roots. This project utilised positional cloning to narrow down the genomic region containing the causal mutant allele behind this phenotype to a 170kb region containing 10 genes. A candidate gene approach will further aim to identify the ina allele. The causal INA gene will be functionally characterised, and its role in signalling to the fungus examined.

P41* Slicing ectomycorrhizas: what can we learn from dissection?

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Within a single infected fine root, different developmental stages of an ectomycorrhiza are expected to coexist. Next to the meristem and the elongation zone without fungal root penetration, a developing zone with ongoing hyphal ingrowth into the rhizodermis is followed by a region with fully developed Hartig net. Regions of older, fully developed structures with former fully functional Hartig net are following.
To get insight in the process of ectomycorrhizal formation and maturation, functional poplar (*P. tremula x tremuloides*) / fly agaric (*Amanita muscaria*) mycorrhizas were isolated from a Petri dish system. After snap freezing, mycorrhizas were dissected piece-by-piece from the tip to the basis about 2 mm wise. For each mycorrhizal section, total RNA was isolated from four individual pools, followed by linear mRNA amplification. Selected marker genes were used to prove mRNA amplification. Out of the first three mycorrhizal sections (meristem + elongation zone, section of developing Hartig net, section containing fully developed Hartig net), three mRNA pools each were hybridized to micro-arrays (Nimble gene, chip version II) containing 60 bp long oligos covering about 56.000 poplar (*P. trichocarpa*) genes. Surprisingly, hardly any difference in gene expression was observed between slices containing developing and fully developed Hartig net, while large differences in transcript levels were observed between the root tip region and any of the following sections. Details of the genome-wide expression analysis are given.

* *Selected flash talk presentations*

P42 The effect on arbuscular mycorrhizal symbiosis of a natural occurring *SYMRK* mutant allele in a Borneo *Trema orientalis* population

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The *Trema* genus represents a group of tropical tree species within the Cannabaceae. Interestingly, five species nested in this lineage known as *Parasponia* can establish rhizobium nitrogen-fixing symbiosis, similar to legumes. *Parasponia* and legumes use a conserved genetic network to control root nodule formation, among which are genes also essential for mycorrhizal symbiosis. However, *Trema* species lost several genes that function in nodulation, suggesting a loss-of the nodulation trait. Strikingly, in a *Trema orientalis* population found in Malaysian Borneo we identified a truncated *SYMBIOSIS RECEPTOR KINASE* (*SYMRK*) mutant allele lacking a portion of the c-terminal kinase domain. In legumes this gene is essential for nodulation and mycorrhization. This raises the question whether *Trema orientalis* can still be mycorrhized. We established quantitative mycorrhization assay for *Parasponia andersonii* and *Trema orientalis*. Plants were grown in closed pots on half strength Hoagland containing 20 µM potassium phosphate in sterilized sand and inoculated with 125 spores of *Rhizopagus irregularis*. Mycorrhization efficiency was determined by analysing the frequency of mycorrhiza (%F), the intensity of the mycorrhizal colonization (%M) and the arbuscule abundance (%A) in the root system. *Trema orientalis* RG33 can be mycorrhized, though with lower efficiency compared to *Parasponia andersonii*. From this we conclude that a functional SYMRK kinase domain is not essential for *Trema orientalis* mycorrhization. In ongoing experiments, we aim to investigate the role of SYMRK in *Parasponia andersonii* mycorrhizaton and nodulation. Two *Parasponia andersonii symrk* CRISPR-Cas9 mutant alleles were created; mimicking the *TorSYMRK*^{RG33} allele and a full *Parasponia andersonii SYMRK* knockout.

P43 Cellular mechanisms of symbiotic phosphate uptake in rice

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At the heart arbuscular mycorrhizal symbiosis are highly branched ephemeral fungal arbuscules that form deep within the root and where host-derived carbon is delivered in exchange for inorganic phosphate (Pi) from the fungus. In the cereal crop rice up to 70% of total Pi is acquired through the mycorrhizal-induced PHOSPHATE TRANSPORTER 11 (OsPT11)[1]. OsPT11 localizes to a host-derived peri-arbuscular membrane (PAM) that envelopes the arbuscule[2], providing a large surface area for symbiotic Pi uptake. Here using deep tissue time lapse live cell imaging to study PAM dynamics, we show OsPT11-GFP localizes to thimble-like foci that coincide with arbuscule fine branching, suggesting arbuscule biogenesis and Pi uptake are coordinated. Moreover, we show that auto-fluorescent vacuolar granules (AFG) accumulate in cereals during AM symbiosis. Transmission electron microscopy showed AFG resemble electron dense acidocalcisomes, poly-P storage organelles that are required for maintaining cellular Pi homeostasis[3]. In conclusion, this study suggests that the Pi uptake dynamics and arbuscule biogenesis are coordinated processes and the potential fate and homeostasis of symbiotic Pi in cereals may resemble a mechanism that is conserved from bacteria to man.

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2. Yang, et al. (2012), The Plant Cell, 24, 4236-4251
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P44 Sub-cellular protein localization in poplar leaves

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We are interested in ectomycorrhiza (ECM) based adaptation of root physiology using poplar as model organism. For a better understanding of cellular function, the sub-cellular localization of protein is important. For this purpose, heterologous expression in tobacco leaves is commonly used, where leaf cells are transformed with constructs expressing *in frame* fusions of the gene of interest and a fluorescent marker. Protein location is than determined by *in vivo* detection of the fluorescence marker by using confocal laser scanning microscopy.
Nicotiana benthamiana belongs, however, to the asteridclade and is thus phylogenetically only distantly related to poplar that belongs to the rosids. We have thus developed a transient expression system for sub-cellular protein localization in poplar leaves. Transient expression has the big advantage over stable transformation that results are generated within a few days. Transgenic poplar leaf cells were obtained after 2-4 days by a combination of either silicium carbide or sonification treatment followed by *Agrobacterium*-mediated transformation. The transformation efficiency showed great differences depending on the treatment, which will be discussed in detail. Nevertheless, by using our approach, we were able to target selected fluorescence markers to the nucleus, peroxisomes and the plasmamembrane. In contrast to tobacco, poplar leaf cells harboring *Agrobacteria* showed a stronger autofluorescence, which was not observed in non-transgenic leaves. For optimal background to signal ratios the expression level of the fluorescent fusion constructs is thus of high importance. While weekly expressed fusion constructs were hardly visible, strong overexpression led to localization artefacts.

P45 Deciphering ectomycorrhiza ontogenesis: from cell wall remodeling towards reciprocal control of both partners.

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Ectomycorrhiza (ECM) are mutualistic interactions occurring between the roots of about 6,000 tree species and hyphae of soil-born fungi of the Basidiomycota and Ascomycota phyla. These interactions are pivotal for nutrient cycling in forest ecosystems and for optimal growth of trees. However, not much is known about the molecular and physiological mechanisms underpinning the establishment of such interactions. Comparative genomics highlight convergent evolution of ECM fungal genomes with reduced numbers of genes encoding plant cell-wall degrading enzymes (PCWDEs) compared and lineage-specific suites of mycorrhiza-induced small-secreted proteins (MiSSPs) (1). In addition to phytohormones production, SSP-mediated communication is also occurring from plants to fungal cells. (2)
This poster will present how the functional study of such proteins, using *Laccaria bicolor-Populus* interaction as a model, can help us deciphering the molecular mechanisms driving the development of mycorrhizal symbioses as well as deciphering tree physiology.

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(2) Plett et al., 2017. Scientific Reports 7. 382

P46 Evolution of the symbiotic gene *RAM2* in land plants

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Arbuscular Mycorrhiza (AM) symbiosis is an association between most embryophytes and soil fungi. It has been proposed as a key event that allowed the colonization of land by plants. The fungus provides water and nutrient to the host plants in exchange for carbon, mainly sugar and lipids. Recent studies provided a deeper understanding of the molecular mechanisms behind AM establishment and exchanges in angiosperm. A symbiotic lipid-biosynthesis pathway expressed in cells colonized by AM fungi have been deciphered, including the Glycerol-3-Phosphate Acyl-Transferase RAM2. However, genetic studies have been so far limited to a few angiosperm species. Here, we used the recently developed model liverwort *Marchantia paleacea* to test whether the mechanisms that allow the transfer of lipid from the plant to the fungus have a deep origin in plants or evolved more recently in Angiosperms. First we conducted a phylogenetic approach to reconstruct the evolutionary history of the genes involved in the production and transfer of lipids to the fungi. This revealed an origin of this pathway in the oldest common ancestor of land plants. Promoter GUS analysis of one of them, RAM2, indicated its transcriptomic induction in arbusculated cells in *Marchantia paleacea.* Trans-complementation assays conducted in *Medicago ram2* using *Marchantia paleacea and fern RAM2* orthologs combined with biochemistry analysis allowed us to demonstrate the conservation of RAM2's biochemical function across land plants. Currently, mutant lines of Marchantia *RAM2* orthologs are being analyzed. Deciphering *Marchantia RAM2* function will allow to determine if the transfer of lipids from host plants to AM fungi is an ancestral trait that participated in the conservation of this symbiosis in embryophytes.

P47* A nucleolar-targeted effector of *Rhizophagus irregularis* potentially affecting host translation

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Recently it has become clear that arbuscular mycorrhizal (AM) fungi secrete an array of putative effector proteins that may be essential to establish a symbiotic relationship with a large range of host plants. Several of these effectors are predicted to translocate to the host nucleus. Here we studied the role of one of such nuclear-targeted AM effector protein, called NLE1 secreted by *Rhizophagus iregularis*. NLE1 is specifically expressed in arbuscules, and its ectopic overexpression leads to a higher fungal colonization level. Expression of GFP-tagged NLE1 in arbusucle-containing cells showed that it specifically localizes to the nucleolus as well as to nuclear speckles. The nucleolus plays important roles in ribosome subunit biogenesis, mediation of cell-stress responses and regulation of cell growth. To study the putative function or NLE1, we searched for putative interacting plant proteins via co-immunoprecipitation coupled with mass-spectroscopy analyses. This identified several putative interactors, including histone 2B and Mediator subunit 36a (MED36a). MED36a is a 2'-O-methyltransferase, also called Fibrillarin, that localizes to the nucleolus where it regulates methylation and processing of rRNA's in addition to a role in mRNA splicing. Preliminary results suggest that overexpression of NLE1 can suppress the methylation level of rRNA. Future experiments aim to test whether NLE1 through its effect on rRNA methylation may affect translation efficiency or specificity in arbuscule containing cells.

* *Selected flash talk presentations*

P48 AP2 transcription factor *CBX1* with a specific function in symbiotic exchange of nutrients in mycorrhizal *Lotus japonicus*

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The arbuscular mycorrhizal (AM) symbiosis depends on reciprocal exchange of phosphorus driven by proton-coupled phosphate uptake into host plants and carbon supplied to AM fungi by host-dependent sugar and lipid biosynthesis. The molecular mechanisms and cis-regulatory modules underlying the control of phosphate uptake and de novo fatty acid synthesis in AM symbiosis are poorly understood. A set of 43 mycorrhiza-regulated transcription factors was screened for binding the CTTC motif. AP2 family transcription factor CTTC MOTIF-BINDING TRANSCRIPTION FACTOR1 (CBX1), a WRINKLED1 (WRI1) homolog, directly binds the evolutionary conserved CTTC motif that is enriched in mycorrhiza-regulated genes and activates *Lotus japonicus* phosphate transporter 4 (*LjPT4*) *in vivo* and *in vitro*. Moreover, the mycorrhiza-inducible gene encoding H^+-ATPase (LjHA1), implicated in energizing nutrient uptake at the symbiotic interface across the periarbuscular membrane, is coregulated with *LjPT4* by CBX1. Accordingly, CBX1-defective mutants show reduced mycorrhizal colonization. Furthermore, genome-wide–binding profiles, DNA-binding studies, and heterologous expression reveal additional binding of CBX1 to AW box, the consensus DNA-binding motif for WRI1, that is enriched in promoters of glycolysis and fatty acid biosynthesis genes. We show that CBX1 activates expression of lipid metabolic genes including glycerol-3-phosphate acyltransferase RAM2 implicated in acylglycerol biosynthesis. Our finding defines the role of CBX1 as a regulator of host genes involved in phosphate uptake and lipid synthesis through binding to the CTTC/AW molecular module, and supports a model underlying bidirectional exchange of phosphorus and carbon, a fundamental trait in the mutualistic AM symbiosis.

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Symbiosis functioning

P49 Carbon to phosphorus exchange rate in relation to the adaptability of single spore line in dual host systems

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The majority of land plants establish mutualistic symbioses with arbuscular mycorrhizal (AM) fungi, that benefit both partners by mediating uptake of essential plant nutrients fueled by host's photosynthates. AM fungi develop extraradical hyphal networks that link different plants. The ability of the same fungal mycelium can colonize different plants at the same time involves an extraordinary level of compatibility and ability to adapt to different hosts by the fungus, though the adaptation to a single host might increase fitness of fungi at the expense of the other host. Our question is how plant and fungal fitness are linked and the genetics behind that. It has been suggested that AM fungi are heterokaryotic, contain genetically distinct haploid nuclei, and in response to a novel plant, the developing fungal hyphae could temporarily segregate nucleotypes in the emerging spores to produce adapted offsprings that have different phenotypes than parents. To study the symbiotic performance of a host-adapted fungus, we will apply ^{33}P isotopes to mesocosms containing one native and one novel plant (C_3 and C_4) that are linked by fungal hyphal network (pure spore lines) to investigate carbon for phosphorus exchange rate through several generations. We analyzed the genomic basis for the initial spores using single cell genomics technique. Plant and fungal fitness, root colonization rate will be estimated. If one host-adapted generation will grow better at the expense of plant growth on the other host, spores generated at harvest will be used as inoculum for the following generation of the novel host.

P50 Biotechnological potential of Arbuscular Mycorrhizal Fungi associated to soybean plants submitted to water deficit period

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The objective of this work was to verify the biotechnological potential of Arbuscular Mycorrhizal Fungi of the genus *Scutellospora* spp., in the physiological response of soybean plants submitted to a water deficit period. Soybean seeds (ANTA82 cultivate) were germinated in pots and grown in a greenhouse, the mycorrhizal inoculant consisted of the fungi *Scutellospora gregaria* and *Scutellospora heterograma*. The plants were inoculated at the seeding orifice with 5 g of inoculum, alone or in mixture, and cultivated until the third trefoil was expanded. Then, the water deficit was applied, where irrigated vessels were maintained with 100% of the field capacity (CC), and in those submitted to the water deficit 60% of the volume of water lost was added every day for 10 days. Afterwards, the plants were rehydrated for seven days and evaluated. The photosynthesis analysis was performed using an infrared gas concentration determination system (IRGA-LI-COR-6800). The experimental design was completely randomized, in a 2x4 factorial arrangement (plants under water deficit and irrigated x control, *Scutellospora gregaria*, *Scutellospora heterograma* and *S. gregaria* + *S. heterograma*). Soybean plants submitted to period of water deficit, and reirrigadas when associated with FMA *Scutellospora heterograma* had higher photosynthetic rate (13.14 μmol CO_2 m^{-2} s^{-1}). Meanwhile, plants without inoculation obtained lower photosynthesis (7.05 μmol $CO2$ m^{-2} s^{-1}). Therefore, it is concluded that the FMA *Scutellospora heterograma* provides increased photosynthetic capacity of soybean plants after a period of water deficit, followed by reirrigation, and drought tolerance, demonstrating the biotechnological potential of this fungus.

P51 Ramf: an R package for analyzing root colonization by arbuscular mycorrhizal fungi

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Ramf is a R package for arbuscular mycorrhizal fungi (AMF) colonization analysis. It can be used with different colonization scoring systems (grid or Trouvelot system), it can save the raw and processed data, make statistical analysis and produce plots with advance annotations. Ramf is a extension of R, one of the best data analysis languages available, completely free, very easy to install and to run. As quite some time is needed to master R we have designed the package for AM researchers who are not familiar with it. It is designed to contain few, well documented functions for easy use.

Almost all AM papers display colonization data in different flavors, with different types of statistical analysis and summary figures. Ramf package aims to introduce a robust statistical platform for consistent data analysis and display.

The Ramf package will be available as open source on a public repository (GitHub). Everyone will be able to open issues, propose and implement new features and ask to incorporate them in the next release of the package.

P52 Polyphosphate localization in *Rhizophagus irregularis* colonizing in a H+-ATPase ha1 mutant of *Lotus japonicus*

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Arbuscular mycorrhizal (AM) associations promote plant P acquisition. Arbuscule-containing cortical cells are sites of nutrient exchange between AM fungus and the host by specialized sets of proteins embedded in host- and fungus-derived membrane. The plant H^+-ATPase *HA1* plays a crucial role in promoting secondary active transport through phosphate transporters by generating proton gradients across periarbuscular membrane [1,2]. In order to investigate AM fungal P metabolism in the mycorrhizal pathway, polyphosphate (polyP) localization in *Rhizophagus irregularis* colonizing *HA1* insertion mutants of *Lotus japonicus* was analyzed. Plants were grown in the two-compartment system that is composed of the root-hyphal and hyphal compartments. The hyphal compartment was supplied with high concentration of phosphate to enhance P translocation via the mycorrhizal pathway. In *ha1-1* mutants, mycorrhizal effects on P uptake were reduced compared to those in wild type (WT). The density of arbuscules in *Ljha1-1* mutant was slight reduced, and the arbuscule size was smaller than that in WT. DAPI staining of polyP showed that polyP was localized in the central region (trunk hypha) of arbuscules but not in fine branches in WT roots. In contrast, fine branches were well stained with DAPI in *ha1-1* mutant. According to immunostaining, polyP signals were observed in cell walls and vacuoles of trunk hyphae colonizing in WT roots. This distribution was contrast to the localization of acid phosphatase activity that was detected in periarbsucular space around fine branches but not trunk hypha in WT roots. We discuss P transfer mechanism based on polyP localization in AM fungi.

[1] Wang et al. (2014) Plant Cell, **26**, 1818-1830.
[2] Krajinski et al. (2014) Plant Cell, **26**, 1808-1817.

P53 Carbon double dependency of AMF in the light of transcriptomic analysis

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AM fungi are obligate symbionts that depend on their host plants for carbon sources. It was estimated that up to 20% of the photosynthetically assimilated C can be allocated by plants to their symbionts [1]. Until recently, the paradigm of trophic exchanges was that the plants provide hexoses to AM fungi in exchange for phosphorus delivered by the fungus. Sugar catabolism was then supposed to fuel the biosynthesis of all fungal carbon components, particularly fatty acids that are abundant in these oleic fungi. The survey of genomic data from *Rhizophagus irregularis* [2] highlighted the absence of Fatty Acid Synthase [3] – FAS, the enzyme complex involved in palmitic acid biosynthesis- thus shifting the paradigm on plant host sugar dependency of AM fungi. Previous and recent publications support that host plants overproduce and transfer acylglycerols to AM fungi[4]. Mirroring this overproduction, we addressed the questions of acquisition/assimilation by AM fungi of fatty acids alongside sugars and probably glycerol. Reciprocal exchanges between symbionts are highly important for the establishment and maintenance of AM symbiosis [5], [6]. To investigate the regulation of carbon exchanges, different biological conditions were produced to investigate global transcriptome variations. The double dependency of AM fungi to sugars and fatty acids opens fascinating questions: how is regulated AM fungal metabolism for these different carbon sources?

[1] Jakobsen et al., *New Phytol.*, **115**, 77–83, 1990.
[2] Tisserant et al., *Proc. Natl. Acad. Sci.*, **110**, 20117–20122, 2013.
[3] Wewer et al., *Plant J.*, **79**, 398–412, 2014.
[4] Rich et al., *Trends Plant Sci.*, **22**, 652–660, 2017.
[5] Helber, et al., *Plant Cell*, **23**, 3812–3823, 2011.
[6] Lahmidi *et al.*, *Plant Physiol. Biochem.*, **107**, 354–363, 2016

P54 Transcriptomic comparison of *Medicago truncatula* during symbiosis

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Plants have evolved to form alliances with microorganisms in the rhizosphere to acquire nutrients that limit their growth. The two most well studied symbionts are arbuscular mycorrhizal fungi and rhizobial bacteria. The arbuscular mycorrhizal symbiosis appeared in the very early stages of plant evolution -about 450 *mya*- while evidence at the rhizobial symbiosis evolving from arbuscular mycorrhizal associations between 60-80 *mya*. The two share many biological aspects in common: capturing nutrients and trading them for carbon with the plant, secreting specific molecules to be recognized, and entering the plant root to directly associate with the plant cells. However there are also key differences: the formation of nodules that form to harbor rhizobia, which are absent in arbuscular mycorrhizal associations. To further understand the differences and similarities, we have collected the transcriptomes during the processes of these two symbioses in *Medicago truncatula*. We have identified the expression levels of genes with RNA-seq for 3 time points after *Rhizophagus irregularis* infection and 15 time points after *Sinorhizobium meliloti* infection. By applying unsupervised fuzzy c-means clustering to the transcriptomes, we were able to unbiasedly classify the gene profiles during symbiosis. Looking more closely into these clusters, we can distinguish both commonalities and differences between these two associations.

P55 Influence of different substrates and arbuscular mycorrhizal fungi on the production of clove lemon saplings

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The main method of propagation of tree species is via transplanting of seedlings, where one of their main challenges is to produce seedlings in quantity and quality, having the ideal substrate as one of their main limiting factors [1]. These seedlings can be directed to the production of rootstocks, a very popular practice in plant propagation [2,3]. The use of agroindustrial residues as raw material for substrate production is an excellent ecological and economical alternative and arbuscular mycorrhizal fungi can increase the efficiency of native seedling establishment [4]. This work aims to evaluate different organic substrates and influence of arbuscular mycorrhizal fungi on seed germination and establishment of rubber tree seedlings. The experimental design was completely randomized in a 7x2 factorial scheme with 8 replicates, where the first factor was constituted by eight substrates and 1: 1 mixtures: Bioplant® commercial substrate; Sugarcane filter cake, Filter Pie with Siderurgica Slag, Filter Pie with sawdust, Sugarcane Bagasse with steel slag; Sugarcane Bagasse and Filter Cake; Slag of Siderurgica with sawdust; The second factor was the treatments: sterile soil with inoculation of spores of mycorrhizal fungi and sterile soil without inoculation of spores. To evaluate the influence of the different substrates and the mycorrhizal fungi on the establishment of seedlings were used seeds of clove lemon. Plant growth analysis was performed by plant height (cm) and stem diameter at 1 cm soil (mm), and the relationship between height. For all the parameters, the treatments with filter cake and the mixture of filter cake with steel slag presented the highest values. The mixture of steel slag and filter cake showed to be a compound with excellent potential, with vegetative development compared to the commercial substrate.

[1] Martins ALM et al. (2017)
[2] Antonio V et al. (2009) Eng Florest.14(3):8–17.
[3] Diniz PFDA (2007)
[4] Filho MT et al. (1980) IEPF;21:21–37.

P56 Glomalin gene as molecular marker for functional diversity of arbuscular mycorrhizal fungi in soil

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Among the ecological services provided by arbuscular mycorrhizal fungi (AMF), the process of soil aggregation is considered to be partially mediated by glomalin, a glycoprotein released by AM fungi into soil during hyphal turnover and after the death of the fungus in the soil [1]. The protein is characterized by abundant production and hydrophobic properties. Although glomalin has been identified in *Rhizophagus irregularis* DAOM197198 [2,3] as a putative homolog of heat shock protein 60, the use of expressed fungal genes encoding glomalin as a marker for functional AMF diversity was never exploited. The present work describes the first attempt to identify the glomalin gene in several AMF species, verify its reliability as gene marker for the identification and discrimination of AMF, and test the possibility to detect its expression in soil. Our goal were a) to design a set of primers set able to amplify many known lineages of AMF glomalin gene, b) to create a new reference glomalin sequence dataset for comparative sequence analyses, including also glomalin gene sequences from transcriptomes and genomes published online, c) to detect with a pilot experiment the expression of the gene in soil. The glomalin reference dataset and its future development will provide the basis to design further primer sets to employ in functional characterization of AMF communities.

[1] Wright et al. (1996) Plant Soil **181**, 193–203.
[2] Gadkar and Rillig (2006) FEMS Microbiol. Lett. **263**, 93–101.
[3] Hammer and Rillig (2011) PLoS ONE **6**, e28426.

P57 Forest fires and mycorrhizal colonization rate in soils under cerrado vegetation

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The main tool used to expand to new areas in the Cerrado is fire. With triggered fires, and in many cases, criminal, new areas are intended for agricultural production [1]. Uncontrolled fire causes loss of nutrients, soil compaction and erosion, degradation of the native biota. All this is more pronounced in the dry season of the year, where the low humidity amplifies the damages of an eventual burn. The objective of this work was to evaluate the influence of the burnings in the Chapada dos Veadeiros National Park on arbuscular mycorrhizal fungi in Cerrado soil. The work area belongs to the Chapada dos Veadeiros National Park, which suffered the largest fire in its history on October 10, 2017. The area affected was 66,000 hectares. Analyzes of spore density and mycorrhizal colonization rate were performed. Plants in burned areas have lower rates of mycorrhizal colonization than preserved areas. The mycorrhizal colonization was normalized in cerrado area after six months of the control of the flames, where the values of colonization approached the preserved areas. When comparing all types of cerrado in the areas under fire influence, it was possible to verify that Campo Limpo, Campo Sujo and Strictu Sensu present higher values of colonization rate, followed by Veredas, with Cerradão with the lowest values. The Cerrado of Campo Sujo, Strictu Sensu, and Veredas presented higher colonization rates than Cerradão and Campo Limpo. When checking the values of mycorrhizal colonization in the preserved and burned areas, all types presented higher colonization rates in the areas without burning than in the areas that suffered fire, except for the clean field type cerrado, which did not present statistical difference among the areas investigated

[1] Danilo Muniz da Silva, et al. (2001) Os Efeitos dos Regimes de Fogo sobre a Vegetação de Cerrado no Parque Nacional das Emas, GO: Considerações para a Conservação da Diversidade. Biodiversidade Bras. 1(2):26–39.

[2] Ribeiro G de FOD. (2017) Como queimadas em diferentes épocas do ano afetam a relação entre gramíneas invasoras e a vegetação nativa de cerrado? [Internet] [PhD Thesis]. Universidade Estadual Paulista (UNESP)

P58 Evolution of the Arbuscular Mycorrhiza Symbiosis

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Most land plant establish a mutualist symbiosis with fungi from the Glomeromycota phylum. The ability to interact and exchange nutrient with a fungal symbiont is found in most extant land plant families and fossil records show the presence of symbiotic structures in plants dating more than 400 million years. This suggests that the last common ancestor of the Embryophyta shared this trait and we can hypothesize that this innovation was a decisive step in the colonization of land.

Using comparative phylogenomics on genomes of plant with or without the ability to mycorrhize, we are able to 1- correlate loss of trait with loss of gene and 2- investigate the origin of those genes. In combination with classical genetics, transcomplementation assays and biochemistry in key plant models such as *Marchantia paleacea*, *Marchantia polymorpha* and *Medicago truncatula*, we can define a core set of genes committed to mycorrhiza through the plant kingdom and hypothesize the mechanisms underlying the ancient co-option of those genes for the establishment of AMS.

P59 Forest fires and glomalin content in soils under cerrado vegetation

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The National Park of Chapada dos Veadeiros, created in 1961, comprises an area of 240,614 ha and is situated in the Brazilian savannah called cerrado [1]. The Brazilian Cerrado has one of the largest biodiversity on the planet, because it is a transitional biome that is in direct contact with other important biomes such as Amazon, Caatinga, Mata Atlântica and Pantanal. It is currently the main frontier of agricultural expansion in Brazil, and it has been the target of several criminal activities, such as fire, with the purpose of deforesting native areas to increase the productive area [2]. The main tool used to expand to new areas in the Cerrado is fire. With burnings provoked, and in many cases, criminal, new areas are intended for agricultural production. Uncontrolled fire causes loss of nutrients, soil compaction and erosion, degradation of the native biota. All this is more pronounced in the dry season of the year, where the low humidity amplifies the damages of a possible burn. The objective of this work was to extract and quantify glomalin-related soil protein (PSRG) in the cerrado biome, to evaluate the interference of fires in the amount of glomalin easily extractable (GFE), and to correlate the amount of glomalin present in the soil as a function of the density of arbuscular mycorrhizal fungi found in the rhizosphere. The presence of fire directly interfered with the amount of glomalin-related soil protein, and higher amounts were found after fire action. The density of mycorrhizal fungi increased after the incidence of fire. It is observed that the higher the density of arbuscular mycorrhizal fungi in rhizosphere of cerrado plants, the higher the GFE rate. Glomalin responds according to the density and conditions of the environment. Areas of Stricto Sensu, Cerradão and Veredas have a greater amount of GFE in areas without the presence of fires. GFE tends to increase as the time passes after the fire, as well as the density of mycorrhizal fungi.

[1]. ICMBio. (2018) ICMBio - Parque Nacional da Chapada dos Veadeiros - PARNA Veadeiros.
[2]. Klink C a., Machado RB. (2005) A conservação do Cerrado brasileiro. Megadiversidade. 1(1):147–155.

P6o* Arbuscular mycorrhizal fungi and rhizobia interactions during the colonization of the same legume root

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Legume plants establish symbioses with both arbuscular mycorrhizal fungi (AMF) and rhizobia. Such symbiotic interactions have been extensively studied over the past years, however, most of these studies are pairwise approaches. In nature, many simultaneous microbial interactions are usually taking place at the same time on the same host. Simulating the natural conditions, where rhizobia and AMF co-exist in soils, we examine the effects of AMF and rhizobium co-inoculations on the establishment of the different symbioses and the general plant performance. Using the well-studied model legume *Lotus japonicus*, we test the implication of the microsymbiont *Mesorhizobium loti* on the colonization of the root by different AMFs, like *Claroideoglomus lamellosum* and *Funneliformis mosseae*, and *vice versa*. According to our results, rhizobia and AMFs do affect one another in different ways. The effect of rhizobium on AMF root colonization was found to differ between AMF strains. We have identified AMF species presenting either auxillary, synergistic or antagonistic to rhizobium effects. On the other hand, all different AMF strains tested were found to have a positive effect in the formation of root nodules, the plant organs that harbor the nitrogen-fixing rhizobia. Interestingly, the co-inoculation of plants with rhizobia and AMF significantly increased the number of nodules formed under heat stress conditions. This study aims to enhance our understanding on how and when the plant chooses and combines its symbionts in order to make a more efficient use of beneficial microbes in crop production.

* *Selected flash talk presentations*

P61 The expression of *Rhizophagus irregularis RiPEIP1* gene in rice promotes plant growth and mycorrhizal colonization

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The *Rhizophagus irregularis RiPEIP1* (*Preferentially Expressed In Planta*) is a fungal gene strongly expressed in the intraradical phase, including arbuscules, in different host plants. When expressed as a GFP fusion in yeast cells, RiPEIP1 localizes in the endomembrane system, in particular to the endoplasmic reticulum, which is consistent with the *in silico* prediction of four transmembrane domains. In the absence of genetic transformation protocols for arbuscular mycorrhizal (AM) fungi, we exploited two different heterologous expression systems. Previous results showed that *RiPEIP1* expression in *Oidiodendron maius*, an ericoid endomycorrhizal fungus, led to enhanced colonization capacity compared to the *O. maius* wild type strain [1]. To gain further insights on the biological role of *RiPEIP1* we also generated rice *RiPEIP1*-expressing lines under the constitutive 35S promoter. A preliminary analysis showed that these transgenic lines have a higher number of crown roots, an increased shoot length, a higher root and shoot biomass, an early flowering phenotype, an increased number of panicles and seeds and a higher AM colonization level compared to wild type plants.
Although the mechanism of action of *RiPEIP1* is still unknown, these findings suggest that *RiPEIP1* expression affects different host metabolic pathways which promote plant growth and mycorrhizal colonization.

[1] Fiorilli et al. (2016) Mycorrhiza, **26**, 609-621.

P62 Growth and ectomycorrhizal development of *Cistus salvifolius* and *Cistus albidus* seedlings inoculated with desert truffles species

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The genus *Terfezia* is considered as one of the most popular taxon in the Ascomycetous desert truffles group. These fungi produce edible hypogeous ascomata growing mostly in arid and semi-arid habitats. They establish mycorrhizal symbioses with specific host plants, belonging mainly to the *Cistaceae* family. In the present study, two Cistaceae species, *Cistus salvifolius* and *Cistus albidus* were inoculated with two mycorrhizal desert truffles, *Terfezia leptoderma* and *Terfezia boudieri* under greenhouse conditions, on soil originating from desert truffle natural habitat in Algeria. The two desert truffles species completely colonized roots of *C. salvifolius* and *C. albidus* seedlings during 7 and 5.5 months growth period respectively, formed an ectomycorrhiza with a thin, less developed sheath on more than 80 percent of short roots and stimulated at least twice as much dry matter production compared to controls seedlings. The formation of a sheathing ectomycorrhiza in *C. albidus* seedlings inoculated with *T. boudieri in in vivo* culture conditions was obtained for the first time. These preliminary results open up the possibility of cultivating some desert truffles species with *Cistus* plants in Algeria.

Multiple interactions

P63 Microbiota dynamics in alfalfa roots after inoculum application: a perspective for sustainable farming

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The interaction with soil beneficial microbes, including the symbiotic ones, positively influences plant's health and productivity, both in wild and cultivated conditions. In the last years, the interest has risen towards the use of plant beneficial microorganisms as natural fertilizers for in field application. As a prerequisite for this approach to be sustainable, the impact of such inocula on the soil native microbiota must be evaluated alongside their effect on the crop performance.
Our ongoing "MicroBOOST" project is making the effort of putting these two needs together, investigating the impact of microbial inocula on the composition of the root-associated microbiota, in the perspective of increasing the quality of the crop used as forage in the dairy chain.
Four experimental Medicago sativa L. (alfalfa) fields are considered, inoculated or not with a commercial mixed (fungal-bacterial) inoculum. During the vegetative period, a time-course sampling of soil, rhizosphere and root is performed, followed by the high-throughput sequencing (MiSeq) of the fungal ITS2 and prokaryotic 16S regions. In parallel, plant-soil properties are assessed, and the forage proximate and fatty acid compositions are evaluated. Special attention is paid to assess whether and to which extent the native microbial communities are shaped by the inoculation, identifying the influence of the treatment on the native microbiota and on the assembly of the residing microbial community. Preliminary data show that the application of the commercial inoculum could increase the soil microbiota diversity and abundance, with a positive impact on the native community of arbuscular mycorrhizal fungi.

P64 Effects of root symbioses and MeJA priming in fenugreek seedlings growing under water deficit conditions

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Trigonella foenum-graecum (fenugreek) is a small seeded annual dicotyledonous legume belonging to the family Fabaceae that is widely distributed throughout the world. Several parts of the plant (leaves, seeds) are consumed and the known beneficial effects can be attributed to its bioactive molecules, e.g. alkaloids such as trigonelline, and saponins such as diosgenin (a steroid saponin). In this work, fenugreek plants were inoculated with an inoculum containing both arbuscular mycorrhizal fungi and rhizobia. After about 6 weeks from inoculation, control (AM-) and inoculated (AM+) plants were subjected to several combined treatments: well irrigated (NS) and not-watered (WS), in combination with methyl jasmonate (MeJA) priming (0µL/L, 50µL/L, 100µL/L, 200µL/L), which was reported to efficiently induce diosgenin biosynthesis in fenugreek seedlings [1]. A number of plant and cellular stress-related parameters were examined, i.e. leaf water potential, MDA, H_2O_2 and proline content indicative of stress levels. Colonization (nodules # and AMF colonization) was verified, in addition to morphometric measurements (i.e. root, stem, fruit dry weight; stem height; fruit number). Moreover, biochemical and metabolite analyses were carried out in leaves (i.e. ABA, IAA, trigonelline and diosgenin). Results showed that biological and chemical priming treatment combinations display a differential impact on plant growth and on production of the considered bioactive molecules. Symbiotic microbes significant improve stem height, diosgenin and trigonelline content, with the two last that showed higher levels following MeJA application under both NS and WS conditions. To support biochemical data, molecular analyses of genes involved in diosgenin and trigonelline metabolism are ongoing.

[1] Chaudhary et al. (2018) IJMS, **16**, 29889–29899.

P65 Arbuscular mycorrhizal fungal inoculation effects on growth-defence trade-offs in grapevine

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During the last years numerous efforts have been done to unveil Arbuscular Mycorrhiza (AM) fungal communities and their functionality in vineyard. It is well known that AM symbiosis provides several ecosystem services leading to plant adaptation in different environmental conditions and positively affect physiological and production features [1]. In this line, although some beneficial effects from the interaction between grapevine and the AM fungi have been already reported, this topic deserves further attention considering also the high diversity of vine material (rootstocks, cultivars and clones). In this study, the potential benefits of an inoculum formed by two AM fungal species (INOQ GmbH, Germany), with or without an oligosaccharide addition, were evaluated on young grapevine cuttings cv. Glera grafted onto 1103P and SO_4 rootstocks. Inoculated and non-inoculated plants were maintained in potted vineyard substrate under greenhouse conditions for two months. At the end of the experiment, engraftment percentage, growth index and the root nitrate uptake were analysed. Preliminary results showed that inoculated plants had higher growth index and engraftment percentage when compared to the non-inoculated ones, independently by the presence of the oligosaccharide. Interestingly, inoculated vines displayed lower nitrate uptake in respect to the non-inoculated. To deepen the outcome of AM fungal inoculation, molecular and biochemical analyses are still ongoing, both on leaves and roots, focusing on the expression of target genes related to hormones and nutrient transporters as well as those linked to defence against pathogens and on the evaluation of the content in hormones, sugars and stilbenoids.

[1] Bedini et al. (2018) Frontiers in Plant Science, **9,** Article 1800.

P66 Metabolomics in plant-microbe interactions: "MetaToul-plant metabolites" platform facilities

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LRSV (Laboratoire de Recherche en Sciences Végétales, University of Toulouse/CNRS)
"MetaToul-plant metabolites" platform is located in LRSV. This facility is one of the four units specialized in metabolomics and fluxomics in Toulouse, and part of the French National Infrastructure MetaboHub.
"MetaToul-plant metabolites" platform, though mass spectrometry facilities, offers global metabolomics and targeted metabolomics.
design new products of natural origin for the health of cultivated plants (biofertilizers, fight against diseases).
As targeted metabolomics, a global approach allows the detection and quantification of about 20 phytohormones and conjugates in a single experiment from plant (roots, aerial samples), or microbes. Classes of plant secondary metabolites are routinely analysable (phytoalexins, alkaloids, phenolic compounds, flavonoids, elicitors derived of amino acids…), others are under way, notably to detect signalling molecules involved in plant-micoorganisms interactions (blumenol, pipecolic acid, BABA…). Specific fungal and bacterial metabolites are analysable (lipochitooligosaccharides (LCOs), chitooligosaccharides (COS) …).

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P67* Imbalance in the mutualistic interaction in the arbuscular mycorrhizal symbiosis affects the fungal microbiota in *Lotus japonicus*

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Most land plants establish mutualistic interactions with arbuscular mycorrhizal (AM) fungi. Intracellular accommodation of fungal symbionts imposes profound remodeling of host traits associated to root morphology and defense, which likely shapes the root microbiota. To understand the effect of arbuscular mycorrhizal symbiosis (AMS) on root microbiota assembly in *Lotus japonicus*, the fungal communities associated with the roots of ten mutants impaired at different stages of AM formation were explored, growing in an agricultural soil under greenhouse conditions. Results showed that host mutants lacking the capacity to form mature arbuscules (arb^-), relative to arb^+ lines, assembled a markedly different root-associated fungal microbiota, characterized mainly by the depletion of Glomeromycota species and the concomitant enrichment of several Ascomycota taxa, including a *Dactylonectria torresensis* taxon. Isolation of *D. torresensis* from *L. japonicus* roots, and re-inoculation of arb^+ and arb^- lines with *D. torresensis* alone or in combination with AM fungus *Rhizophagus irregularis* indicated displacement of *D. torresensis* by the AM fungus, implying tripartite interactions between the host plant, its root microbiota and AM fungi. Our results demonstrate that AMS have effect on root-associated microbial assemblages only to a limited extent, but AMF specifically exclude several Ascomycota taxa.

* *Selected flash talk presentations*

P68 Fungal diversity affecting the production of the desert truffle *Terfezia claveryi* Chatin in productive and non-productive plants by metagenomics approach

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Recently, some strategies focused on the control of climatic parameters or the study of beneficial native bacteria, have been carried out in order to improve the desert truffle cultivation between *Helianthemum almeriense* x *Terfezia claveryi* [1]. In order to increase the field production of ascocarps is necessary to know how the microorganisms interact with the soil and rhizosphere, and whether they interfere in the symbiosis and in the fruiting of this fungus. Currently, the development of new technologies, such as high-performance sequencing technologies and new computational methods to collect and extract information from complex data sets, has allowed us to achieve our purpose through metagenomics approach. This approach has already been applied to unravel the fungal and bacterial biodiversity associated with edible black and white truffle, *Tuber magnatum* and *Tuber melanosporum*, respectively [2], but there is not information on the same aspect in desert truffles. In this study, a total of 62 samples from soil and root in productive and non-productive plants of *H. almeriense* inoculated with *T. claveryi*, in a plantation 4-years-old (Murcia, Spain), were evaluated and fungal diversity has been analyzed using the sequencing of ITS2 rDNA region. Preliminary results are discussed accordingly.

[1] Morte et al. (2017) Mycorrhiza - Eco-Physiol., Second. Metab., Nanomater., **2**, 23-42
[2] Zampieri and Mello (2017) Ital. J. Mycol., **46**, 1-7.

P69 Influence of diazotrophic bacteria on arbuscular mycorrhizal fungi on leguminous plants in the cerrado

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Green fertilization is an agricultural practice that aims to protect the soil against erosion with the vegetation cover and subsequent incorporation of cultural remains to the soil in order to increase the organic matter and improve the nutritional conditions of the next crop [1]. Organisms that promote growth and plant health may potentiate the productivity of cover crops [2,3]. This work aimed to evaluate the influence of inoculation with nitrogen-fixing bacteria of the genus rhizobium on arbuscular mycorrhizal fungi in legumes in the Cerrado. The experiment was carried out in the experimental area of the Faculdade Evangelica de Goianésia. The experimental design was completely randomized with treatments arranged in 8x2 factorial scheme with five replicates. The first factor was composed of 8 leguminous species: calopogonium (*Calopogonium muconoides*), Crotalaria juncea, Crotalaria spectabilis, bean (Canavalia ensiformis), pigeon pea (*Cajanus cajan*), dwarf pigeon (*Cajanus cajan cv persimmon*), gray mucuna (*Mucuna nivea*) and black mucuna (*Mucuna pruriens*). The second factor was composed by the treatments: With Inoculation of *Rhiziobium sp* and without inoculation. No chemical fertilization was performed. Guandu dwarf had the highest rate of mycorrhizal colonization and gray mucuna presented the lowest rate among the legumes analyzed. Pigeon bean and pigeon pea presented the highest values of spore density. The inoculation of diazotrophic bacteria does not influence mycorrhizal interactions in legumes

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P70 Nitrogen-fixing bacteria and arbuscular mycorrhizal fungi in the production of vegetables

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Vegetable production is the main source of subsistence income in family agriculture, which is responsible for supplying urban centers in South America [1]. The use of inexpensive technology with no damage to the final product and no environmental impact is an excellent alternative for this production medium [2]. The use of arbuscular mycorrhizal fungi together with biological nitrogen fixation is already a dominated management technique used in the production of vegetables [3]. This work aimed to evaluate the influence of the use of nitrogen fixing bacteria of the genus Azospirillun on the interactions between arbuscular mycorrhizal fungi in eight species of vegetables under Cerrado soil. The experiment was carried out in the experimental area of the Faculdade Evangelica de Goianésia. The experimental design was completely randomized with the treatments arranged in a 7x4 factorial scheme with five replicates, the first factor being 8 species of vegetables: Lettuce, Green Onion, Carrot, Coriander, Cabbage, Spinach and Radish. The second factor was composed by the treatments: Inoculation of Azospirillum, Application of Bovine Spittle and Inoculation of Azospirillum with application of Bovine Spittlement and Control. The use of diazotrophic bacteria does not influence the mycorrhizal interactions in vegetables. It was verified superior values in chives, coriander and cabbage, when compared to lettuce, radish, carrot and spinach. The spore density was higher in lettuce, radish, cabbage and spinach than in chives, carrots and coriander.

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[2] Batista N da S (2016). Diversificação de cultivos de hortaliças associada ao uso de insumos para a fertilidade do solo, em sistema orgânico de produção.
[3] Pagano MC et al. (2009). Hortaliças Folhosas Comerciais e suas Associações Micorrízicas em Minas Gerais, Brasil. Cad Agroecol;4(1).

P71 The genome of *Gigaspora margarita* offers hints on ancient plant-fungal-bacterial interactions

Francesco Venice[1], Stefano Ghignone[2], Alessandra Salvioli[1], Joëlle Amselem[3], Mara Novero[1], Xie Xianan[4], Kinga Sliedslevska[5], Hassine Radhouane Khouja[1], Emmanuelle Morin[6], Bernard Henrissat[7], Francis Martin[6], Paola Bonfante[1]

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Despite their importance in establishing mutualistic symbiosis with 72% of land plants, Arbuscular Mycorrhizal Fungi (AMF, Glomeromycotina) are still enigmatic from a biological point of view, since our knowledge on the genetic mechanisms regulating AM symbiosis mostly mirrors a plant-centric point of view. In addition to being members of the plant microbiota, some AMF also possess their own microbiota, harboring uncultivable endobacteria inside their cytoplasm and thus interacting with plant and bacterial kingdoms. Differently from the AMF so far sequenced, *Gigaspora margarita* may host different endobacterial populations, as well as viral sequences, representing a good model to investigate such interkingdom interactions. The genome of *G. margarita* is the largest fungal genome so far annotated (773.104 Mbp) with a relevant presence of transposable elements (more than 60%). It possesses several unique traits, such as the presence of secondary metabolites gene clusters, the expansion of some carbohydrate active enzymes families (i.e. chitinsynthases and secreted multicopper oxidases), the expression of a high number of genes involved in Pi metabolism, and potential horizontal gene transfer events. In the context of a comparative genome analysis, the sequencing of *G. margarita* reveals evolutionary pathways, which may have allowed early-diverging fungi to interact with both plants and bacteria. We provide insights into *G. margarita* biotrophic life style, reveal the importance of its immunity system, which has to guarantee interactions with both bacterial and plant cells, and advance hypothesis on the biological meaning of its expanded genome.

P72° Is auxin signaling part of the RAM1-regulated arbuscocyte developmental program?

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The exchange of nutrients in arbuscular mycorrhiza symbiosis between plants and glomeromycotan fungi is performed by fungal broccoli-shaped structures, called arbuscules (1,2). *REDUCED ARBUSCULAR MYCORRHIZA1* (*RAM1*), encoding a GRAS transcription factor, was identified to be a core regulator of arbuscule development (3,4,5). The *ram1* mutant displays a stunted arbuscule phenotype (3,4,5). Arbuscule branching also requires auxin signaling (6). We wondered how auxin signaling is placed relative to RAM1 in a signaling network regulating arbuscule development. We found in *L. japonicus* that the auxin reporter *DR5*:*GUS* was active in arbuscocytes (arbuscule containing cells) in the wild type but not in *ram1*, indicating that *RAM1* is required for *DR5*:*GUS* induction in these cells. Furthermore, ectopic expression of *RAM1* induced *DR5*:*GUS* in a patchy pattern in absence of the fungus. In addition, some AM-induced auxin response genes were not induced in the *ram1* mutant. In addition, auxin biosynthesis inhibitor almost blocked the colonization between wild type roots and fungi. Taken together, this indicates that activation of auxin signaling or biosynthesis in arbuscocytes may involve RAM1.

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° *this abstract belongs to the session on Cellular and molecular aspects of mycorrhizal interactions*

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