SwissPLANT symposium – Early Career Meeting 2023

22–23 January 2023, Les Diablerets, Switzerland

Organizer:

S**wiss Society of Plant Biology with financial support by SCNAT**

Organizing committee:

Henry Janse van Rensburg, Dept of Environmental Sciences, University of Basel
Wojciech Wietrzynski, Biozentrum, University of Basel

Mentors:

Christian Fankhauser, University of Lausanne, President of the Swiss Society of Plant Biology
Klaus Schläppi, University of Basel

SwissPLANT symposium - Early Career Meeting 2023
22–23 January 2023, Les Diablerets, Switzerland

Sunday, 22 January 2023

14:00	**Welcoming**		
14:15	Daniele Albertini	ETH Zürich	Non-cell autonomous RNA silencing restricts *Turnip Yellows Virus* (TuYV) spread in *Arabidopsis thaliana*
14:30	Robin E. Bautzmann	U Bern	Effects of biodegradable micro- and macroplastic on *Zea mays* L. growth, transcriptome and the associated root and soil microbiome
14:45	Kevin Bellande	U Neuchâtel	Conserved developmental trajectories channelling lateral root primordium morphogenesis.
15:00	Kyle Bender	U Zürich	Functional organization of plant immune receptor kinase complexes
15:15	Anja Boss	U Bern	Bacterial engineering of symbionts of entomopathogenic nematodes to enhance biological control on the western corn rootworm in maize crops
15:30	Joaquín Clúa	U Lausanne	A cytochrome-containing protein plays a dual role in phosphate starvation responses and iron homeostasis
15:45	Nasim Farahani Zayas	U Lausanne	A lignin-like polymer is deposited in the outer cell wall of the *Arabidopsis* root epidermis
16:00	**Coffee break/poster session**		
17:00	Natalia González Gaarslev	U Lausanne	Harnessing genetic interactions driving inflorescence complexity in tomato
17:15	Xiaoyu Hou	U Zürich	The Arabidopsis PP2Cs regulate LRX1-mediated cell wall integrity sensing
17:30	Lena Hyvärinen	U Geneva	Temperature plasticity of a seed coat apoplastic barrier promotes seed dormancy in *Arabidopsis thaliana*
17:45	Jonatan Isaksson	U Zürich	Insight into the molecular mechanisms of the AvrPm3-SvrPm3 effector protein interactions derived from the wheat powdery mildew pathogen
18:00	Léa Jacquier	U Geneva	Symplastic connections in differentiated Arabidopsis roots
18:15	Henry Janse van Rensburg	U Basel	A receptor protein mediates soil microbial feedbacks in *Arabidopsis*
18:30	**Dinner**		
20:00	**Poster session and drinks**		

Monday, 23 January 2023

07:00	**Breakfast**		
08:00	Session chair		
08:15	Darina Koubínová	U Neuchâtel	Phylogenetic resolution of deep nodes of the Ophioglossaceae ferns
08:30	Aime Jaskolowski	U Lausanne	Cross-talk between biotic and abiotic stress: Arabidopsis response to phosphate starvation and *Botrytis cinerea* infection
08:45	Miguel Loera-Sánchez	ETH Zürich	Efficient monitoring of plant genetic diversity changes in multispecies meadows
09:00	Beatrice Manser	U Zürich	The wheat zinc finger protein TaZF acts as third component in the NLR-mediated powdery mildew effector recognition
09:15	Sandi Paulisic	U Lausanne	Shade induced changes of chromatin architecture in Arabidopsis thaliana
09:30	Aditya Nayak	ETH Zürich	Functional Variation of Naturally Evolved Plant Meiotic Axis Protein ASY1
09:45	**Coffee break/poster session**		
10:15	Kinga Rutowicz	U Zürich	Linker histone H1 as a facilitator of trascriptomic reprogramming during diurnal rhythm.
10:30	Mayank Sharma	ETH Zürich	MAR-binding filament protein-1 determines the location of starch granule initiation in chloroplasts
10:45	Edouard Tourdot	U Zürich	Investigate the functional role of 3D chromosome folding in nuclear defense systems and molecular actors involved using reverse ChIP approach
11:00	Pallavi Vetal	U Lausanne	Investigating post-translational control and trafficking of Arabidopsis PHOSPHATE1 (AtPHO1)
11:15	Florent Waltz	U Basel	Investigating mitochondrial molecular organization across photosynthetic organisms using cryo-electron tomography
11:30	Lei Wang	U Bern	Immature leaves are the volatile sensing organs of maize
11:45	**Closing/voting**		
12:00	**Lunch**		

Non-cell autonomous RNA silencing restricts *Turnip Yellows Virus* (TuYV) spread in *Arabidopsis thaliana*

Daniele Albertini and Olivier Voinnet

Department of Biology – Institute of Molecular Plant Biology, ETH Zürich

Remarkably, plant RNA silencing can act non-cell autonomously. This, combined with its key role in antiviral defense, has prompted speculations that a virus-derived silencing signal emitted from infected cells might move ahead of the infection and confer sequence-specific immunization to as-yet-uninfected cells. Indirectly supporting this notion, recombinant tombusvirus devoid of the P19 silencing suppressor is impaired in phloem unloading, but not in vascular replication. Additionally, certain silencing components seem to specifically act systemically. Recently, we showed that sRNA duplexes can serve as mobile signals. There is, however, no direct evidence that these molecules have an immunization potential, and nor is there any indication of their physical range-of-action and, ultimately, impact on virus distribution *in planta*. Testing this hypothesis is difficult, because it requires uncoupling viral spread from sRNA movement. Here, we exploited the natural phloem restriction of TuYV to investigate these issues in Arabidopsis roots. We show that mobile virus-derived (v)siRNA duplexes move and indeed confer immunization well over 50μm without relay-amplification, both radially (into cells surrounding the vasculature), but also longitudinally up to the quiescent center in which vsiRNAs were physically captured.

Effects of biodegradable micro- and macroplastic on *Zea mays* L. growth, transcriptome and the associated root and soil microbiome

Robin Bautzmann[1], Jan Wälchli[2], Klaus Schläppi[2] and Doris Rentsch[1]

[1]*Institute of Plant Sciences, University of Bern, Bern, Switzerland*
[2]*Department of Environmental Sciences, University of Basel, Basel, Switzerland*

Email: robin.bautzmann@ips.unibe.ch, jan.waelchli@unibas.ch, klaus.schlaeppi@unibas.ch, doris.rentsch@ips.unibe.ch

The awareness about plastics polluting the environment has been growing in the past years, while so far, there is little information on the consequences of plastic pollution in soils. Evidently, plastic contamination has the potential to effect crop plants and its associated soil biota, which ultimately could affect food security.
A pot experiment was conducted to investigate the effects of biodegradable (BD) micro- and macroplastic on *Zea mays L.* W22 growth, its transcriptome and the associated root and soil microbiome. Since the main component of BD plastic is thermoplastic maize starch, a corn starch treatment was included. Moist sand and silt loam soil were used as substrates and mixed with 0 to 10% (v/v) contaminant, respectively. The plants were cultivated for two weeks in a growth chamber under controlled conditions. Analysis showed that shoot and root fresh weight and chlorophyll content of maize leaves were reduced in a dose dependent manner when BD micro- or macroplastic was added. The effects were more pronounced in the sand substrate. Bacterial communities of both substrates and the roots shifted with the addition of BD plastic. Alpha diversity decreased in both substrate types, possibly due to a decrease in low abundant phyla. Opposite effects were detected in roots where the alpha diversity increased. The addition of starch showed similar effects. Recent results from RNA-seq analyses will also be presented.

Conserved developmental trajectories channelling lateral root primordium morphogenesis.

Cristovao De Jesus Vieira Teixeira[1], **Kevin Bellande**[1], Thomas Badet[1], Anne C. Roulin[2] and Joop E.M Vermeer[1,2]

[1]Laboratory of Cell and Molecular Biology, Institute of Biology, University of Neuchâtel
[2]Department of Plant and Microbial Biology, University of Zurich

Email: cristovao.dejesus@unine.ch; kevin.bellande@unine.ch; thomas.badet@unine.ch; anne.roulin@botinst.uzh.ch; josephus.vermeer@unine.ch

Understanding root development and its adaptative behaviours to changing environments is a major goal of the 21st century. The root system anchors the plant to the soil and absorbs water and nutrients to sustain plant development. Secondary root formation, such as lateral roots (LR), are major agronomic traits and significantly contribute to the adaptative nature of the root system architecture. LRs originate primarily from pericycle tissue in angiosperm species. The pericycle comprises a single cell layer surrounding the vascular tissues and is overlaid by the endodermis, cortex, and epidermal tissues. To emerge, the LRs need to grow through these neighbouring cell layers. Our current knowledge about these spatial accommodation mechanisms shaping LR development is still incomplete and is mostly restricted to the dicotyledon model species *Arabidopsis thaliana.* Using an inducible system to synchronise the LRs development, we generated the first time-course LR RNAseq in the non-cultivated grass model plant *Brachypodium distachyon*. In parallel, the natural variation of LR traits in wild accessions of *B. distachyon* is currently being used for a genome-wide association study (GWAS). Comparison with published transcriptome datasets from other plant species will be used to identify conserved core, but also new modules that have been recruited to channel root branching in grasses.

Functional organization of plant immune receptor kinase complexes

Kyle W. Bender and Cyril Zipfel

Department of Plant and Microbial Biology – University of Zürich

Email: kyle.bender@botinst.uzh.ch, cyril.zipfel@botinst.uzh.ch

Receptor kinases (RKs) fulfill central roles in regulating developmental programs and environmental responses in plants. Although it is widely recognized that ligand-perceiving RKs are regulated by a range of accessory RKs, it is unclear how the organization of RKs within the plasma membrane contributes to the control of RK-mediated signal transduction. Using the immune RKs FLAGELLIN SENSING 2 (FLS2) and ELONGATION FACTOR Tu RECEPTOR (EFR), we performed an affinity proteomics analysis to characterize, broadly, the composition and organization of cell-surface immune receptor complexes containing these RKs. We observed that receptor complexes containing FLS2 or EFR are remarkably static; in our approach, SERK co-receptors and – paradoxically – BIR proteins are the only components recruited to the receptor complex upon ligand stimulation. We find that FLS2 and EFR are associated with a core set of accessory RKs, all of which have known or predicted immune functions, suggesting the existence of a core immune-activating complex with the capacity to perceive multiple, distinct ligands. Receptor clustering is a well-known phenomenon in non-plant eukaryotes and has different biochemical and physiological consequences depending on the specific receptor. Current work aims to compare EFR/FLS2 receptor complexes to those containing 'non-immune' RKs such as BRI1. We are additionally exploring the physiological role of immune receptor clustering by characterizing immune signaling outputs using different ligand combinations.

Bacterial engineering of symbionts of entomopathogenic nematodes to enhance biological control on the western corn rootworm in maize crops

Anja Boss[1], Szabolcs Tóth[2], Stefan Toepfer[2,3], Matthias Erb[1] and Ricardo Machado[4]

[1]*Institute of Plant Sciences, University of Bern*
[2]*Plant Protection Institute, Szent Istvan University, Hungary*
[3]*CABI, Delémont*
[4]*Institute of Biology, University of Neuchâtel*

Maize plants synthesize a variety of secondary metabolites which play important roles in the plant's life cycle, including defence and plant-insect interactions. One particular secondary metabolite of maize are benzoxazinoids which act as allelochemicals and natural pesticides. However, the specialist insect *Diabrotica virgifera virgifera* is able to sequester benzoxazinoids and use these plant toxins to defend themselves against entomopathogenic nematodes and their bacterial symbionts, limiting their biocontrol potential. Therefore, we wanted to understand the genetic mechanisms of benzoxazinoid resistance in symbiotic *Photorhabdus* bacteria. For that reason, we isolated 27 *Photorhabdus* symbionts from different nematodes all over the world and increased their benzoxazinoid resistance through experimental evolution. Benzoxazinoid resistance evolved through multiple mechanisms, including a mutation in a multidrug efflux pump. We reintroduced benzoxazinoid-resistant *Photorhabdus* strains, as well as their non-selected ancestors, into two strains of *H. bacteriophora* nematodes and identified four nematode–symbiont pairs that were able to kill benzoxazinoid-sequestering *D. virgifera virgifera* larvae more efficiently under lab conditions. Tested in the field, the control efficacy could not exceed the potential of the already commercially available nematode products, but our results suggest that modification of bacterial symbionts and targeting candidate genes to engineer better biocontrol agents might provide a successful and time-efficient strategy for a variety of crops in the future.

A cytochrome-containing protein plays a dual role in phosphate starvation responses and iron homeostasis

Joaquín Clúa, Jonatan Montpetit and Yves Poirier

Department of Plant Molecular Biology, University of Lausanne, Biophore Building, CH-1015 Lausanne, Switzerland

Phosphate is an essential macronutrient required for plant growth and development. However, it is present at suboptimal levels in many terrestrial ecosystems. To ameliorate this limitation, plants have evolved developmental and physiological mechanisms known as phosphate starvation responses (PSR). One of the main PSR in *Arabidopsis thaliana* is a deep restructuration of the root system architecture, which includes a reduction in primary root growth resulting in a shallower root system better adapted to explore the nutrient-rich topsoil. Intense research over the last years has shown that this developmental change is dependent on the accumulation and redistribution of iron (Fe) at the root tip, which in turn, participates in Fenton reactions and generates reactive oxygen species that affect meristem function and cell elongation. We have recently identified and characterized a CYBDOM (chytochrome b561 and DOMON domain) protein in *A. thaliana*, named CRR, which is involved in the primary root growth response to phosphate starvation. We determined that CRR is an ascorbate-dependent ferric-reductase whose expression levels modulates iron distribution pattern in the root, affecting meristem function and cell elongation. Moreover, this activity also has shown to be critical for iron toxicity tolerance since CRR determines the transport rate of iron from root to shoot. Our results, showed for the first time the biological role that CYBDOM proteins play in plants.

A lignin-like polymer is deposited in the outer cell wall of the *Arabidopsis* root epidermis

Nasim Farahani Zayas[1], Damien De Bellis[1,2] and Christiane Nawrath[1]

[1]*Department of Plant Molecular Biology - University of Lausanne*
[2]*Electron Microscopy Facility - University of Lausanne*

During evolution, plants developed diffusion barriers to isolate themselves from the outside environment. In several species, including onion and soybean, modifications of the outer cell wall of the root epidermis with a “diffuse”, non-lamellated form of suberin that can be stained with lipid dyes, such as fluorol yellow (FY), have been described.
Here we report that in *Arabidopsis* the outer epidermal wall is reinforced by molecules that can be stained with Auramine O (AO), but not with FY. Pharmacological inhibition of the phenylpropanoid pathway reduces the intensity of AO staining, while exogenous application of lignin monomers and other phenylpropanoids lead to increased AO staining of the outer cell wall. *PAL2* and *CH4*, key genes of the phenylpropanoid pathway, are expressed in the root epidermis, as are several *RBOH* genes, which are required for the formation of reactive oxygen species (ROS). Analysis of higher-order mutants in monolignol biosynthesis and in ROS formation and as well as the application of peroxidase inhibitors indicate the deposition of a lignin-like polymer in the outer root epidermis. Since the lignin stain Basic Fuchsin stains weakly the outer root epidermis, but the lignin stain Safranine O stains as strongly as AO, we hypothesize that an atypical lignin is deposited in the outer cell wall of the root epidermis of *Arabidopsis*.

Harnessing genetic interactions driving inflorescence complexity in tomato

Natalia Gaarslev and Sebastian Soyk

Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

The phenotypic diversity of inflorescences is determined by the genetic networks underlying meristem maturation. In tomato, inflorescence architecture is shaped by genetic interactions between the MADS-box transcription factor genes *JOINTLESS 2 (J2), ENHANCER OF JOINTLESS 2 (EJ2)*, and *LONG INFLORESCENCE (LIN)*, homologs of *Arabidopsis SEPALLATA 4*. It has been demonstrated that MADS-box gene dosage negatively correlates with inflorescence complexity. However, how differences in MADS-box gene dosage led to quantitative changes in inflorescence complexity remains poorly understood at the molecular level. To tackle this challenge, we sequenced transcriptomes from a collection of single and higher-order MADS-box mutants to investigate the transcriptional networks orchestrated by *J2*, *EJ2*, and *LIN* during meristem maturation. Coexpression analysis on significantly deregulated genes on the mutant collection allowed associating different degrees of inflorescence complexity, including those of an additive and synergistic nature, to specific expression clusters. Moreover, we found transcription factor families previously suggested to be involved in the regulation of reproductive development, including B3-domain and MADS-box genes. Targeting two previously uncharacterized MADS-box genes by genome editing suggests additional genetic interactions and a role in inflorescence development. Our approach sheds light on gene networks and the interaction among genes regulating inflorescence complexity. Understanding the developmental pathways guiding meristem maturation in determining inflorescence architecture may allow fine-tunning inflorescences, which can be exploited for crop improvement.

The Arabidopsis PP2Cs regulate LRX1-mediated cell wall integrity sensing

Xiaoyu Hou, Garbor Kadler, Shibu Gupta, Amandine Guérin, Caroline Levasseur and Christoph Ringli

Institute of Plant and Microbial Biology, University of Zurich, Zurich 8008 CH

For plant cell growth to take place, control mechanisms that closely survey cell wall remodeling are necessary. LRR-extensins (LRXs) of Arabidopsis are regulators of cell wall development. LRXs are high-affinity binding sites for RALF peptide hormones that are involved in cell growth regulations by contracting cell wall acidification. The transmembrane protein kinase FERONIA (FER) functions in cell wall integrity sensing and interacts with RALFs and LRXs. Therefore, we propose an LRX-RALF-FER module that regulates cell wall development. In Arabidopsis, LRX1 and LRX2 are predominantly expressed in root hairs and mutations in these genes cause defects in root hair development. Our group uses Arabidopsis root hairs as a model system to study LRX-mediated cell wall integrity sensing.

To decipher the regulatory mechanism of LRX1-mediated cell wall integrity sensing, we screened for repressor of lrx1 (rol) mutants that reconstitute the root hair development in the root hair defective mutant background lrx1. One of the characterized mutants, rol23, has a mutation in a gene encoding for a type 2C protein phosphatase. Protein phosphatases have been shown to regulate signaling pathways by controlling kinase turnover. This study aims to understand how the induction and retention of LRX1-mediated cell wall integrity sensing signaling are regulated by type 2C protein phosphatase.

Temperature plasticity of a seed coat apoplastic barrier promotes seed dormancy in *Arabidopsis thaliana*.

Lena Hyvärinen[1], Anne Utz-Pugin[1], Christelle Fuchs[1], Kay Gully[2], Christiane Nawrath[2], Sylvain Loubéry[1] and Luis Lopez-Molina[1]

[1]*Department of Plant Sciences, University of Geneva*
[2]*Department of Plant Molecular Biology, University of Lausanne*

Seed dormancy is an adaptive trait whereby germination is blocked under favourable conditions to avoid germination out of season. Over time mature dry seeds lose dormancy and gradually acquire the capacity to germinate. Dormancy levels, i.e., the time required to release dormancy of the newly produced dry seed, are influenced by the environment of the mother plant and particularly by cold temperatures, which increase dormancy levels. It is also known that the seed coat, a maternal dead tissue, is important to keep seeds dormant over time, probably by shielding the seed living tissues from atmospheric oxygen.

Biochemical and genetical evidence previously established that cold promotes polyester accumulation in the seed coat and mutant seeds deficient in polyester biosynthesis have low dormancy and viability. However, it is unclear which seed coat structures, such as apoplastic barriers, are remodeled or created *de novo* in response to cold during seed development.

Combining histological and genetical approaches, I discovered a previously uncharacterized polar apoplastic barrier located in the outer side of the outer integument 1 (oi1) cell layer. This barrier is strongly reinforced by cold and this process requires a MYB transcription factor (TF), specifically expressed in oi1 cells. I showed that mutants lacking the MYB TF, lacking the barrier, are less dormant, thus providing direct evidence that this barrier promotes dormancy in Arabidopsis.

Insight into the molecular mechanisms of the AvrPm3-SvrPm3 effector protein interactions derived from the wheat powdery mildew pathogen

Jonatan Isaksson[1], Matthias Heuberger[1], Milena Amhof[1], Lukas Kunz[1], Kaitlin McNally[1] and Beat Keller[1]

[1]*Institute of Plant and Microbial Biology, University of Zurich*

Agriculture relies heavily on the use pesticides to prevent pathogen growth and disease. There are increasing concerns about their effects on the health of the environment, livestock and humans. This is leading to regulations to decrease the use and risk of these chemicals and to use genetically based resistance in plant breeding as an alternative strategy for crop protection. In cereal crops, powdery mildew disease is caused by the obligate biotrophic fungus *Blumeria graminis* (*B.g.*) which can be divided into subgroups of *formae speciales* based on the specific grass species that they infect. Disease caused by wheat powdery mildew (*B.g. tritici*) is largely controlled by intracellular nucleotide-binding leucine-rich repeat (NLR) proteins. The wheat NLR *Pm3* resistance gene consists of an allelic series that provides race-specific resistance and host-specificity against *B.g. tritici* by recognizing small secreted fungal proteins called effectors. Previously, three of these avirulence effectors (*AvrPm3*s) were identified that are recognized by distinct alleles of *Pm3*. Furthermore, a suppressor of *Pm3* triggered immunity (*SvrPm3*) was identified in powdery mildew. In our studies we reveal that AvrPm3 effectors, although sequence divergent, are structurally related proteins that can biochemically interact with each other to form hetero- and homomeric complexes. Along with this, our studies of AvrPm3 recognition specificity by Pm3 provides new insight into the molecular mechanism that governs the Pm3-AvrPm3-SvrPm3 interactions.

Symplastic connections in differentiated Arabidopsis roots

Léa Jacquier, Linnka Legendre Lefebvre and Marie Barberon

Department of Plant Sciences, University of Geneva, Switzerland

The differentiated root of Arabidopsis is characterized by endodermal barriers which are essential for tight regulation of water and nutrients. These barriers correspond to specialized structures, Casparian strips and suberin lamellae, formed at the periphery of endodermal cells. The differentiation of the endodermis is progressive along the root, first forming the Casparian strips (state I of differentiation), and second the deposition of suberin lamellae (state II of differentiation). It is well characterized that the Casparian strips form an apoplastic barrier and play an important role in nutrients homeostasis. Once the solutes enter a cell, they can theoretically use either the transcellular pathway or the symplastic pathway. In this context, the cell-to-cell connections via plasmodesmata are poorly characterized.
We aim to study the symplastic pathway (1) in the context of differentiated roots, (2) along the differentiation gradient and (3) across the different cell types of the root. For this purpose, we are tracking the movements of mobile fluorophores across the root using cell-types specific promoters. This cell-to-cell analysis of symplastic movements reveals a directional movement at some interfaces. This also seems to be established along the differentiation gradient of the root. We are currently studying the mechanisms behind this directionality using cellular biology approaches, electron microscopy and a genetic screen approach. The mutants obtained via the genetic screen will potentially help us understand how this directionality is established and its function for nutrient acquisition. This work will provide a better understanding of cell-to-cell communication in the context of differentiated roots.

A receptor protein mediates soil microbial feedbacks in *Arabidopsis*

Henry Janse van Rensburg[1], Katja Stengele[1], Niklas Schandry[2], Claude Becker[2] and Klaus Schlaeppi[1]

[1]*Plant Microbe Interactions, Department of Environmental Sciences, University of Basel*
[2]*Faculty of Biology, Institute of Genetics, Ludwig Maximilian University of Munich*

Plants exude a diverse array of compounds into the soil and thereby they condition the surrounding soil and its microbiome. Such soil conditioning results in microbial feedbacks on the performance of the next plant generation. Benzoxazinoids (BXs) are abundant bioactive compounds in the root exudates of important crops like maize and they selectively structure the rhizosphere microbiota of maize. *Arabidopsis thaliana* expresses positive growth feedbacks when grown on BX conditioned soil. While the ecology and the agronomic impact of plant-soil feedbacks are well described, little is known about the underlying mechanisms of plant responses to soil microbiomes. Using a Genome Wide Association Study performed on 410 *Arabidopsis* accessions, we identified an uncharacterized toll-interleukin (TIR) nucleotide-binding site (NBS)-leucine-rich repeat (LRR) receptor, possibly involved in plant defense responses. A particular allele variant of this receptor locus was associated with accessions that expressed a positive growth feedback on BX conditioned soils, while other variants associated with negative growth feedbacks. Mutants of the receptor have lost the strong positive growth feedback on BX-conditioned soil, confirming the involvement of the identified receptor in microbiome-driven growth feedbacks. We have identified a novel receptor gene mediating responsiveness to microbial feedbacks and our results suggest a growth-defense trade-off during plant-soil feedbacks.

Phylogenetic resolution of deep nodes of the Ophioglossaceae ferns

Darina Koubínová[1], Li-Yaung Kuo[2] and Jason R. Grant[1]

[1]*Institut of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland*
[2]*Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu City, Taiwan*

Ophioglossaceae is a small, eusporangiate fern family distributed worldwide in temperate and tropical regions. It represents one of the most ancient fern lineages. There are currently more than 100 species, about 12-14 genera and 4 subfamilies recognized. Many of the species are widespread, others are limited to small areas. Similarly, some of the main genera contain tens of species, while some are monotypic. Compared to other plant families, a solid backbone phylogeny, i. e. at the generic level, is still not available in the Ophioglossaceae.

Former attempts to infer phylogenetic tree structures used only limited number of plastid regions or contained missing data. In the two available phylogenomic analyses, plastome datasets were used but the number of included genera was very small and a simplified substitution model was applied. Moreover, some new genera were discribed only recently and were not not included in the previous studies.

To resolve the Ophioglossaceae phylogeny at the generic level, we used a phylogenomic approach with adding more subfamily representatives to cover all currently recognized genera and analyzed both plastome and mitogenome coding sequences (CDS) datasets. We tested different partition and substitution models. Our phylogemonic results overall supported a novel, previously uncovered topology which presented the most solid infrafamily backbone for Ophioglossaceae.

Cross-talk between biotic and abiotic stress: Arabidopsis response to phosphate starvation and *Botrytis cinerea* infection

Aime Jaskolowski and Yves Poirier

Department of Plant Molecular Biology, University of Lausanne

Compelling evidence shows that plants respond in a specific manner when faced with more than one stress simultaneously, and the outcome cannot be predicted based on the plant's responses to each individual stress mainly due to the complex interactions existing between defense signaling pathways. Phosphate (Pi) is one of the most limiting nutrients in both natural and agricultural ecosystems. Pi deficiency has been associated with the induction of the defense hormones salicylic acid (SA) and jasmonic acid (JA), as well as of secondary defense metabolites. *Botrytis cinerea* is a necrotrophic fungus with a very wide host range and is one of the major pathogens responsible for important losses in agriculture and JA synthesis is the main defense signaling pathway activated upon necrotroph infection. Here we report that, although most of the expected defense responses against *Botrytis* are being properly induced, such as transcriptional activation of defense genes and JA and camalexin biosynthesis, the Arabidopsis Pi-deficient mutant *pho1* is more susceptible to *Botrytis* infection than a wild type plant. Our data suggest that this phenotype is due to a defect in the early defense mechanisms, mainly callose deposition. We found that this phenotype is associated to the elevated abscisic acid (ABA) levels in the *pho1* mutant, as the inhibition of ABA biosynthesis in *pho1* restores callose deposition and response to *Botrytis*.

Efficient monitoring of plant genetic diversity changes in multispecies meadows

Miguel Loera-Sánchez, Bruno Studer and Roland Kölliker

Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich

Email: miguel.loera@usys.ethz.ch, bruno.studer@usys.ethz.ch, roland.koelliker@usys.ethz.ch

Plant genetic diversity (PGD) influences ecosystem services of grasslands. It also comprises the genetic resources necessary for forage plant breeding. However, the ecosystem-level effects and dynamics of grassland PGD remains largely understudied, mainly because suitable monitoring methods are lacking.

We developed a cost-effective, amplicon sequencing method for PGD assessment that has the potential for large-scale applications. The method is based on the targeted sequencing of genomic loci that are genetically diverse within the most economically relevant forage grass and legume species. In five key species (*Lolium perenne* L., *L. multiflorum* Lam., *Trifolium pratense* L., and *T.* repens L.) the overall nucleotide diversity of the amplicons ranged from 5.19×10^{-3} to 1.29×10^{-2}, a similar range to that of flowering-related genes. This amount of genetic variation was enough to assess the genetic differentiation of cultivars of *L. multiflorum*, which were grown in pure stands and in mixtures.

The method will now be upscaled and tested in the field. For this, we oversowed 60 meadow plots to simulate changes in grassland PGD. We will evaluate sampling strategies and DNA processing conditions needed for an accurate representation of the PGD in meadow plots. In addition, we are testing the performance of the method using multispecies samples.

The wheat zinc finger protein TaZF acts as third component in the NLR-mediated powdery mildew effector recognition

Beatrice Manser, Stephanie Bräunlich, Jonatan Isaksson, Thomas Wicker and Beat Keller*

Department of Plant and Microbial Biology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland

Email: beatrice.manser@botinst.uzh.ch, stephanie.braeunlich@agroscope.admin.ch, jonatan.isaksson@botinst.uzh.ch, wicker@botinst.uzh.ch, bkeller@botinst.uzh.ch

Plant defense responses to pathogenic microbes are driven by the direct perception of microbial effector proteins and indirectly through their modifications of host proteins. It remains largely elusive, how immune receptors indirectly monitor the activity of fungal effectors. The wheat immune receptor and nucleotide-binding leucine-rich repeat receptor (NLR) Pm2a confers race-specific resistance against the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*) and recognizes the AvrPm2 effector. Here we identify the wheat zinc finger TaZF as a third component involved in indirect Pm2a-mediated AvrPm2 recognition. TaZF interacts with both Pm2a and AvrPm2 forming a Pm2a-TaZF-AvrPm2 complex, which localizes to the wheat nucleus. Silencing of *TaZF* in wheat leads to impaired Pm2a-mediated *Bgt* resistance. Using truncated proteins, we identified the Pm2 LRR domain as the mediator of the interaction with TaZF and indirectly also with AvrPm2. Our findings illustrate how plant immune receptors can detect a fungal effector through binding to additional host proteins, such as transcription factors and highlights the importance of understanding effector function for the identification of indirect NLR-mediated effector recognition.

Shade induced changes of chromatin architecture in Arabidopsis thaliana

Sandi Paulisic, Alessandra Boccaccini and Christian Fankhauser

Center for Integrative Genomics, University of Lausanne, Switzerland

Light provides energy and information about the surrounding environment to plants. In dense plant communities, proximity of neighboring vegetation can limit light availability for photosynthesis by outgrowing and shading the plant. This requires a fast developmental adaptation. Arabidopsis usually responds by elongating its hypocotyls and stems, repositioning the leaves to better capture the light, and accelerating flowering. Vegetation proximity and shade, are sensed by phytochromes as a reduction of red to far-red light ratio, resulting in phytochrome inactivation and allowing PHYTOCHROME INTERACTING FACTORs to promote a wide scale genome reprograming event. A major expression cascade is initiated, particularly of genes involved in auxin biosynthesis and signaling, and cell elongation. Gene expression is not entirely dependent on the binding of transcription factors to the DNA, but also on the chromatin conformation. The essential structural element of chromatin, the nucleosome, can facilitate or repress the action of transcriptional machinery. Accessible chromatin regions, lacking nucleosomes, are highly accessible to the transcriptional machinery and are major sites of DNA regulatory motives. This work tried to identify chromatin architectural changes, especially of the accessible chromatin regions during shade avoidance response and its effect on the regulation of gene expression. Using ATAC-seq to profile the chromatin landscape of Arabidopsis under short and long shade exposure we identified chromatin conformational changes in an important shade regulator *HFR1*.

Functional Variation of Naturally Evolved Plant Meiotic Axis Protein ASY1.

Aditya Nayak and Kirsten Bomblies

Institute of Molecular Plant Biology – ETH Zurich

The meiotic axis, a multiprotein complex, holds sister chromatids together at the base to begin meiotic prophase. The axis also provides a structural framework for synapsis and recombination during meiosis. It contains HORMA domain-containing proteins (HORMADs), which oligomerize via their C termini closure motifs. ASY1 is one such meiotic HORMAD protein which also contains a SWIRM domain and through alphafold2 based structural prediction, I discovered that ASY1 also contains a winged Helix-Turn-Helix (wHTH) domain. While the HORMA domain and its role in meiotic axis formation has been largely characterized, there is very little information about the role of the wHTH and SWIRM domains in the formation of meiotic axis.
Based on these predicted structural models, I hypothesized that the wHTH and SWIRM domains might have DNA reeling functions during the early stages of meiosis. To probe this hypothesis, I took an integrated biochemical and biophysical approach to understand the DNA interaction activity and function of these domains and the full length protein.
Furthermore, to understand the role of these proteins in successful polyploid meiosis, I used the ASY1 gene variants (from diploid and tetraploid *Arabidopsis arenosa*) to investigate their role in oligomerization and DNA binding. I found that naturally occurring evolution can modify these properties and might be playing a role in how tetraploids cope with the challenge of polyploidy.

Linker histone H1 as a facilitator of trascriptomic reprogramming during diurnal rhythm.

Kinga Rutowicz[1], Diana Zörner[1], Marc Schmidt[1], Maciej Kotliński[2], Tomasz Bieluszewski[3] and Célia Baroux[1]

[1]Department of Plant and Microbial Biology, University of Zürich, Zurich, Switzerland
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[3]Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poland

Email: kinga.rutowicz@uzh.ch

Long thought to be only chromatin bricks, H1s are revealing a facet in epigenetic regulations in both plant and animal cells. Our long-term objective is to elucidate the function of linker histones in nuclear organization and transcriptional reprogramming during cellular differentiation and cellular responses to developmental and environmental cues. We have shown that Arabidopsis H1 are dynamically regulated at developmental transitions and play the role of fine-scale
chromatin architects at the micro- and nanoscopic scale. (Rutowicz et al., 2019). While H1 appeared largely dispensable for plant growth under stable conditions, we observed that challenging conditions revealed unsuspected roles of H1 in physiological and transcriptional adjustments.
Here we present a project aiming at investigating the hypothesis that linker histones are molecular hubs for diurnal- based reprogramming in plants, taking Arabidopsis as model system.
Our working model is that H1-mediated chromatin organization offers an optimized arrangement facilitating reprogramming under developmental or environmental cues, securing thereby the robustness of cellular and transcriptional responses. What fuels our hypothesis is the observation that H1-depleted plants, such as in the *3h1* triple mutant, shows different phenotypes under diurnal and continuous light regimes at the level of root development and tissue regeneration.

MAR-binding filament protein-1 determines the location of starch granule initiation in chloroplasts

Mayank Sharma, Melanie R. Abt, Simona Eicke, Chun Liu, Theresa Ilse, Barbara Pfister and Samuel C. Zeeman

Institute of Molecular Plant Biology, Department of Biology, ETH Zurich, 8092 Zurich, Switzerland

The biogenesis of starch granules, one of the major carbohydrate compounds in plants, can be subdivided into two sequential processes: 1., the formation of granule initials and 2., their subsequent expansion. While much is known about the mechanisms of starch biosynthesis, ultimately leading to granule expansion, the phenomenon of granule initiation is still being explored. Recently, several coiled-coil domain containing proteins have been implicated in the process of starch granule initiation. Mutant plants lacking one or more of these proteins produce fewer starch granules per chloroplast compared to those of wild-type plants. Amongst these, MAR-binding filament protein 1 (MFP1) is the only protein directly associated with the thylakoids and regulates the membrane association of its partner Protein targeting to starch 2 (PTST2), another essential protein involved in granule initiation. To test if MFP1 also determines the location of starch granule formation, we designed and expressed a series of MFP1 variants 'mis'targeted to distinct chloroplast sub-compartments in Arabidopsis. The location of starch granules was examined in the leaf chloroplasts with electron microscopy-based approaches. The results are presented here and signify that MFP1 is indeed one of the main factors responsible and obligatory for the formation of starch granules at specific locations within the chloroplasts.

Investigate the functional role of 3D chromosome folding in nuclear defense systems and molecular actors involved using reverse ChIP approach

Edouard Tourdot and Stefan Grob

Department of Plant and Microbial Biology – University of Zürich

Invasive DNA elements, such as viruses and transposable elements (TEs) are ubiquitous major contributors to evolution in all organisms. Their mobilisation can support adaptation to environmental challenges but can also have catastrophic consequences by triggering chromosomal rearrangements, such as deletions, inversions, transpositions, and translocations. Thus, invasive element mobilization directly affects the host's fitness. To safeguard the genome from this potential threat, a multitude of mechanisms have evolved to prevent mobility of invasive elements. The defence must comprise two major steps: 1) The identification of foreign DNA and 2) the transcriptional or post-transcriptional silencing of the invasive element. Elucidating how invaders are detected bears great potential, as it addresses the fundamental question: How can cells distinguish between endogenous and exogenous genetic material? Stefan Grob have discovered a 3D nuclear structure termed the KNOT; this structure is shaped out by specific genetic elements called KNOT Engaged Elements (KEE). This 3D nuclear structure is a novel genome defence system, termed KNOT-linked silencing (KLS). KLS induces ectopic 3D chromosomal contacts between the transgene insertion site and the KNOT. During my postdoc I aim at identifying potential proteins interacting within the KNOT with the KEEs and potentially responsible of their capacities of recognising foreign DNA. To do so, I will develop and challenging methods, for example a reverse Chromatin ImmunoPrecipitation named Cas9 locus-associated proteome (CLASP).

Investigating post-translational control and trafficking of Arabidopsis PHOSPHATE1 (AtPHO1)

Pallavi Vetal and Yves Poirier

Department of Plant Molecular Biology, University of Lausanne

Arabidopsis PHOSPHATE 1 is a transporter expressed in the root xylem parenchyma and involved in exporting Pi from root to shoot. It has a cytosolic SPX domain followed by six transmembrane spanning α-helices, ending with a domain called EXS. PHO1 is localized at the Golgi/trans-Golgi network and the EXS domain is important for this localization. Considering the role of PHO1 as a Pi exporter to the apoplast, we investigated if PHO1 can be transiently associated with the PM by inhibiting the most common endocytic pathway, the clathrin-mediated endocytosis (CME). We show that interference with CME leads to PHO1 localization at the PM, indicating that PHO1 is internalized from the PM via endocytosis. PHO1 endocytosis is independent of canonical Adaptor Protein complex 2. Moreover, the EXS domain of PHO1 fused to GFP is also stabilized at the PM by inhibiting CME, indicating that this domain is crucial for its sorting to/from the PM. Reduction in ^{33}P efflux from root to shoot in PM-stabilized PHO1 lines indicate that recycling of PHO1 from the PM to TGN is important for its Pi export activity. Current efforts aim at identifying the region of PHO1 responsible for its trafficking at the PM.

Investigating mitochondrial molecular organization across photosynthetic organisms using cryo-electron tomography

Florent Waltz and Benjamin D. Engel

Biozentrum, University of Basel

Mitochondria are essential components of eukaryotic cells that act as metabolic hubs and powerhouses, producing energy through aerobic respiration. Recent studies have revealed that the main molecular complexes found in mitochondria, involved in gene expression and metabolism, are strikingly different across eukaryotic species. In the Engel lab, we use cryo-electron tomography (cryo-ET), a cutting-edge technique that unravels the native organization of molecular complexes directly inside the cell. We apply cryo-ET to investigate organelle biology (nucleus, ER, Golgi, chloroplast), specifically on photosynthetic organisms.

Using a combination of biochemistry, high resolution cryo-EM and cryo-ET I study the molecular diversity of mitochondrial complexes across the photosynthetic lineages and how this diversity impacts mitochondria ultrastructure.

Recently, we have been investigating the mitochondrial ribosome of the green alga *Chlamydomonas reinhardtii*. Combining biochemistry, single particle cryo-electron microscopy, and *in situ* cryo-ET, we report the 3D structure and functional analysis of the *C. reinhardtii* mitoribosome, revealing an extreme example of ribosome evolution and species-specific adaptation. Our structure revealed the high divergence of this ribosome, composed of reduced and fragmented rRNAs stabilized by species-specific r-proteins, and our cryo-ET data unraveled the native organization and structure of these mitoribosomes inside mitochondria. I am now expanding this approach to plants and other microalgae.

Immature leaves are the volatile sensing organs of maize

Lei Wang, Simon Jäggi, Jamie Waterman, Mario Walthert and Matthias Erb

Institute of Plant Sciences, University of Bern

Plants perceive herbivore-induced volatiles and respond to them by upregulating their defenses. So far, the organs responsible for volatile perception remain poorly described. Here, we show that responsiveness to the herbivore-induced green leaf volatile (Z)-3-hexenyl acetate (HAC) is largely constrained to younger maize leaves. Older leaves are less sensitive to HAC, but more sensitive to non-volatile elicitors. In a given leaf, responsiveness to HAC is high at immature developmental stages and drops off rapidly during maturation. Neither stomatal conductance nor leaf cuticle composition explain this pattern, suggesting perception mechanisms upstream of canonical defense signaling as driving factors. In conclusion, our work identifies immature maize leaves as dominant volatile sensing organs. The tight spatiotemporal control of volatile perception may facilitate within-plant defense signaling to protect young leaves, and may allow plants with complex architectures to explore the dynamic odor landscapes at the outer periphery of their shoots.

List of participants (alphabetical):

Daniele	**Albertini**	ETH Zurich
Robin Eva	**Bautzmann**	U Bern
Kevin	**Bellande**	U Neuchâtel
Kyle	**Bender**	U Zurich
Anja	**Boss**	U Bern
Joaquin	**Clua**	U Lausanne
Christian	**Fankhauser**	U Lausanne
Nasim	**Farahani Zayas**	U Lausanne
Natalia	**González Gaarslev**	U Lausanne
Xiaoyu	**Hou**	U Zurich
Lena	**Hyvärinen**	U Geneva
Jonatan	**Isaksson**	U Zurich
Léa	**Jacquier**	U Geneva
Henry	**Janse van Rensburg**	U Basel
Aime	**Jaskolowski**	U Lausanne
Darina	**Koubínová**	U Neuchâtel
Miguel	**Loera Sánchez**	ETH Zurich
Beatrice	**Manser**	U Zurich
Aditya	**Nayak**	ETH Zurich
Sandi	**Paulisic**	U Lausanne
Kinga	**Rutowicz**	U Zurich
Klaus	**Schlaeppi**	U Basel
Mayank	**Sharma**	ETH Zurich
Edouard	**Tourdot**	U Zurich
Pallavi	**Vetal**	U Lausanne
Florent	**Waltz**	U Basel
Lei	**Wang**	U Bern
Wojciech	**Wietrzynski**	U Basel

www.spsw.ch