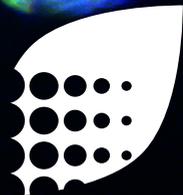




BREAKTHROUGHS IN PLANT SCIENCES

PSC SYMPOSIUM DEC 5TH 2018

Zurich-Basel Plant Science Center
www.psc2018.ethz.ch



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Zurich-Basel Plant Science Center (PSC)

Publisher

Zurich-Basel Plant Science Center

Tannenstrasse 1

8092 Zürich

Editors

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Cover picture

Powdery mildew thriving on a wheat leaf.

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Print

ETH Zurich

20th Anniversary of the
Zurich-Basel Plant Science Center

Breakthroughs in Plant Sciences

PSC Symposium
5th December 2018
ETH Zurich

The Symposium is made
possible through funding
by the Zurich-Basel Plant
Science Center.

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Organization

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→ www.plantsciences.ch

Venue

ETH Zurich, Auditorium Maximum (HG F30)

Rämistrasse 101, 8006 Zurich

Symposium website

→ www.psc2018.ethz.ch

Admission is free of charge.

Program

Wednesday, December 5th, 2018

09.00 Opening remarks by
Prof. Beat Keller, U Zurich and Zurich-Basel Plant Science Center

Session 1 PLANT SIGNALING AND DEVELOPMENT

09.30 -

11.00 FERONIA and the regulation of receptor kinase-mediated
 signaling

Prof. Cyril Zipfel, U Zurich, Switzerland

Decoding the Compatible Pollen Response Pathway in the
Brassicaceae Stigma

Prof. Daphne Goring, U Toronto, Canada

Auxin integrates growth and stomatal response during drought

Prof. Mark Estelle, U California, San Diego, USA

11.00 -

11.30 Coffee break and poster session

Session 2 PLANT ECOLOGY AND EVOLUTION

11.30 -

13.00 Using Arabidopsis thaliana to understand climate-driven
 adaptation

Prof. Detlef Weigel, MPI Tübingen, Germany

Hydrogen isotopes in plant organic compounds as indicator for
plant metabolic processes?

Prof. Ansgar Kahmen, U Basel, Switzerland

Integrating macro- and micro-evolutionary perspectives on plant diversity: breakthroughs from genomic data at multiple hierarchical levels

Prof. Elena Conti, U Zurich, Switzerland

13:00 Lunch and poster session

Session 3 INNOVATIONS FOR PLANT IMPROVEMENT

14.30 -

16.00 Improving photosynthesis efficiency in plants, dream or reality?

Prof. Mark Aarts, U Wageningen, The Netherlands

Natural and induced plant genomic instability

Prof. Luca Comai, U California, Davis, USA

Genome editing with programmable nucleases in crop plants

Prof. Caixia Gao, CAS and U Copenhagen, Denmark

16.00 Keynote

Global perspective on the importance of plant science research

Dr. Susanne Brink, Editor of Trends in Plant Sciences

16.30 - 17.00 Closing remarks and poster prizes by **Prof. Samuel C. Zeeman**,
Chair of the Zurich-Basel Plant Science Center

Invited speakers

in speaking order

FERONIA and the regulation of receptor kinase-mediated signaling

Cyril Zipfel

University of Zurich, Switzerland

Cyril Zipfel is the Chair of Molecular & Cellular Plant Physiology at the University of Zurich (Switzerland) (since June 2018). Before that, he was Senior Group Leader and Head of The Sainsbury Laboratory (TSL) in Norwich (UK) where he had his group since 2007. He performed his doctoral (2001-2005) and post-doctoral research (2005-2007) in the laboratories of Profs. Thomas Boller (Friedrich-Miescher Institute, Basel, Switzerland) and Jonathan Jones (TSL Norwich, UK) (supported by an EMBO Long-Term Post-Doctoral Fellowship), respectively. He is a pioneer and leader in the field of plant innate immunity and receptor kinases. His work is focused on understanding the molecular basis of plant innate immunity mediated by surface-localized immune receptors, as well as the application of this research to engineer disease resistance in crops. He was awarded competitive European Research Council grants in 2012 and 2018, is a Highly Cited Researcher (since 2014), and was awarded Charles Albert Shull Award from the American Society of Plant Biologists in 2015 and the 4th Tsuneko & Reiji Okazaki Award from Nagoya University in 2018. He was elected to the European Molecular Biology Organisation in 2018.

Abstract

Plants genomes encode hundreds of cell surface-localized receptor kinases (RKs) that control almost all aspects of plant life, ranging from reproduction, growth to responses to the external environment. Using RKs that function as immune receptors by perceiving microbial elicitors, we are studying the molecular basis of plant immunity, but also more generally how plant RKs work at the mechanistic level. Using the leucine-rich repeat RKs FLS2 and EFR (which perceive bacterial flagellin and EF-Tu, respectively) as model systems, we are investigating how plant RKs function as part of multimeric protein complexes at the plasma membrane – often in complex with other RKs, which act as regulatory proteins. Our recent work uncovered the importance of these regulatory RKs and RK-associated proteins in controlling the assembly of functional heteromeric receptor complexes. These observations also raise the inherent question of how these dynamic receptor complexes get formed and organized at the plasma membrane. Notably, we have recently uncovered an important role of the *Catharantus roseus* RLK1-like (CrRLK1L) FERONIA (FER) as a regulatory RK controlling immunity. Building on this recent published work, I will present our unpublished work that sheds light on the mechanisms underlying ligand perception by FER, and on the more general role that FER may play in the regulation of multiple signaling pathways beyond immunity and reproduction.

Decoding the compatible pollen response pathway in the Brassicaceae stigma

Daphne Goring

University of Toronto, Canada

Daphne Goring is a Professor in the Department of Cell & Systems Biology at the University of Toronto. She is the Vice-President of the Canadian Society of Plant Biologists and a Fellow of the American Association for the Advancement of Science. Daphne Goring's research investigates the cellular mechanisms that discriminate between compatible and self-incompatible pollen grains in the Brassicaceae to regulate sexual reproduction. More specifically, her research explores the cellular signalling pathways in the Brassicaceae pistils that control the recognition and rejection of self-pollen to prevent inbreeding (self-incompatibility pathway), and the delivery of resources to compatible pollen to promote pollen hydration and germination (compatible pollen response pathway).

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Abstract

In Brassicaceae species (*Brassica*, *Arabidopsis*), the first step towards sexual reproduction is initiated when pollen grains are deposited on the stigmatic papillae at the top of the pistil. The characteristic Brassicaceae dry stigmas enable a rapid control over the fate of pollen grains following pollination. It is at this stage, that the well-known self-incompatibility response is activated to reject self-pollen. The key regulators of self-incompatibility have been identified as the pollen SP11/SCR ligand and the stigma S Receptor Kinase, and some downstream signalling events have been defined. Lesser known is how compatible pollen is recognized and distinguished from foreign pollen, to elicit requisite cellular responses in stigmatic papillae. This pollen-stigmatic papillar communication is needed for pollen hydration, germination and pollen tube entry into the stigma. Following this, the pollen tube continues its journey down the pistil to deliver the sperm cells to an ovule for fertilization. Small peptide ligands on the pollen surface, such as the PCP-Bs, are proposed to mediate the initial compatible pollen recognition, but the corresponding receptors in the stigmatic papillae are unknown. We are currently investigating a number of candidate signalling proteins that may be responsible for initiating compatible pollen responses in the stigma. Our previous work has also uncovered a role for vesicle trafficking via the exocyst complex in the stigma and we are now investigating the involvement of other vesicle transport machinery in this pathway. Finally, we are interested in gaining a better understanding of the cellular dynamics that take place in the stigmatic papillae following the simultaneous activation of the self-incompatible and compatible pollen response pathways.

Auxin integrates growth and stomatal response during drought

Mark Estelle

University of California, San Diego, USA

Mark Estelle received his Ph.D. in Genetics at the University of Alberta in 1983 where he worked on the regulation of gene expression in *Drosophila*. After postdoctoral work with Chris Somerville at Michigan State University, he established his own lab at Indiana University studying the important plant hormone auxin. He is now the Tata Chancellor's Endowed Professor in Cell and Developmental Biology at the University of California San Diego. Mark Estelle's lab continues to explore the mechanism of auxin action as well as the role of ubiquitin-dependent protein degradation in cellular regulation. His research group uses the genetically tractable plant *Arabidopsis thaliana* to identify key proteins in auxin signaling including the auxin receptor. His recent work is focused on understanding the specificity of auxin response in different cellular contexts.

Abstract

The plant hormone auxin controls growth and diverse physiological processes through a complex transcriptional network that includes thousands of genes. Auxin regulates gene expression by promoting the degradation of transcriptional repressors called Aux/IAA proteins. The 29 Aux/IAA genes in *Arabidopsis* exhibit unique but partially overlapping patterns of expression. Although some studies have suggested that individual Aux/IAA genes have specialized functions, genetic analyses of the family have been limited by the lack of loss-of-function phenotypes. Recently, we demonstrated that several Aux/IAA proteins are required for drought tolerance in *Arabidopsis*. In our latest work, we show that the Aux/IAs act by maintaining glucosinolate (GLS) levels in drought conditions. Further, we show that GLSs, normally associated with resistance to insect herbivory, also promote stomatal closure. Based on our work we propose that auxin acts to integrate growth and stomatal response during drought. GLSs are a relatively recent adaptation that are found primarily in the Brassicaceae. Our results suggest that in addition to their role in defense, they are recently evolved signaling molecules.

Using *Arabidopsis thaliana* to understand climate-driven adaptation

Detlef Weigel

MPI Tübingen, Germany

Prof. Detlef Weigel, a German-American scientist, is currently a director at the Max Planck Institute for Developmental Biology. He is a member of the US National Academy of Sciences, the German National Academy of Sciences Leopoldina and the Royal Society, and recipient of several scientific awards. The first major finding from his lab was that an *Arabidopsis thaliana* gene could dramatically accelerate flowering of trees; this established a proof of concept for *Arabidopsis* genetics as a platform for biotechnological discoveries. His group later discovered the first plant microRNA mutant and the factor that turned out to be the long sought-after mobile flower-inducing signal. In the past decade, his work has come to incorporate aspects at the interface of evolution and ecology, including an international effort to sequence the genomes of many natural *A. thaliana* strains (The 1001 Genomes Project). Detlef has an extensive record of service to the scientific community, having served on a series of editorial and advisory boards. He is a forceful advocate of open access publishing and founding Deputy Editor of eLife. He is a co-founder of three biotech startups.

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Abstract

My group is addressing fundamental questions in evolutionary biology, using both genome-first and phenotype-first approaches: (i) Where do new genetic variants come from? (ii) Why are some variants maintained for a much longer time than others? (iii) And why are some combinations of variants incompatible with each other? The background for these questions is our population genomic work in *Arabidopsis* and the related genus *Capsella*. In collaboration with Bergelson, Ecker, Mott, Nordborg, Schmid and others, including Monsanto, we have been describing whole-genome variation in wild isolates of *A. thaliana* (<http://1001genomes.org>). This has, for example, led to the discovery of a Neanderthal-like group that has apparently survived since the Last Glacial Maximum. A similarly surprising finding that emerged from the *Capsella* work with Neuffer, Slotte and Wright is the ubiquity of long-term balancing selection, specifically at immunity loci. On the other end of the spectrum, we are analyzing new DNA mutations and epigenetic variants that have arisen under laboratory conditions or in a natural mutation accumulation experiment. The latter studies take advantage of an *A. thaliana* lineage that was apparently introduced to North America in historic times and accounts for about half the population there (with Bergelson and Burbano labs). We have been able to support what we see in the extant North American population by whole-genome sequencing of herbarium samples from the 19th century.

The ultimate goal of our top-down studies is to understand how genetic and epigenetic variation interact with natural selection to shape geographic patterns of diversity. One example is our efforts to predict which *A. thaliana* populations will and which populations will not be able to adapt to climate change. In my talk, I will focus on a rainfall manipulation experiment carried out in Spain and Germany with over 500 *A. thaliana* accessions with complete genome information. Integration of fitness data with climate metrics reflecting where genetic variants occur geographically allows us to deduce the strength of selection driven by local climate across the range of the species. With rapidly increasing droughts and rising temperatures in Europe over the coming century, we forecast a wave of new directional selection forces moving North, causing genetic turnover and decimation of native *A. thaliana* populations. Additional information about our work can be found on our website, <http://weigelworld.org>.

Hydrogen isotopes in plant organic compounds as indicator for plant metabolic processes?

Ansgar Kahmen

University of Basel, Switzerland

Ansgar Kahmen is an associate professor at the University of Basel and group leader of the Physiological Plant Ecology unit since 2013. The overall motivation of his research is to investigate the ecophysiological processes in plants that determine the fluxes of water, nitrogen and carbon in ecosystem. With his research, Prof. Kahmen seeks to understand how plants function in the context of a changing environment. While ecophysiological processes in plants are at the heart of his interest, his investigations have a strong link to research in agriculture, biogeochemistry and earth-system sciences. Key tools for his research are stable isotopes that his groups uses to characterize ecophysiological processes and as recorders of changes in the environment. To date, his work has resulted in 57 peer-reviewed publications in multidisciplinary and leading ecological and plant sciences journals. He was awarded the the Strassburger Prize of the German Botanical Society for outstanding research in 2007 and the highly-regarded Dr.-Karleugen-Habfast Award of the German Stable Isotope Association for outstanding research using stable isotopes in 2012.

Abstract

In contrast to the stable carbon, nitrogen and oxygen isotope composition of plant materials, the hydrogen isotope composition of plant organic compounds has not yet been established as a tool in ecological or biogeochemical research. With the development of new analytical instruments that allow the hydrogen isotope analysis of selected plant compounds there is, however, growing interest to explore the power of hydrogen isotopes as tools for ecological and biogeochemical research. In my talk I will present our recent work indicating that the hydrogen isotope composition of plant organic compounds reflects the carbon and energy metabolism in plants. Our experiments revealed that that autotrophic and carbon-autonomous plant tissue is ^2H depleted while heterotrophic or non-carbon-autonomous tissue is ^2H enriched. We can show that these patterns apply for various levels of organization: across plant species (e.g. heterotrophic parasitic plants and their autotrophic hosts), across different organs within an individual plant (e.g. autotrophic leaves vs. heterotrophic roots), and even within a plant organ (e.g. following the transition of a leaf from a carbohydrate sink to a carbohydrate source during ontogeny). As such, the hydrogen isotope composition of plant tissue seems to be a potentially powerful proxy for the carbohydrate metabolism in plants that could serve thus as an important new tool in plant ecology, plant breeding, biogeochemistry, and paleoecological applications.

Integrating macro- and micro-evolutionary perspectives on plant diversity: breakthroughs from genomic data at multiple hierarchical levels

Elena Conti

University of Zurich, Switzerland

Elena Conti received her undergraduate degree from the University of Bologna (Italy) and Ph.D. from the University of Wisconsin-Madison (USA), followed by post-docs at Washington State University and Harvard. Back in Europe, she has been a Professor at the University of Zurich (Switzerland) for 18 years and was Chair of the Department of Systematic and Evolutionary Botany for six years. She served as elected member of the scientific committee of the UNESCO Diversitas-bioGENESIS program for several years and has been recently elected as Councilor for the Society of Systematic Biologists. She is also Associate Editor for *Annals of Botany*. At the recent International Botanical Congress in Shenzhen (China) she organized a symposium titled "Evolution of plant reproductive systems: from ecology to genomics". Elena Conti studies the origin and evolution of biological diversity at different hierarchical scales, from phylogenomics to hybridization and introgression. Her current research program focuses on: 1) Evolution of plant reproductive strategies and their associated floral morphologies, with emphasis on heterostyly in primroses; 2) Biogeography of alpine-arctic and Mediterranean island plants.

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Abstract

One of the biggest challenges in biology is the integration of knowledge at different hierarchical levels, but current breakthroughs in sequencing technology, computational infrastructure, and analytical methods make this goal more attainable than ever before. Recent reviews have assessed the state of our knowledge on angiosperm evolution, focusing primarily on macroevolutionary patterns. However, the link between macroevolutionary outcomes and the processes that generate them is still largely missing. For example, once we identify a morphological, ecological, or genomic change that appears to be correlated with a shift of diversification rates in a phylogeny, can we discover how that change shaped the dynamics of speciation and extinction? What are the genetic underpinnings of complex phenotypic traits, for example heterostyly, and how do they affect evolutionary trajectories? In this talk, I argue that a fruitful approach to connecting macro- and microevolution is by combining studies at multiple hierarchical scales in focal taxa, de facto turning them into model lineages for evolutionary biology. I will refer primarily to my work on primroses, where I strive to link macro- and micro-evolution, phenotype and genotype. In primroses, I investigate the interconnections between hierarchical levels by integrating evidence from phylogenetics, population genetics, comparative genomics, experimental and functional studies, and morphological and modeling analyses. I will conclude by highlighting current challenges to a more holistic understanding of evolution before suggesting possible ways forward.

Improving photosynthesis efficiency in plants, dream or reality?

Mark Aarts

Wageningen University, The Netherlands

Mark Aarts is full professor at the Laboratory of Genetics of Wageningen University (The Netherlands). At Wageningen University, he also obtained his PhD with a thesis describing the development of a heterologous transposon system in *Arabidopsis*, which was successfully used for the isolation of tagged mutants and the corresponding genes. In his post-doc he applied this system for the analysis of disease resistance loci against downy mildew. As a researcher at the PRI in Wageningen, he investigated programmed cell death related to plant development. Upon moving to the group of Maarten Koornneef at Wageningen University, he set up a new research line on the evolution of heavy metal adaptation in the Zn/Cd/Ni hyperaccumulator *Noccaea caerulescens*. Since a few years, he is interested in the genetics of plant photosynthesis. He is currently heading a research group investigating different aspects of natural variation in plants (*Arabidopsis thaliana*, *Noccaea caerulescens* and other Brassicaceae and *Gomphrena* sp./Amaranthaceae), related to adaptation to the abiotic environment (drought, cold, nutrient deficiency, heavy metal exposure, varying light regimes), with a strong focus on the environmental effects on photosynthesis.

Abstract

The genetics of photosynthesis efficiency is not much investigated, even though breeding for photosynthesis would be interesting to maintain increases in crop yields. One of the reasons is the notorious difficulty in adequately phenotyping photosynthesis parameters for genetic research. We developed the Phenovator, a phenotyping platform for high-throughput imaging of light use efficiency of photosystem II electron transport (Φ_{PSII} or F_q'/F_m') through chlorophyll fluorescence measurements. It has been used to phenotype *Arabidopsis* recombinant inbred line populations, a full set of 49 reciprocal cybrids (novel cytoplasm-nucleotype combinations) and a diversity panel of around 350 genetically diverse accessions for genome wide association analysis. Plants were phenotyped several times per day, at optimal conditions and abiotic stressful conditions. The observed genotypic variation was used to identify quantitative trait loci (QTL) and corresponding candidate genes as well as genes involved in cyto-nuclear interactions.

Natural and induced plant genomic instability

Luca Comai

University of California, Davis, USA

Luca Comai is professor of Plant Biology at the Genome Center of the University of California at Davis. He has B.S. equivalent from the Università di Bologna, Italy, and a Ph.D. in plant pathology from UC Davis. In his career, he has worked on bacterial plasmid genetics, plant biotechnology (glyphosate resistance via alteration of EPSP synthase), and genetics and genomics of polyploidy. He co-developed TILLING, a method to identify targeted mutations. Since joining UC Davis in 2006, he has focused on function and regulation of chromosomes in polyploid genomes and on stress-induced genome instability. Dr. Comai teaches the foundation genetics course at UCD using a flipped approach. He has authored over 130 publications, has an H impact factor of 69, is a Fellow of the American Association for the Advancement of Science. In 2017 he received the ASPB Innovation Prize for Agricultural Technology.

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Abstract

Genome integrity is dependent on balancing DNA replication, recombination, and repair. We are studying genome instability resulting from stresses. One is haploid induction, a sexual process resulting in uniparental progeny with a haploid genome complement, which we are studying in *Arabidopsis* and potato. Haploid induction is often associated with genome elimination, such as when modification of CENH3, the major epigenetic determinant of centromere function, results in chromosome mis-segregation. Another stress results when protoplasts are regenerated into plants. The resulting “somaclonal variation” has uncertain causes. By characterizing the structural outcome of instability, our results identify potential similarities between these processes and provide a framework for understanding and engineering of plant genomes.

Genome editing with programmable nucleases in crop plants

Caixia Gao

Chinese Academy of Sciences and University of Copenhagen

Caixia Gao is Principal Investigator of the Institute of Genetics and Developmental Biology (IGDB), Chinese Academy of Sciences. Prior to joining IGDB in 2009, she served as Research Scientist of DLF's biotechnology group in Denmark, where she worked in plant genetic transformation and molecular biology. Her current research area mainly focuses on developing a highly efficient and robust CRISPR platform in plant cells to enable targeted genome editing and the application of the resultant methods in improving plants traits for high-quality, disease resistance and stress tolerance in crop species.

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Abstract

Crop improvement requires the constant creation and use of new allelic variants. Conventional breeding can be limited in providing the genes and alleles required to meet the agricultural challenges. In the past decade, Genome editing can accelerate plant breeding by allowing the introduction of precise and predictable modifications directly in an elite background. The most promising utilization of the CRISPR/Cas9 system can be used to generate targeted genome modifications including mutations, insertions, replacements and chromosome rearrangements. The use of CRISPR in agriculture should be considered as simply a new breeding method that can produce identical results to conventional methods in a much more predictable, faster and even cheaper manner.

Keynote

Global perspective on the importance of plant science research

Susanne Brink

Editor of Trends in Plant Sciences

Susanne Brink is the editor for *Trends in Plant Science*, a monthly review journal with broad coverage of basic plant science. Before moving into scientific publishing, Susanne worked at the bench for over ten years. The overarching topic of her research was aimed at understanding of protein movements in the cell. In particular she studied targeting signals and sorting mechanisms for nuclear encoded chloroplast and peroxisomal proteins on their route to their final destination in the cell. Susanne holds a Masters in Biology from the University of Goettingen, Germany and a PhD in Biology from the University of Wuerzburg, Germany. She moved to England in March 1995 with a long-term EMBO fellowship to research the targeting of thylakoid proteins at the University of Warwick. In March 1997 she moved to the University College London (UCL) to broaden her interest in protein targeting mechanisms by shifting the focus of her research from plant cells to mechanisms in animal cells. In September 2000 Susanne left bench work behind to accept the position as editor for *Trends in Plant Science*, which is published by Elsevier as part of a series of 15 *Trends* journals, and belongs to the wider Cell Press publishing group. Since 2002 she has also been involved with people management tasks in the *Trends* journal group and currently holds the title of Senior Managing Trends Editor.

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Poster abstracts

Beyond catalysis: the role of starch synthase 5 in the biogenesis of starch granules

Melanie R. Abt, David Seung, Barbara Pfister, Samuel C. Zeeman

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Transitory starch is a plant storage compound produced as granules in the chloroplasts during the day, and degraded at night to provide energy and carbon sources to sustain metabolism. Starch consists of two glucose polymers that differ in structural complexity; amylopectin and amylose. Whereas amylose is synthesized by a single granule-bound starch synthase (GBSS), amylopectin synthesis requires the activity of several soluble starch synthases (SSs), branching enzymes and debranching enzymes. In *Arabidopsis thaliana*, four isoforms of SSs, SS1-SS4, have been characterized so far. All of them transfer the glucose moiety of the substrate ADP-Glucose to a growing glucan chain; however, SS4 is special since it is also a major determinant of starch granule initiation. An additional, previously uncharacterized protein is present in many species that shares similarities with canonical SSs - particularly SS4 - but also has unique features. We call this protein starch synthase 5 (SS5) although bioinformatic and biochemical analyses suggest that it is catalytically inactive. The phenotypes of single *ss5* mutants, and of double mutants also lacking other enzymes involved starch biosynthesis, indicate that SS5 also acts to promote the process of starch granule initiation. We are using combinatorial approaches to obtain information ranging from individual protein properties to protein-protein interactions networks, and to the analysis of epistatic behaviors of genes. This work is shedding new light on aspects of starch metabolism that have remained in the dark so far.

Trait-dependent resemblance of flowering phenology and floral morphology in the allopolyploid *Cardamine flexuosa* to the parental diploids in natural habitats

Reiko Akiyama, Stefan Milosavljevic, Matthias Leutenegger, Rie Shimizu-Inatsugi

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Allopolyploids possess complete sets of genomes derived from different parental species and exhibit a range of variation in diverse traits. Reproductive traits may play a key role in reinforcing reproductive isolation between allopolyploid and its parental species, influencing how allopolyploids thrive. However, empirical data comparing variation in reproductive traits in allopolyploids with parental species are still rare, especially from natural habitats. Here, we documented flowering phenology and floral morphology of the allopolyploid wild plant *Cardamine flexuosa* and its diploid parents *C. amara* and *C. hirsuta* in their native range in Switzerland. Flowering of *C. flexuosa* peaked at intermediate timing with flowering period overlapping with parents. *Cardamine flexuosa* resembled *C. hirsuta* in the size of flowers and petals and the length/width ratio of flower and petal, while it resembled *C. amara* in the length/width ratio of flower. The results provided empirical evidence of trait-dependent variation of allopolyploid phenotypes in natural habitats at local scale. They also suggest that variation in some reproductive traits in *C. flexuosa* to be associated with self-fertilization. Consideration of the mating system can therefore contribute to the understanding of the processes that may have formed trait variation in polyploids in nature.

The life style of *Colletotrichum lupini* during plant colonization

Joris Alkemade^{1,2}, Monika M. Messmer¹, Maria R. Finckh³, Ralf T. Vögele², Pierre Hohmann¹

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The fungal pathogen *Colletotrichum lupini* causes anthracnose disease in lupin, preventing the crop's successful re-introduction in the temperate and humid regions of Europe. In order to develop relevant resistance assays and identify successful disease control strategies, the life style and cycle of this pathogen need to be further understood. The *Colletotrichum* genus contains a wide variety of plant pathogens with distinctive life styles. These life styles can be broadly classified as necrotrophic, hemibiotrophic, quiescent and endophytic, with hemibiotrophism being the most common. Typical for a hemibiotrophic life style is the switch from a biotrophic lifestyle into a necrotrophic one. Switching between life styles throughout host colonization makes detection complicated, as asymptomatic plants might have to be considered sources of infection as well. Primary infection of *C. lupini* originates from infected seeds. However, plants originating from infected seeds do not show obvious disease symptoms until later stages of plant development. Determining the relative fungal biomass in distinctive plant tissues during successive stages of germination and plant development showed significant fungal development without obvious disease symptoms or a decrease in plant growth. This suggests that *C. lupini* maintains an endophytic or weak biotrophic life style, prior to switching to necrotrophism. Healthy looking seeds and young plants can therefore be sources of disease inoculum later in the growing season. This highlights the importance of quantifying *C. lupini* in seeds and improving seed health in order to reduce primary disease inoculum.

Regulation of plant immune signaling by receptor kinase phosphorylation

Kyle W. Bender

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Plasma-membrane (PM) localized receptor kinases (RKs) and receptor-like proteins fulfill central roles in both plant development and response to environmental cues. In particular, members of the plant leucine-rich repeat (LRR) RK family are integral to the activation of plant immunity following perception of pathogen derived molecular patterns (PAMPs). Among the earliest responses to PAMP perception is receptor phosphorylation, however, the functional importance and mechanistic basis for control of immune signaling by RK phosphorylation remains largely undescribed. Using ELONGATION FACTOR Tu RECEPTOR (EFR) as a model RK to understand the impact of phosphorylation, we reveal that site-specific phosphorylation of EFR differentially impacts distinct branches of the immune signaling pathway. Additionally, we demonstrate that the protein kinase activity of EFR is dispensable for immune signaling. Collectively, our physiological analyses suggest that EFR does not play a direct role in signal propagation by phosphorylation of downstream substrates. We propose that EFR phosphorylation controls the dynamics of receptor complex assembly following stimulation by its cognate ligand. To address this hypothesis, we are developing approaches using labelled ligands to isolate both active and inactive PM-localized EFR complexes for characterization by mass spectrometry.

The transcriptional landscape of pattern-triggered immunity

Marta Bjornson¹, Thorsten Nürnberger², Cyril Zipfel¹

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24 One of the best-studied aspects of plant response to pathogen attack is the perception of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by plasma membrane-localized pattern recognition receptors (PRRs). While transcriptional responses to PAMPs/DAMPs have often been studied individually, it is still unknown whether plant cells differentiate the origin or nature of the perceived PAMPs/DAMPs, and what the master regulators of major, rapid immune transcriptional changes are. To address these questions, we have undertaken a transcriptomics study focusing on early timepoints, using a panel of PAMPs/DAMPs selected to represent a diversity of molecular makeup, originating organism, and corresponding PRR class. This has revealed a large set of genes induced rapidly by all assayed PAMP/DAMPs, most but not all of which are also induced by multiple abiotic stresses, representing a general stress response (GSR). A known key GSR regulator is required for PAMP-induced disease resistance, and gene regulatory network analysis has implicated several other transcription factors as novel GSR regulators. Finally, despite the strong GSR, many genes are also induced in a PTI-specific or elicitor-specific manner, and ongoing work is investigating the role of these genes in plant responses to pathogens. Thus, combining a panel of elicitors with an early-focused time series has defined the importance of the GSR in PTI, revealed genes responsive specifically to PRR signalling, and implicated new PTI responding and regulating genes with roles in plant immunity.

Multidimensional imaging reveals fusion and anisotropic growth of semi-crystalline starch granules

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Starch is a plant storage carbohydrate that plays a central role in buffering the diel patterns of photosynthetic carbon assimilation. Although composed of simple glucose polymers, starch forms as discrete, semi-crystalline granules, with defined locations, numbers and shapes within the chloroplast. Understanding starch biosynthesis requires knowledge of both its biochemistry and its cell biology. While much biochemical progress has been made, there is little cell biological knowledge. To address this we combined several complementary imaging techniques. Serial block-face scanning electron microscopy revealed either single or clustered granule initials form in stromal pockets between the thylakoid membranes. By combining $^{13}\text{C}_2$ stable isotope labelling with nanometer-scale secondary ion mass spectrometry (nanoSIMS) we demonstrate coalescence of starch granule initials and subsequent anisotropic granule growth, resulting in their characteristic lenticular shape. These insights provide a new conceptual framework for understanding the synthesis of this vital plant product.

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Breeding dandelions as a new source of natural rubber

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Natural Rubber (NR) is an essential commodity for our society. The EU is totally dependent on imports. >99% of production of natural rubber comes only from one source, the rubber tree (*Hevea brasiliensis*). Its cultivation is geographically limited to tropical zones, mainly in South-Asian countries, and is nowadays threatened. Therefore the EU has recently included NR in its Critical Raw Materials list, as the only material of biological origin. Finding an alternative rubber source, also suitable for European cultivation, is an urgent issue. The rubber dandelion, *Taraxacum koksaghyz* (TKS), is one the most promising alternatives. However, this wild plant is small and does not produce sufficient rubber to be economically profitable. We developed a breeding protocol for the selection, that allowed us to increase the rubber production of TKS and obtain breeding lines which yield is more than 4 times higher than the wild dandelion. The results of this research, combined with the work of many other stakeholders at different stages of production chain, lead us to conclude that the establishment of this novel industrial crop will enable the EU to become less dependent on the import of natural rubber and at the same time to respond to the global rubber shortage.

Guard cell sugar metabolism for stomatal movement

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Stomata are tiny pores on the leaves' surface that allow the plant to exchange gases and photosynthetic byproducts with the atmosphere. Guard Cells (GCs) are specialized cells that regulate stomatal movements by altering their turgor in response to environmental stimuli, accumulating inorganic ions and organic osmolytes in their vacuoles. Here we show that hexose transport across the plasma membrane by Sugar Transport Proteins (STPs) plays a major role in *Arabidopsis* GC's ability to accumulate starch and execute their physiological function. Similarly, we investigate the role of cell wall invertases (cwINVs) in supplying GCs with hexoses.

Research into the function of RubisCO in green seeds

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This work aims to test genetically a CO₂ recycling pathway proposed to operate during the development of green seeds, including many that are agronomically important. RubisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) is famous for its role in CO₂ assimilation as part of the Calvin-Benson cycle in leaves, which generates carbohydrates for export to the sink tissues. Energy-rich lipids, which are stored in many seeds, are derived from these transported carbohydrates. During the synthesis of fatty acids for lipid production, one molecule of CO₂ is released for every two carbon atoms incorporated, representing significant carbon loss. Previous work on developing *Brassica napus* L. embryos proposed that RubisCO, working outside of the context of the Calvin cycle, could increase the efficiency of lipid synthesis by reincorporating CO₂. This pathway was supported by models and radioactive labelling experiments, but not tested genetically. I use mutants of *Arabidopsis thaliana*, - a relative of *B. napus* L. that also has green, lipid-rich seeds - to reduce the flux through RubisCO in a seed-specific way. Interestingly, abolishing the RubisCO pathway in seeds leads to a reduction in lipid content greater than predicted by the proposed recycling pathway and a reallocation of carbon into other pools.

High-density linkage map enables chromosome-scale assembly of the *Marchantia polymorpha* genome

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M. polymorpha has recently become a prime model for investigation in many research fields, from cell biology to evolutionary ecology. Due to its phylogenetic position and the assumption that the earliest land plants were liverwort-like, it is frequently used to investigate how plants colonized and adapted to the terrestrial environment and how increasing overall complexity has evolved throughout the land plants. Here we present a chromosome-scale assembly of the *M. polymorpha* genome using a high-density linkage map with a total map length of 712 cM and an average marker density of 0.1 cM. Using the linkage map, we arranged 90% of the scaffolds of the v1.3 assembly into eight linkage groups corresponding to the eight autosomes of *M. polymorpha* covering 208 Mbp of the genome. We found that overall genome structure of *M. polymorpha* is in some aspects different from that of the model moss *Physcomitrella patens*. Specifically, recombination rates vary considerably across the chromosomes and correlate both with genetic polymorphisms.

Spatiotemporal dynamics of nutrient capture in mixed cropping systems

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As agricultural systems have become more intensive, biodiversity in crop cultivation has declined while artificial inputs to control pests and weeds and to enhance soil fertility have increased. Counteracting this development is necessary to prevent further detrimental effects on surrounding environments. Agroecology aims to do just that: sustainable and efficient crop production with less chemical inputs by increasing the diversity of the system. Mixture cropping is a promising agroecological concept, where at least two crop species are grown simultaneously in close proximity and the increase in biodiversity drives the productivity of the system. This increase in productivity has often been related to a better use of resources, where complementarity (different plants use spatially, chemically or temporally different resource pools) or facilitation (different plants facilitate resource uptake of neighbouring plants) effects are enhanced by biodiversity. Our understanding of the mechanisms underlying complementarity and facilitation effects in cropping systems are still limited but crucial to successfully plan future agroecological systems.

The aim of this project is to broaden our understanding of nutrient cycling (nitrogen, phosphorous, carbon) and resource use (nutrients, light) in mixture cropping systems compared to monoculture systems. Recent work has stressed the importance of including temporal dynamics of plant-plant interactions to better understand complementarity effects, as most experimental assessments of such dynamic plant-plant interactions are based on single time-point measurements of the final yield and consequently ignore dynamic processes preceding the harvest. Repeated measurements of plant N (nitrogen) and P (phosphorous), biomass and light interception during the growing season will shed light on the dynamic coupling between nutrient capture, growth and aboveground competition for light of plants grown in mixture and monoculture systems. To detect facilitative effects and niche differentiation processes the percentage of plant N derived from different sources (soil, seed, atmosphere) and interspecific P facilitation with the help of the enzyme acid phosphatase will be observed throughout the growing season. Similarly, aboveground partitioning of light will be measured throughout the growing season as it has been suggested from previous work that belowground resource partitioning may not be the main driver for enhancing productivity in diverse communities. The experimental work will be conducted in an experimental garden in Spain with a semiarid climate on sandy, nutrient-poor soil as facilitative effects have been observed mainly in such stressful settings.

Management-induced differences in the bacterial community composition of permanent grasslands are temporally stable over a growing season

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Agricultural management is a strong driver of microbiome composition in permanent grassland agro-ecosystems. It is not currently clear, however, how temporally stable such management-induced differences are over the course of a growing season. 3 permanent grassland management types were used for this study; intensive, low intensive and extensive, with a sown grassland-to-maize rotation being used as a control. 10 separate fields of each management type were selected from around the region of Zurich, Switzerland from which soil samples for microbiome analysis were taken seven times over the 2017 growing season (April to October). The bacterial 16S rRNA gene was PCR amplified from each sample and an amplicon-based Illumina Miseq sequence analysis was conducted. Agricultural management had a highly significant effect on bacterial community composition ($P < 0.001$), with each of the four management types harboring significantly distinct bacterial communities. No significant difference in the bacterial community was seen between the seven sampling events in either the intensive, low intensive or extensively managed permanent grasslands ($P > 0.05$). There were, however, significant differences seen between the first two samplings and the later sampling events in the maize rotation ($P < 0.05$). Such changes were associated with the ploughing of the sown grassland. Further work will examine the fungal community composition. These results highlight the stability of management-induced differences in bacterial community composition in permanent grasslands. They will inform a larger European study investigating the influence of both agro-climatic region and management type on the microbiome composition in such grasslands.

Improving southern anthracnose resistance in red clover

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Red clover (*Trifolium pratense* L.) is one of the most important forage legumes in temperate climates. It is an excellent feed for cattle due to its high protein content and digestibility, and its ability to fix atmospheric nitrogen improves and maintains soil fertility. In recent years, losses in red clover due to southern anthracnose caused by *Colletotrichum trifolii* increased, increasing the demand for resistant cultivars.

F1 progenies of four reciprocal bi-parental crosses were spray inoculated with a single-spore isolate of *C. trifolii*. Survivor plants were again spray inoculated using a mixture of seven additional isolates, and survivor plants were again recorded. Single isolate inoculation yielded a segregation ratio of 1:1 and 1:0, respectively, indicating that one dominant gene was governing resistance. Inoculation with the isolate mixture strongly suggested the existence of at least one additional resistance. Genotyping by sequencing (GBS) of pooled leaf samples revealed potential candidate genes involved in basal defense against fungal pathogens.

The knowledge gained to date will be enhanced by the phenotyping and the genotyping of 400 red clover accessions. Anthracnose resistance will be assessed in the greenhouse and in the field at different locations. The data collected will be used for genome wide association mapping. This study will provide insight and tools to support genomics-assisted breeding strategies that accelerate breeding progress and result in superior red clover cultivars.

TTL genes are essential signaling components required for cellulose biosynthesis under stress conditions

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As sessile organisms, plants require mechanisms to sense and respond to the challenging environment, that encompass both biotic and abiotic factors that result in differential development. In these conditions is essential to balance growth and stress responses. As cell walls shape plant growth, this differential growth response because alterations to the plant cell wall and cellulose is a major component. Therefore, understanding the mechanisms that regulate cellulose biosynthesis is essential to develop strategies to improve plant production. Previous studies have shown that the GSK3 kinase BIN2 modulate cellulose biosynthesis through phosphorylating cellulose synthases and that the expression of cellulose synthases are regulated by brassinosteroids. Our previous work reveals that the tetratricopeptide-repeat thioreoxin-like (TTL) TTL1, TTL3, and TTL4 genes, in addition to their reported role in abiotic stress tolerance, are positive regulators of BR signaling. We observe association of TTL3 with most core components in transducing BR signalling, such as LRR-RLK BRI1, BIN2 and the transcription factor BES1 that positively regulate cellulose biosynthesis. We show that *ttl* mutants are affected in cellulose biosynthesis, particularly in osmotic stress conditions. Furthermore, TTL3 associates with LRR-RLKs that have been shown to be important for cellulose biosynthesis such as FEI1 in the FEI1/FEI2/SOS5 pathway. We aim to investigate the mechanisms by which TTL proteins regulate cellulose biosynthesis using a combination of genetics, biochemical, and molecular and cell biology approaches.

This work was supported by grants from: (1) Ministerio de Ciencia e Innovación BIO2014-55380-R, BIO2014-56153-REDT; (2) Ministerio de Economía, Industria y Competitividad (BES-2015-071256); (3) Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech.

Identification of a RNA-binding protein as a novel negative regulator of plant immunity in *Arabidopsis thaliana*

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Immune responses are often under tight control to avoid excessive or untimely activation of cellular responses, which could otherwise be detrimental to the homeostasis of host cells. Despite increasing knowledge in animals, how innate immunity is fine-tuned in plants remains largely unknown. A forward-genetic screen was performed to identify *Arabidopsis thaliana* mutants that regained immune signaling in the immunodeficient genetic background bak1-5. This screen led to the identification of 10 modifier of bak1-5 (mob) mutants, which regained immune signaling and resistance to the bacterial pathogen *Pseudomonas syringae*. We mapped the mob7 mutation to a gene encoding a protein of unknown function. Interestingly, MOB7 is conserved among land plant species and is not induced by elicitors. In addition, recent studies found that MOB7 is a novel RNA-binding protein, which interacts with the eukaryotic translation initiation factor, eIF4E. Ongoing work will identify MOB7-associated RNAs and proteins in order to elucidate its function in the regulation of plant innate immunity.

Identifying key factors involved in *Marchantia polymorpha* sexual reproduction using tissue-specific transcriptome and methylome data

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The alternation of generations, involving a multicellular sporophytic and gametophytic phase, is a hallmark of the plant life cycle. This process, characterized by gamete formation, fertilization and the associated ploidy change, has a common evolutionary origin and thus includes many pathways and regulatory factors that are shared across the land plants [Bowman et al. (2016) *Ann. Rev. Genet.* 50, 133]. Although several factors have been described that play a role in sexual reproduction, our understanding of the complex network regulating this complex process is far from complete.

The liverwort *M. polymorpha* has become a model organism for both evo-devo studies and functional genetic analyses, due to its basal evolutionary position among land plants and its streamlined genome. Making use of different publicly available *M. polymorpha*, transcriptomic datasets, in combination with our in-house methylome tissue atlas and our on-going transcriptome profiling data of reproductive cells and tissues, we attempt to identify some of the key factors that play a role in gamete differentiation and embryo development. With this analysis we intend to confirm the role of previously described players in cell specification and how they are conserved across evolution, but also to propose new candidates that may play crucial roles for the sexual reproduction of *M. polymorpha*.

Understanding the effects of allopolyploidy on genetic diversity and selection using *Arabidopsis kamchatica*

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Allopolyploidization is the result of hybridization and whole genome duplication (WGD) and is relatively common in plants, including many crop species. Comparison studies between diploid parents and their derived subgenomes in polyploids are essential for understanding the genomic consequences of WGD. Here, we provide empirical evidence for theoretical long-term effects of polyploidy on the evolution of genomes. *Arabidopsis kamchatica* is a self-compatible, natural allotetraploid derived from the hybridization of self-incompatible *A. halleri* subsp. *gemmifera* (found in East Asia) and *A. lyrata* subsp. *petraea* (from Far East Russia). Demographic modeling reveals timing of bottleneck and subsequent expansion and, in addition to phylogenetic support, multiple hybrid origins. About 60% of SNPs in the subgenomes were shared with their corresponding parent. Furthermore, genome wide diversity of the two subgenomes was less than half of either diploid parent, which is an expected consequence of a bottleneck and transition in mating system. The distribution of fitness effects showed the subgenomes of *A. kamchatica* contained higher proportions of neutral mutations than in the respective diploid parental genomes, consistent with the lower effective population size estimated by demographic analysis. The proportion of adaptive substitutions (α) was positive for the three species, but lower in the allopolyploid. Taken together, we begin to characterize the conserved and divergent genomic patterns to help identify novel adaptations that have arisen after polyploidization and describe the impact of redundant gene copies on the evolution of plant genomes.

LRX proteins regulate cell wall development by interacting with FERONIA and RALF

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Each plant cell is surrounded by a rigid yet flexible cell wall that protects and provides stability to the cell. In order to coordinate cell growth and cell wall composition, numerous participants are involved. Among them are the *Catharanthus roseus* receptor-like kinases (CrRLK) including FERONIA (FER), which consist of an extracellular malectin-like domain, a transmembrane domain and a cytoplasmic kinase domain. FER regulates cell growth via interaction with extracellular RALF (rapid alkalinisation factor) hormone peptides. In the extracellular space are the LRR-extensin (LRX) proteins, involved in regulating cell wall development. Recent results show that in vegetative tissue, LRXs interact with FER and RALF1, suggesting that these three proteins are acting in the same process to regulate cell wall integrity and cell growth. This hypothesis is supported by the observation, that a multiple *lrx* mutant is phenotypically very similar to a *fer* mutant. Both show a dwarf phenotype and collapsed root hairs.

The LRX proteins consist of an extensin domain, which seems to insolubilize the protein into the cell wall. Further, they contain a cysteine rich domain, an LRR (leucin-rich repeat) domain, an N-terminal domain and a signal peptide. The LRR domain has been shown to be involved in protein-protein interactions with FER and RALF. Domain swap experiments showed functional equivalency of the vegetative expressed LRX proteins (LRX1,2,3,4 and 5) and partial functional equivalency of the LRR domain of the pollen expressed LRX8. However, the LRR domains seem to interact with the RALF peptide in a rather specific manner. It remains to be elucidated how the interaction specificity of the RALF peptides with the LRRs fit with their functional equivalency.

Two-source $\delta^{18}\text{O}$ method for g_m estimation

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Mesophyll conductance (g_m) significantly and variably limits photosynthesis through its limitation on the supply of CO_2 from the intercellular airspaces to the sites of carboxylation. The g_m can be estimated by coupling theoretical models to measurements of gas exchange and isotopic fluxes of either ^{13}C or ^{18}O . Uncertainties in the ^{13}C -model have been explored previously, but there has been little testing undertaken to determine the reliability of g_m estimates from the ^{18}O -model. In this study, we exploited the action of carbonic anhydrase in equilibrating CO_2 with leaf water and manipulated the observed photosynthetic discrimination ($\Delta^{18}\text{O}$) according to the isotopic composition of the source gas CO_2 and water vapour. We developed a 2-source $\delta^{18}\text{O}$ method, whereby two measurements of $\Delta^{18}\text{O}$ were obtained for a leaf with its gas exchange characteristics otherwise unchanged. Despite manipulating the $\Delta^{18}\text{O}$ by over 100%, in most cases we observed consistency in the calculated g_m , providing confidence in the theoretical model. Where there were differences in g_m estimates between source-gas measurements we explored uncertainty associated with micro-scale variation in leaf water isotopic composition and sub-saturation of the internal vapour pressure; we found evidence for both. We also provide experimental guidelines to minimise the sensitivity of g_m estimates to measurement errors.

POLAR-guided signaling complex assembly and localization drive asymmetric cell division

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Stomatal cell lineage is a prime example of asymmetric cell division (ACD) necessary for plant survival. In *Arabidopsis thaliana*, the GLYCOGEN SYNTHASE KINASE3 (GSK3)/SHAGGY kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) phosphorylates both the mitogen-activated protein kinase (MAPK) signaling module and its downstream target, the transcription factor SPEECHLESS (SPCH) to promote and restrict ACDs, respectively, in the same stomatal lineage cell. Yet, the mechanisms allowing for the coexistence of such mutually exclusive activities remain unclear. Here, we identified the plant-specific protein POLAR as a stomatal lineage scaffold for a subset of GSK3-like kinases, confining them to the cytosol and, subsequently transiently polarizing them together with BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) prior to the ACD. As a result, the MAPK signaling is attenuated allowing for SPCH to drive ACD in the nucleus. Moreover, GSK3-mediated phosphorylation of POLAR was required for its turnover. Our study uncovers an unknown mechanism, by which a scaffolding protein ensures GSK3 substrate specificity that might serve as a paradigm for understanding GSK3 regulation in plants.

Plant endocytosis and immune responses induced by *Fusarium oxysporum*

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40 Endocytosis is an essential process to control protein turnovers at the plasma membrane (PM), thus regulating signalling and other protein functions at the cell surface. In plant-microbe interaction, the plant immune response is initiated by the perception of microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) through PM-bound pattern recognition receptors (PRRs). Many PRRs are known to be internalized via clathrin-mediated endocytosis (CME) upon recognition of specific MAMPs or DAMPs. However, investigation of the role and dynamics of CME during immune response activation is limited. To shed light on this field, we use the model pathosystem *Arabidopsis thaliana*-*Fusarium oxysporum* 5176. *F. oxysporum* is an agronomically important plant pathogen that infects more than 100 crops by penetrating the root vasculature, blocking water transport, and resulting in wilt disease. By studying CME, PRRs involved in defense against early stages of *F. oxysporum* infection could be discovered.

In my work, I observed decreased CME adaptor lifetimes at the PM correlating with an increased endocytosis activity upon *F. oxysporum* perception (with germinated spores and elicitor mixture). Besides, CME adaptor mutants exhibited enhanced susceptibility to *F. oxysporum* infection compared to wild-type plants. These data support the essential role of CME in plant defense against this pathogen and open interesting questions regarding the molecular mechanisms behind. In addition, I applied a novel proteomic strategy to identify proteins internalized upon *F. oxysporum* infection. Following endocytic trafficking, I isolated organelles from the infected plants and analyzed the proteins immunoprecipitated with markers residing at these organelles. Among several candidates from the IPs, I focus on a receptor, Resistance to *Fusarium oxysporum* 7 (RFO7), which possibly recognizes *F. oxysporum*. Rfo7 mutants showed enhanced susceptibility compared to control plants, which supports the role of RFO7 as a PRR of *F. oxysporum*. The detailed molecular characterization of RFO7 function will help to deepen our understanding of early plant defense signalling in response to *F. oxysporum*.

Receptor-mediated resistance to *Fusarium oxysporum*: the story of RESISTANCE TO FUSARIUM OXYSPORUM 1 (RFO1)

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Improvements made to crop breeding and pesticide usage techniques during the 20th century are largely accredited for diminishing crop losses and increasing global food security. Even so, current average crop losses due to pathogens alone can be as high as 15% worldwide. *Fusarium oxysporum* spp. is one of the most important fungal pathogens of plants, infecting over 100 different crop species. *F. oxysporum* is a hemibiotrophic filamentous fungus that grows intercellularly and actively evades plant innate immune responses. To protect crops from such pests it is first necessary for plants to detect these invaders, for this, plants employ extra- and intracellular receptor proteins.

Here we aim to characterize the *Arabidopsis* plasma membrane receptor, RESISTANCE TO FUSARIUM OXYSPORUM 1 (RFO1) for application in future crop protection. Previous work has identified RFO1 as a defense receptor necessary for full resistance to *F. oxysporum* infection. However, it is not known what RFO1 detects and how it acts in mediating defense responses. Preliminary data presented here begins to reveal the nature of RFO1 including the range of *F. oxysporum* strains it defends against, its potential ligand(s) and changes in intracellular dynamics during infection.

A new regulator for protophloem differentiation: JUL1 (RBP2)

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Phosphoinositides are very important membrane lipid signalling molecules that are known to regulate various cellular processes. A tight balance between Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) and Phosphatidylinositol-4-phosphate (PI(4)P) is crucial for proper protophloem development. Two phosphatases mediating the conversion of PI(4,5)P₂ to PI(4)P are COTYLEDONVASCULAR PATTERN 2 (CVP2) and its partially redundant homologue CVP2 LIKE 1 (CVL1). In the *cvp2 cvl1* double mutant the protophloem strand has some undifferentiated cells, the so-called gap cells. This phenotype comes together with an impaired post-embryonic root growth and discontinuous vein pattern in cotyledons.

To identify new factors controlling protophloem differentiation, our group performed a suppressor screen using the *cvp2 cvl1* double mutant. This screen indicated that knockdown of JUL1 (JUL, a.k.a. RBP2) partially rescues the root phenotypes of *cvp2 cvl1* double mutant. It has been recently shown that JUL1 directly binds and induces RNA G-quadruplexes in the 5' UTR of SUPPRESSOR OF MAX2 1-LIKE4/5 (SMXL4/5), which are key promoters of phloem differentiation. We are now interested in characterising the role of JUL1/RBP2 in the context of CVP2/CVL1 mediated protophloem differentiation. Accordingly, we will analyse whether it binds to and regulates other important players in protophloem development which are differentially regulated in *cvp2 cvl1* mutants.

Is there a trade-off between pollen number and other traits in *Arabidopsis thaliana*?

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Recently, we identified REDUCED POLLEN NUMBER 1 (RDP1) as controlling pollen number gene by using GWAS and molecular functional analysis in *Arabidopsis thaliana*. We showed that null mutant of RDP1 produced about half of the number of pollen grains of the wild-type. Next we asked is there a trade-off between pollen number and any traits in *A. thaliana*? To answer this question, first we measured ovule number between the wild-type and RDP1 mutant. Unexpectedly, ovule number was also reduced in the RDP1 mutant. RDP1 is expressed not only in pollen cells but also in ovule cells and RDP1 which might affect ovule number also. Then, we measured candidate trade-off traits using F1 complementation cross lines (RDP1Uod-1/rdp1Bor-4 and RDP1Bor-4/RDP1Uod-1). These lines have the same background (one chromosome comes from small pollen number accession Uod-1 and another chromosome comes from large pollen number accession Bor-4) but have a different RDP1 allele (one line only has a functional RDP1 allele from Uod-1 and another line only has a functional RDP1 allele from Bor-4). We found that pollen number in plants with RDP1Uod-1 was significantly lower than in plants with RDP1Bor-4. In this study, we measured ovule number, flower organ sizes (sepal, petal stamen and pistil length), flowering time, rosette size, dry weight and seed weight using these lines and no trade-off was detected. These results suggest that allelic difference of RDP1 only affect the pollen number.

Receptor-Like Protein Kinase 2 (RPK2) acts as a negative regulator of phloem identity in *Arabidopsis thaliana*.

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44 Two specialized tissues form the vascular system of plants: xylem and phloem, each functioning to transport water and photoassimilates respectively. The regulated timing between cell proliferation and cell differentiation allows for constant growth within the apical root meristem, a stem cell niche located at the tip of the root. The phloem cell lineage is formed by an initial periclinal division in the sieve element/procambium precursor cell, resulting in a SE precursor and a procambium cell. A second periclinal division in the SE precursor produces two different sieve elements, proto- and meta- phloem where protophloem is actually the first tissue to differentiate within the root meristem. Previous studies have identified two enzymes involved in the phosphoinositide biosynthetic pathway, COTYLEDON VASCULAR 2 (CVP2) and CVP2 LIKE1 (CVL1) as regulators of vascular tissue differentiation and vein patterning. These enzymes catalyze the conversion of phosphatidylinositol (4,5) bis-phosphate (PIP₂) into phosphatidylinositol 4-phosphate (PIP). The *cvp2 col1* double mutant exhibits impaired post-embryonic root growth, a reduction in the number of the sieve element formative periclinal divisions, undifferentiated protophloem cells and a discontinuous vein pattern. A screen performed to identify mutations that suppress all root and vascular phenotypes observed in *cvp2 col1* identified RECEPTOR LIKE PROTEIN KINASE 2 (RPK2) as a potential candidate. In particular, we observed that a mutation in the kinase domain of RPK2 rescues *cvp2 col1* impaired root growth by restoring protophloem specification and thus differentiation. RPK2 is known to have a role in cell specification during embryogenesis and in regulating the maintenance of stem cell pools in the shoot as well as in the root meristems. In addition, the BARELY ANY MERISTEM 3 (BAM3) and CLAVATA3/EMBRYO SURROUNDING REGION 45 (CLE45) module is one example of a receptor-ligand pair known to function in the inhibition of sieve element specification. We have found *rpk2* to be resistant to the inhibitory effects of protophloem formation after exogenous treatment with CLE45 and other CLE peptides, suggesting that RPK2 is involved in various CLE peptide signaling pathways. The molecular characterization of RPK2 using cell fate markers and confocal microscopy has allowed us to discover its role as a negative regulator of vascular identity acting to restrict protophloem specification. We are further working on understanding the mechanism by which *rpk2* rescues the vascular defects observed in *cvp2 col1*.

Evaluation of a novel rice iron biofortification strategy involving facilitated iron loading onto phloem

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Over a billion people worldwide suffer from iron (Fe) deficiency. Among diverse measures to tackle human Fe deficiency, an approach to intrinsically increase the Fe concentration in the edible parts of the crop, known as Fe biofortification, is a promising way to ameliorate Fe deficiency. Rice (*Oryza sativa* L.) is an attractive crop for Fe biofortification because its area of cultivation largely overlaps with the distribution of human population affected by Fe deficiency. Since there is only a little genetic variation for the grain Fe concentration present in rice germplasm, genetic engineering rather than conventional breeding is a more realistic measure to improve polished rice Fe concentration. To date, only a few transgenic biofortification strategies that can barely reach the target polished rice Fe concentration in a limited number of genetic backgrounds have been reported. Therefore, efforts to further expand the pool of options to biofortify diverse rice cultivars more easily must be made. In our project, we aim to evaluate a potential novel genetic engineering strategy for rice Fe biofortification, which involves facilitation of Fe loading onto phloem. Rice phloem is a more efficient channel than xylem for nutrient loading onto the developing rice grains. Thus, we hypothesized that facilitating xylem-to-phloem Fe transfer by expressing an Fe transporter gene in rice stem nodes, which are a hub for intervascular nutrient exchange, leads to increased grain Fe loading. In addition, we will combine this strategy with constitutive expression of NICOTIANAMINE SYNTHASE, which boosts the synthesis of Fe chelators involved in Fe acquisition and internal transport, and/or endosperm-specific expression of iron storage protein gene FERRITIN. The polished grain Fe concentration and agronomic performance of the resultant transgenic lines will be evaluated in the subsequent generations.

Cellulose synthesis and biotic stress

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Plants are essential to cover our demand for food, fibre and biofuels. Pathogens cause extensive yield losses of crops and an improved understanding of plant defense mechanisms against pathogens is therefore of pressing importance. One of the most relevant plant pathogen is the soil fungus *Fusarium oxysporum* that causes high losses in more than one hundred crops worldwide. The high impact that *F. oxysporum* can have on crop plants is best represented by the near extinction of the first exported banana cultivar 'Gros Michel'. Despite this agronomic and therefore economic importance, very little is known about its recognition by the plant and further host adaptation to the infection.

Here we show that a plant cell already reacts within minutes to a fungal attack by shutting down cellulose synthesis and therefore growth. Furthermore, we show that the response is initiated by an immediate apoplastic pH change that perturbs the proton motive force of a cell.

Molecular basis of convergent evolution: Parallel reduction of the sporophyte phase in mosses

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Convergent evolution is the process whereby organisms independently evolve similar traits. Whether evolution of convergent morphological traits is achieved by similar genetic mechanisms is subject of much debate. We aim to provide detailed insights into the molecular mechanisms of convergent evolution by studying the repeated evolution of reduced sporophyte phenotypes in the moss family Funariaceae.

In bryophytes the dominant haploid gametophytic phase alternates with a diploid sporophyte phase. While the gametophyte remains largely unchanged within Funariid mosses, the morphology of the sporophyte differs drastically. Recent research on the phylogeny of the family has shown that a highly reduced sporophyte evolved multiple times independently. This repeated morphological reduction together with the simple structure of the sporophyte phase and their amenability for reverse genetic work makes the Funariaceae family an ideal model system to study convergent evolution in a set of closely related species.

To address the question how reduced sporophytes are established we gathered transcriptomic data of four developmental stages of sporophytes from *Funaria hygrometrica* and *Physcomitrella patens*. While *F. hygrometrica* represents the complex sporophyte phenotype, the sporophyte of *P. patens* is reduced to a simple spherical capsule without dehiscence mechanisms. By statistical and custom network analysis of the expression data we identified candidate genes that are potential key regulators of sporophyte architecture. The function of these candidate genes will be verified by reverse genetic approaches. Identification of genetic changes leading to a reduced sporophyte will allow us to expand research on other family members and compare molecular mechanisms between them.

Ecosystem services assessment of arable land in response to cropping system and drought

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Agroecosystems are nowadays often confronted with ecosystem degradation due to unsustainable intensification and climate change. One reason for intensification is that the projected demand for food and feed is increasing due to global population growth and dietary changes. Additionally, drought events, which are projected to increase in both frequency and severity in the future, will potentially have strong effects on the ecosystem services provided by agroecosystems.

Organic farming has been proposed to better cope with recent agricultural challenges. However, whether organic farming can solve the trade-off between production and non-production services remains unclear. Likewise, conservation tillage might provide considerable ecological benefits compared to conventional tillage, but the contribution of different tillage methods to the resilience of agroecosystems towards drought needs further investigation.

The objective of this study is to assess the response of ecosystem services in organic and conventional cropping systems with conventional and conservation tillage to simulated drought. Several provisioning, supporting and regulating ecosystem services are being measured. We collect data on aboveground biomass yield, crop quality, crop performance as provisioning services; litter decomposition, nutrient retention, symbiotic nitrogen fixation, soil fertility, nitrogen availability as supporting services; and nitrate leaching risk, plant infection and soil erosion risk as regulating services.

First results show drought effects on litter decomposition, an important measure of nutrient cycling, assessed via the Tea Bag method in a pea and barley mixture during 2018. The outcomes of this research will provide information on the performance of the two cropping system under drought.

Resilience of biodiversity to human disturbance is mediated by species invasion: a prickly issue

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Human activity is causing unprecedented environmental change, leading to more rapid losses of plant and animal diversity during the last 50 years than at any time in human history. Resilience is necessary to sustain desirable ecosystem states in variable environments and uncertain future. However, we have only limited understanding of the processes that determine the extent to which an ecosystem recovers from disturbance or transitions into a new state. We studied the resilience of biodiversity to human-driven ecological disturbance. Combining large-scale natural experiment with local-scale field experiment, we assessed whether and how plant diversity could recover to combination of overgrazing disturbance and species invasion and we analyzed the interactions among plants, invader species and livestock. The resilience of biodiversity to overgrazing disturbance was inhibited by species invasion. Under overgrazing pressure, the invader species protects and supports otherwise vulnerable biodiversity. Meanwhile, in absence of livestock, invader–plant interactions turn into negative thus inhibiting the resilience of biodiversity once overgrazing pressure ceased. Our study indicates that complex interactions among species invasion, biodiversity and livestock drive the resilience and recovery of the ecosystem to human activity.

Alteration of plant volatile emissions by plant-associated pseudomonads

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50 Plant odors can convey ecologically relevant information to other organisms. For example, changes in plant volatile emissions induced by insect feeding damage frequently serve as foraging cues for the natural enemies of insects. Such volatile-mediated interactions have important implications for the ecology of natural communities, as well as for applied efforts to manage herbivorous pests via the release of natural enemies. It is also increasingly clear that root-associated microbes, such as rhizobacteria and mycorrhiza, can influence the constitutive and induced emission of plant volatiles. However, we are only beginning to understand the implications of such effects for plant-insect interactions. This project investigates the potential of plant-associated pseudomonads to alter the constitutive and induced volatile emissions of tomato plants. Specifically, we are exploring (1) whether different *Pseudomonas* strains influence plant volatile emissions in different ways and how this is effecting natural enemy recruitment; (2) whether these effects differ when plants are attacked by two herbivore species with different degrees of feeding specialization; and (3) whether different *Pseudomonas* strains that influence herbivore-induced volatiles also alter floral emissions, with potential implications for pollinator attraction. Here we present preliminary findings from chemical analyses of headspace volatile collections and insect behavioural assays. This work will enhance our understanding of the ways in which root-associated microbes influence plant-insect interactions and explore their potential to increase the efficiency of biological pest control in agricultural ecosystems.

A Golgi-localized glycosyltransferase mediates the response of plant cells to cellulose perturbations

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The ability of plants to adapt to various stresses relies on rapid perception and subsequent response to external stimuli. Remodeling of the primary cell wall is essential for growth as well as adaptation to these external stresses. Cellulose, the main component of plant cell walls, is synthesized at the plasma membrane as glucan chains extruded into the apoplast, which assemble into paracrystalline microfibrils via interactions with other cell wall polysaccharides. This paracrystalline structure is important for tensile strength, cellular expansion, and response to invading pathogens. Recent works have demonstrated a role for apoplastic proteins in the maintenance of this cellulose paracrystalline structure, such as the chitinase-like protein CTL1/POM1. Mutations of CTL1 result in cellulose-deficient plants characterized by a dwarfed phenotype in all developmental stages and altered response to various stresses. Regulation of perception and response to cellulose perturbations is a major driver towards balancing optimal plant development and stress response activation. To identify proteins involved in this equilibrium, we screened for suppressors of *ctl1-2* in adult stage (*sca*). We isolated a mutant in a gene encoding for a Golgi-localized glycosyltransferase (SCA18) that reverts *ctl1-2* phenotypes back to wild-type (WT)-like. Mutations in SCA18 attenuate growth inhibition and ectopic lignification in *ctl1-2* plants, but did not affect WT, resembling the phenotype of THESEUS1 mutants. To better understand the structure and function of SCA18, we are currently employing cell biology, glycoproteomics, biochemistry and spectroscopy approaches. Our data opens the possibility of a regulatory role of SCA18 in cell wall integrity maintenance pathways by activating cell-wall-integrity sensors, such as THESEUS.

New workflow for whole genome bisulfite sequencing data analysis in plants with application to rapid adaptation of artificially synthesized polyploids

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Whole genome duplication (WGD) events are common in plants and polyploidization is considered an important motive force to produce diversity. Allopolyploidy is a WGD event where a new species arises after the crossing of two different species. The resulting polyploid species possesses both full genomes of the parents.

Most of the studies about allopolyploidization focus on the genetic aspect of this event to understand allopolyploids' success. In contrast, fewer studies focused on epigenetics. Recently it has been suggested that epigenetics may play a major role in short-term evolution as opposed to genomic changes playing a major role over longer timescales.

The allopolyploid *Arabidopsis kamchatica* ($2n=4x$) and its parental diploids *A. halleri* ($2n=2x$) and *A. lyrata* ($2n=2x$) provide an excellent model system to study both epigenetic changes over short timescales and their relation(s) with rapid evolution. The available genomic information of *A. halleri* and *A. lyrata* combined with the possibility of making artificially synthesized *A. kamchatica* individuals allows us to study DNA methylation in an early stage of polyploid speciation.

Currently, many tools are available to analyze DNA methylation at a whole genome level, but no official standard tools exist. The situation becomes more difficult when considering non-model species, because most of the existing tools have been used and optimized for model species.

The aim of this project is then twofold: the first is to develop a reproducible and optimized workflow to analyze whole genome bisulfite sequencing data from polyploids, and the second is to use the workflow to investigate epigenetic changes in "freshly" synthesized *A. kamchatica* individuals.

Wheat powdery mildew has a dynamic effector repertoire

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Blumeria graminis f.sp. tritici (*B.g. tritici*) is the causal agent of the wheat powdery mildew, an economically important disease of wheat. As obligate biotrophic fungi, powdery mildews modify the host cells to feed on them while at the same time avoiding the host's defense system. For this fine-tuned interaction, *B.g. tritici* relies on an arsenal of effector proteins. The highly fragmented *B.g. tritici* genome available so far has prevented a systematic analysis of effector genes that are known to be involved in host adaptation. To study the diversity and evolution of effector genes we produced a chromosome-scale assembly of the *B.g. tritici* genome and found that this highly repeat rich (>85%) genome encodes for 844 candidate effector genes, >40% more than previously reported. Candidate effector genes have characteristic local genomic organization such as gene clustering and certain transposable element families. A large group of 412 candidate effector genes shows high plasticity in terms of copy number variation in a global set of 36 isolates and of transcription levels. Our data suggest that copy number variation and transcriptional flexibility are main drivers for adaptation in *B.g. tritici*. The high repeat content may play a role in providing a genomic environment that allows rapid evolution of effector.

Pathotype-specificity of angular leaf spot in common bean – Genome wide association studies and implication for resistance breeding in Latin America and Africa

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54 Common bean (*Phaseolus vulgaris* L.) is an important food security crop because of its high protein level and affordable price. However, beans are frequently attacked by pests and diseases. One of the most devastating fungal bean diseases in the tropics is angular leaf spot (ALS), responsible for yield losses of up to 80%. Although pathotype-specificity of ALS resistance has been reported in common bean, little is known about the efficiency of resistance loci against different pathotypes. Here we conducted GWAS for ALS resistance in a large diversity panel of common bean. Surprisingly, only two of the five previously described resistance loci were found to be significantly associated with ALS resistance when tested with pathotypes from Colombia and Uganda in the field and greenhouse. The resistance locus on chromosome 4 was effective against one particular pathotype, while the resistance locus on chromosome 8 was effective in all trials. The latter locus was further dissected to characterize the haplotype-specific effect on ALS resistance: Of the eleven haplotypes at this locus, one haplotype was conferring broad-spectrum resistance and six haplotypes were showing pathotype-specific effects.

To the best of our knowledge, our study is the first to describe ALS resistance in such a diverse panel of common bean, tested with an extensive diversity of pathogen isolates on two continents. Our research also highlights the importance of understanding ALS pathotype-specificity to enable durable resistance management strategies for common bean. Molecular markers co-segregating with resistance loci or haplotypes will facilitate breeding for pathotype-specific ALS resistance.

Unravelling the genomics governing the hornwort-cyanobacteria symbiosis

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After an era of monocultures, agricultural research is now looking into diversity to improve current practices. This also takes the microbiome into account which surrounds the plant, supports the uptake of nutrients but is unique to each plant. Providing Nitrogen to plants, the symbiosis partner *Nostoc punctiforme*, a cyanobacterium shows great prospects to be translatable onto crop plants. However, many studies have been published about the cyanobacterial side of the symbiosis lacking information about the plant host. In Péter Szövényi's lab, we currently conduct a study to investigate the plant side of the mutualistic association using *Anthoceros agrestis*, a hornwort recently introduced as a model organism. During my Ph.D., I will dissect the genetic control of the symbiotic association through time course RNA-seq experiments. A systems approach will be taken to discover and prioritize candidate genes from both partners, and their functional involvements validated by reverse genetics. In addition, the hornwort orthologs of the genes of the common symbiosis pathway (CSP), which play a role in both rhizobia and arbuscular mycorrhiza (AM) symbioses, will be examined to test whether a conserved module exists that regulates all three forms of plant-microbe symbioses. This research will provide an overview on the interaction between hornworts and cyanobacteria, from the early communications to the symbiotic maintenance. The outcome will greatly complement what is known about plant interactions with rhizobia and AM fungi, and address whether the CSP also extends to cyanobacteria symbiosis. In addition, given the multiple origins of cyanobacteria symbiosis in plant tree of life, future work can build upon this hornwort study to examine other plant lineages, thereby testing if there is a unified molecular mechanism behind plant-cyanobacteria symbiosis. Furthermore, the essential genes and metabolites identified through this research will lay the foundation for future efforts to assess and design crop-cyanobacteria symbiosis.

Parentally inherited adaptations that may broadening habitats of allopolyploids: heavy metal hyperaccumulation and gene expression in *Arabidopsis kamchatica*

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Allopolyploids inherit entire sets of chromosomes from their diploid parents and have the potential to combine or merge parental adaptations. This may provide allopolyploids with a generalist strategy that can increase species distributions beyond the parental species' habitats and also provide enhanced abilities to tolerate extreme condition. The allopolyploid species *Arabidopsis kamchatica* is a natural hybrid of the diploid parents *A. halleri*, a heavy metal hyperaccumulator, and *A. lyrata*, a non-hyperaccumulating species. *A. kamchatica* plants collected from natural populations showed high levels of cadmium and zinc accumulation in leaves even at sites with low to moderate levels of these heavy metals in the soil. Using hydroponic growth chambers, all plant genotypes were able to hyperaccumulate and we detected significant quantitative variation in leaf and root accumulation of zinc. *A. kamchatica* requires higher treatment concentrations to achieve similar levels of leaf accumulation to *A. halleri*, indicating that the diploid has higher transport efficiency when treated with low amounts of zinc. We measured homeolog expression ratios of genes involved in hyperaccumulation using pyrosequencing and found a strong bias in the halleri-derived copies in most genes. Our results confirm that merging parental adaptations such as metal tolerance and hyperaccumulation may provide a generalist strategy in polyploids to inhabit diverse environments.

Wheat small RNA response to powdery mildew disease

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In the last decade small RNAs have been shown to play a key role in host-microbe interactions. In 2013, the study of Weiberg et al. had a major impact on what was known about cross-kingdom exchanges of small RNAs and provided the first example of fungal secreted small RNAs that were capable of silencing the plant's defense genes. These findings open a new scenario where closely living species might use RNA interference (RNAi) as an additional layer of interaction. In the case of a host-pathogen system, this would represent an "extension" of the more classic and wide studied Effector- (ETI) and pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) models. Wheat miRNAs are already known to be involved in basal cell biology and to be expressed in response to (a)biotic stresses. Therefore, we believe that such a unique and durable host-pathogen relationship could drive the evolution of a cross-kingdom RNAi mechanism that would complement or even regulate the already known ETI and PTI defense mechanisms. In this study, we want to uncover the existence of specific wheat-derived miRNAs that can be expressed in response to powdery mildew infection in order to 1) be secreted and down-regulate virulence genes that are important for the pathogen or 2) to regulate important resistance genes in the host (positive "feedback response"). The final goal of the study is to widen the understanding of the forces that are driving the interaction between wheat and mildews, an agronomically important groups of biotrophic fungal pathogens.

Real-time floral and mating system evolution mediated by pollinators and herbivores

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Pollinators can impose strong selection on floral traits, however in nature herbivory by insects is an important selection factor too. Nevertheless, our understanding of how floral traits evolve under different pollinator and/or herbivory scenarios is still limited. Using an experimental evolution approach, we investigated floral and mating system evolutionary changes in *Brassica rapa* subjected to four treatments: bumblebee-pollination (*Bombus terrestris*) and hand-pollination, with and without herbivory (*Pieris brassica* larvae); each treatment was replicated three times, each comprising 36 plants for a total of 8 generations. With this full factorial design we evaluated the impact of bumblebee-pollination (P), herbivory (H) and their interaction (H x P). We found that plants under selection by bee-pollinators evolved increased floral attractiveness, mainly due to a higher emission of the volatile compounds benzaldehyde, benzyl nitrile and p-anisaldehyde. On the other hand, plants that underwent herbivory during the experiment evolved an increased in their chemical defenses despite there were no longer herbivores triggering induced defenses (concentrations of glucosinolates in leaves and nectar). Plants under selection of both, bee-pollinators and herbivores simultaneously evolved higher degrees of autonomous selfing and reduced spatial separation of sexual organs (herkogamy). In conclusion, our experimental evolution approach was successful in addressing fundamental questions regarding adaptive floral evolution under different pollination and herbivory scenarios.

***Marchantia* MpFERONIA reveals ancient role of CrRLK1L function during both vegetative and reproductive development**

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Unlike in animals, the life cycle of all land plants alternates between two multicellular generations, the haploid gametophyte and the diploid sporophyte. Throughout the course of land plant evolution, the sporophyte replaced the gametophyte as the dominant generation, and both development and mechanism of sexual plant reproduction fundamentally changed. Whereas fertilization in more primitive plants requires spermatozoa that swim from the male to the female gametophyte, flowering plants deliver their immotile sperm cells to the embryo sac (female gametophyte) inside the growing pollen tube (male gametophyte). In the flowering plant *Arabidopsis thaliana*, many developmental aspects of fertilization, such as pollen tube growth and reception, are dependent on the joint functions of several members of the *Catharanthus roseus* RLK1-like (CrRLK1L) receptor-like kinase subfamily. The CrRLK1L family, which also controls a variety of developmental processes in vegetative development, comprises 17 members in *A. thaliana*. Individual members can have very distinct and sometimes opposing developmental functions, making it difficult to assess the primary or original function of the CrRLK1Ls. To reduce the genetic complexity, we explored the genome of *Marchantia polymorpha*, probably the most ancestral of land plants still extant today. Based on sequence homology, we identified a single CrRLK1L gene, which we named MpFERONIA (MpFER). Characterization of lines with reduced MpFER activity indicate that MpFER is involved in the vegetative development of *M. polymorpha* and controls rhizoid formation, overall growth, and cell size. These phenotypes indicate a conserved and basal function of the CrRLK1L family in cell expansion. In addition, spermatogenesis and male fertility are impaired in these lines during reproductive development. Analysis of the MpFER expression pattern suggests further potential functions in female gametangia development, as well as sporophyte development. Thus, the CrRLK1L gene family originates from a single gene that is involved in cell shape control and has a conserved role in the vegetative and sexual development of the gametophyte. During land plant evolution, this ancestral gene was recruited, diversified, and specialized to fulfill new developmental roles in the formation of both gametophytic and sporophytic structures in the angiosperm life cycle.

Adaptation to metalliferous soils: no gene, but gene-network convergence?

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Higher plants are rooted to the spot and obtain nutrients from the soil where they grow. However, some soils provide hazardous nutrient combinations, for example distorted concentrations of macronutrients or high concentrations of trace metal elements (TME). Such extreme soil environments require plants to adapt in order to survive.

We are interested in the genetic basis of adaptation to soils rich in TME (metalliferous) in two sibling species, *Arabidopsis arenosa* and *Arabidopsis halleri*, that have adapted in parallel to metalliferous soils. To identify the genomic regions that are most diverged between metallicolous (populations from metalliferous sites) and non-metallicolous populations, we sampled and whole-genome re-sequenced 65 individuals from metalliferous and paired non-metalliferous soils. In addition to divergence scans seeking loci under strong divergent selection, we performed environmental association analyses, linking genetic variation to TME concentrations.

Here we show that different sets of genes have been selected on metalliferous soils in the two sibling species. Even within species there is only limited evidence for gene-based convergence between site pairs, highlighting the complexity of adaptation to metalliferous soils.

Ongoing work aims at identifying gene regulatory networks mediating polygenic adaptation to metalliferous soils in both species and at functionally linking adapted genotypes to phenotypes in *Arabidopsis arenosa*.

Moisture stress tolerance in tef (*Eragrostis tef*)

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Tef [*Eragrostis tef* (Zucc.) Trotter] is a vital cereal crop in the Horn of Africa where it is annually cultivated on over three million hectares of land. It is a staple food crop for over 60 million people in Ethiopia. The grain of tef is nutritious as it contains high level of proteins and iron. Tef is also considered as a healthy food since its grain is gluten-free.

Despite its importance tef productivity is extremely low. The main causes for inferior yield are lodging (displacement of the stem due to rain and wind) and moisture stresses (waterlogging and drought).

In this study we investigate molecular mechanism altering the tolerance or susceptibility of tef plants to extreme moisture regimes. Our studies on waterlogging and drought are based on hydroponic systems. One part of the study is the investigation of morphological and physiological parameters (e.g. stomatal conductance, proline content and aerenchyma in adventitious roots). In addition, the expression on several genes known to be regulated in drought and waterlogging in other cereal crops will be investigated with quantitative PCR.

Root system of seabuckthorn

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Seabuckthorn (*Hippophae rhamnoides*) a fascinating berries producing plant has a root system with several interesting traits. We studied how availability of mineral nutrients affected root development in experiments at the Swedish University of Agricultural Sciences using plants cultivated in greenhouse, growth chamber and in vitro. Low P and Fe resulted in a special trait with more lateral roots and densely positioned rootlets having determinate growth similar to what is called a cluster root. Plants evolved at different origins showed different root morphologies. *H. rhamnoides* spp turkestanica originating in a natural population in northern Pakistan formed four times more cluster roots and a more branched root system compared to plants of *H. r. ssp mongolica* originating from breeding in rich black earth soil. Different Metabolites and gene expression patterns were observed during cluster root development. Formation of shoots from roots called root suckers were observed at the groove of lateral roots in natural population as well as in our in vitro cultivation system. Treatment with high P and auxin (IAA) gave highest production of shoots from root. Fluorescence microscopy of roots stained with 4',6-diamidino-2-phenylindole suggested meristem activation in the pericycle between endodermis and vascular tissue. The in vitro system allows studies of root traits and applications of propagation of seabuckthorn.

Biological control: fighting below ground insect pests with *Pseudomonas* bacteria

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Below ground insect pests are a yet unsolved problem not only in organic, but also in conventional crop production because they are difficult to target and the few effective chemical pesticides are already or will be banned in near future due to raising concerns for environmental and consumer safety. So far, mostly *Bacillus thuringiensis* was used in biological control of insect pests but resistance against major Bt toxins has been reported. This project aims at developing a new approach for the biological control of soil-dwelling pest insects compatible with organic production. We evaluate the potential of a specific group of plant-beneficial fluorescent *Pseudomonas* bacteria with entomopathogenic activity (EPP) for insect control as a new non-*Bacillus* bacterial biocontrol agent with a different mode of action. We further investigate whether below-ground insect biocontrol can be improved by combining EPP with entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN), which are already well-established biocontrol agents (BCA's) used in organic production. We will test promising EPP-EPN-EPF combinations in greenhouse and on-farm field trials against the cabbage root fly *Delia radicum*, a pest causing increasing losses in the production of brassicacean crops and for which no satisfactory control measures exist. This project will give exciting new insights into complex interactions between agriculturally important members of the soil and rhizosphere ecosystem. We hope to provide new methods based on the combined application of beneficial soil organisms for the control of an important insect pest in organic and conventional vegetable production, which may be adapted to other problematic soil pests.

Feasibility and adaptation of genome editing in *Malus domestica*

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Apple is the most important fruit in Switzerland and the second most important fruit worldwide. The outcrossing nature of apple, the long breeding cycle and the high demands on fruit uniformity and quality make it challenging to establish novel cultivars that are better adapted to changing environmental conditions. Genome editing enables the incorporation of beneficial genes into established cultivars, thereby shortening the time required to improve an existing cultivar with e.g. a resistance gene. The aim of this doctoral thesis is to adapt genome editing to apple, increase the efficiency of homology directed repair (HDR) and to obtain a powdery mildew resistant Gala cultivar without the introduction of foreign DNA during the breeding process. In plants, DNA-free genome editing requires the production of protoplasts. Apple protoplasts will be transiently transfected with purified ribonucleoproteins to induce targeted double strand breaks. A short DNA fragment will be provided to serve as repair template for HDR. I will use a stably transformed apple GFP line as a reporter line (GFP to BFP = HDR; GFP to non-fluorescence = non-homologous end joining (NHEJ); GFP to GFP = absence of editing event) to evaluate the efficiency of HDR-enhancing strategies. Such strategies include different structure of the DNA template, e.g. ssDNA or dsDNA or the use different ribonucleoproteins, e.g. Cas9 or Cpf1. After the editing event, I will establish a protocol for protoplast regeneration. A functioning protocol for DNA-free genome editing and apple protoplast regeneration will have wide implications on the apple breeding process and will enable to adapt existing cultivars to changing environmental conditions.

Environmental, evolutionary and spatial context-dependence of plant-plant interactions in crop systems, and effects on ecosystem diversity and functioning

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Ecology and agriculture are usually considered and treated by scientists as separate disciplines. However, bringing the two fields together could be doubly useful: indeed, applying ecological concepts to agriculture has the potential to increase the sustainability of our current food production systems while offering the opportunity to shed light on some ecological questions. In this project, we therefore propose to use an agricultural context to investigate crucial questions in ecology related to biodiversity-ecosystem functioning relationships. Specifically, we are using a biodiversity experiment with eight different crop species to study changes in plant-plant interactions in mixtures and monocultures under different environmental and evolutionary conditions, and their effects on several ecosystem services. In a first step, I will disentangle and quantify facilitation and niche differentiation/competition in crop communities of varying species richness and composition, under different levels of stress and with different coexistence histories. To do that, we will set up the biodiversity experiment in two European countries that differ drastically in terms of climate (Spain vs Switzerland), where we will repeat the setup in fertile and infertile soils, and with seeds coming from different coexistence backgrounds. This will allow us to understand the environmental and evolutionary context-dependence of plant-plant interactions. In a second step, I will use spatially-explicit ecological interaction models derived from interaction experiments to determine how crop productivity is affected by spatial patterns in intercropped systems and how to optimise the spatial configuration for highest productivity. This research will lead to a better understanding of the spatial dependence of plant-plant interactions and be useful for farmers to optimise their sowing patterns. Finally, I will investigate how diversity and the subsequent changes in plant-plant interactions and productivity scale up to the levels of weed and soil microbial communities. Specifically, in each experimental plot I will measure weed species richness and biomass, as well as soil microbial diversity through DNA analyses and soil microbial respiration as a proxy for microbial activity. Considered as a whole, the proposed project will lead to a better understanding of the environmental, evolutionary and spatial context-dependence of plant-plant interactions, and how these interactions scale up to weed and soil communities in an applied agricultural context.

Interplay between auxin and phosphoinositides in regulating proteases vacuolar trafficking during xylem differentiation in *Arabidopsis thaliana* roots

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66 Plant vasculature is formed by xylem tissues, which delivery water and nutrients from the root to the photosynthetic organs, and phloem elements responsible for the transport of synthesized sugars throughout the whole plant body¹. Xylem differentiation is a complex process encompassing several steps, from secondary cell wall formation (SCW) and lignification to eventual programmed cell death (PCD).² During PCD, xylem cells clear their cellular content via the release of proteolytic enzymes that are released into the cytosol after vacuolar degradation and rupture. XYLEM CYSTEINE PROTEASE 1 (XCP1) and its homologue XCP2 are two cysteine proteases that are loaded into the vacuole, participating in vacuolar micro-autolysis and in cellular macro-autolysis in the late stages of PCD². However, the mechanism that regulates the loading of this proteases into the vacuole remains unclear. Phosphatidylinositol (4,5) bis-phosphate [PtdIns(4,5)P₂] is a lipid compound virtually present in all cell membranes, recently described as a regulator of vacuolar trafficking during xylem differentiation³. We are exploring the hypothesis that XCP1 and XCP2 vacuolar loading is mediated by PtdIns(4,5)P₂, in an interplay with the phytohormone auxin, a known regulator of vacuolar morphology⁴.

Plant water relations under drought in organic and conventional farming systems

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Due to the changing climate, agricultural production systems will be progressively subjected to more frequent severe weather events, such as prolonged summer drought. Thus, there is an increasing need for a better understanding about water use of crop plants during water shortage. Adaptations of farming practices to climate change require assessing and improving the resilience of agricultural systems to ensure food security.

In Switzerland, the main farming systems are organic and conventional farming, with different tillage strategies i.e., intensive and conservation tillage (reduced tillage in organic farming and no-tillage in conventional farming). In order to evaluate the performances of different Swiss farming systems under drought, we assessed (1) how crop water relations significantly change under different farming systems given different soil water availabilities, and (2) if crop drought resistance was higher under organic compared to conventional farming, and under conservation tillage compared to intensive tillage.

Drought periods were simulated with portable rainout shelters. Water stress was estimated using a combination of leaf water potential measurements in the field and plant vulnerability to xylem embolism of pea and barley under different farming systems. Preliminary results indicate that (i) pea was more resistant to drought than barley showing a lower degree of xylem embolism under drought; (ii) the grown pea-barley mixture was generally more resistant to drought under intensive compared to conservation tillage, and in organic compared to conventional farming. Outcomes of this work will help to inform farmers and other stakeholders about necessary adaptations of soil and crop management to future climatic conditions.

Herbivores as drivers of demographic and evolutionary change in a plant invader under global warming

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68 Invasive alien plants together with their natural enemies from the native range used as biocontrol agents are ideal study system to address questions of whether and how fast organisms adapt to changing environments. Our study aims to get insights into the demography and evolvability of the European plant invader *Ambrosia artemisiifolia* to a recently introduced and potential biocontrol insect, *Ophraella communa*. Combining a species distribution model with underlying mechanistic processes, we are able to predict the abundance of the *O. communa*, and thus its impact on *A. artemisiifolia* across the area suitable in Europe for both the plant and its insect herbivore. Besides these ecological interaction studies, we presently also explore potential evolutionary changes in this plant-herbivore interaction under climate change. In an ongoing field selection experiment in N-Italy, using artificial populations of *A. artemisiifolia* exposed to *O. communa* under two temperature conditions, we assess the evolutionary changes of *A. artemisiifolia* populations. Pooled samples from each of the experimental populations will be analysed over four years (a) for their genetic composition using next-generation sequencing (pool-seq) (b) for metabolomic profiles and (c) in various bio-assays. By now, we collected seeds from the offspring generations from all four field treatments (warming by *Ophraella* herbivory) and grew them alongside their parents in growth chambers, in the quarantine, and greenhouse to assess the beetle preference and plant performance. I will present preliminary results of the quarantine preference and performance studies with *O. communa* on offspring plants from the field selection populations, and of the competition ability of offspring plants from greenhouse studies. I then will present the top differentiated SNPs (0.1%) among two generations from pool-seq analyses. These studies will improve forecasting of the biocontrol efficiency, and of the spread of invasive alien plants in a changing world.

Hornworts, a new window into early land plant evolution

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The monophyletic group of hornworts is believed to represent the immediate sister group of all vascular land plants. However, this traditional view is still debated and cannot be satisfactorily resolved owing to the lack of detailed knowledge on the general biology and genomic features of hornworts. Until now, advancement in this field was primarily hindered by the lack of genomic resources for a hornwort model species. Here we provide an update on the efforts towards a high-quality genome draft of the model hornwort, *Anthoceros agrestis*, and some of its relatives. We show that *A. agrestis* has a remarkably small genome, with few recent paralogs, which makes it appropriate for genetic analysis. We also provide an overview of the *A. agrestis* gene space and a preliminary gene expression atlas which shed light on the regulation of morphological and developmental traits that are either shared with other embryophytes or unique to hornworts. Furthermore, we report our first achievements on the genetic transformation of *A. agrestis* using various techniques. Finally, we summarize our achievements and provide a list of issues that need to be resolved in the future.

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Detecting alterations in starch granule morphology by high-throughput screening methods in a barley population

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70 Barley (*Hordeum vulgare* L.) is one of the major cereal crops worldwide and is used for feed and food purposes as well as a raw material for brewing beer. In particular, storage starch from barley seeds functions as the carbohydrate source for the yeast to produce alcohol. Starch is stored in discrete, semi-crystalline, water-insoluble granules, which differ in shape and size depending on the botanical origin. Barley shows a bimodal distribution of starch granules, where small, spherical shaped granules (\varnothing 1 – 8 μm) account for the vast majority of the total starch granule number, but only for a small fraction of the starch weight. Accordingly, the large, lenticular granules (\varnothing 8 – 25 μm) account just for a small amount of the total starch granule count but contribute most to the total starch weight. While comprehensive knowledge about the chemical composition of starch and the enzymes involved in its synthesis is available, the explanation of how the bimodal distribution of granules is established is still lacking. To identify involved genes, a screening for granule characteristics is conducted in a population carrying induced variation using flow cytometry and high-throughput microscopy. Through these approaches, mutants exhibiting changes regarding the bimodal size distribution of starch granules can be identified. This will provide a basis for further studies on the genes underlying starch granule initiation and for crop quality improvement.

Diversity in insect interactions within *Pseudomonas protegens* and *P. chlororaphis* strains isolated from roots and arthropods: adaptation to different hosts?

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Pseudomonas are versatile bacteria with the ability to adapt to many different ecological niches and, part of them, are known to promote plant-growth and control soil-borne pathogens. Furthermore, fluorescent pseudomonads belonging to the species *P. chlororaphis* and *protegens* are also able to colonize and persist in insects upon oral uptake, and, in some cases, to even cause lethal systemic infections. Among other factors, the Fit toxin was demonstrated to contribute to insecticidal activity in *Pseudomonas*. However, it is still unclear whether *P. chlororaphis* or *P. protegens* are commonly associated with insects in nature and whether there is an adaptation to root- or insect-associated lifestyles. In this study, we isolated *Pseudomonas* strains from roots and arthropods collected in agricultural fields and undisturbed areas. Isolates were tested for presence of the fitD gene, for systemic insecticidal activity and were phylogenetically classified using Multilocus-Sequence-Analysis. To investigate whether there is a certain niche adaptation, a selection of *P. protegens* and *P. chlororaphis* root and insect isolates were tested for oral insecticidal activity, root-colonization capacity and for the ability to control fungal root diseases. First results show that fit-harboring insecticidal pseudomonads are not only commonly present in the rhizosphere but also in arthropods, which implies that these animals are an additional ecological niche for certain pseudomonads. All investigated isolates can colonize roots and cause lethal systemic infections in Lepidoptera upon injection, but some *P. chlororaphis*, interestingly isolates from arthropods, could not efficiently infect and kill insects upon oral uptake. Our findings indicate that *P. chlororaphis* might be a more versatile group than previously thought, but also rises evolutionary and ecological questions concerning the interactions between pseudomonads bacteria and arthropods in nature.

Genetic pathways controlling meristematic activity in hornwort-sporophytes

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One major change that accompanied the evolution of embryophyte land plants from a haplontic ancestor was the elaboration of the sporophyte and parallel reduction of the gametophyte phase. Flowering plants have established complex branched sporophytes, which grow through the continuous activity of a multicellular meristem located at the tip of the shoot. Genetic developmental pathways controlling the activity of this shoot apical meristem have been researched extensively in flowering plants. In contrast, bryophytes, the most basal group of extant land plants, have subordinate, unbranched, monosporangiate and upright sporophytes that remain attached to the gametophyte generation. Bryophyte-sporophytes exhibit multicellular meristems that contribute to sporophyte growth, most notably the intercalary meristem of moss-sporophytes and the basal meristem of hornwort-sporophytes. Yet, regulatory mechanisms controlling the activity of these meristematic regions are not known. Comparison of regulatory gene networks controlling sporophyte development could help to resolve the evolutionary-developmental trajectory between bryophyte-sporophytes and the more complex sporophytes of vascular plants. Therefore, we are currently working to provide a detailed account on the regulatory mechanism governing sporophyte development in bryophytes. To this end, we established transcriptomic profiles for five different sporophyte tissues of the hornwort *Anthoceros agrestis*, using laser-assisted microdissection coupled with RNA-sequencing. Analysis of differential gene expression across the five tissues allowed us to establish a first hypothetical model for the regulatory mechanisms governing sporophyte development in hornworts. Future work will focus on describing fine-scale spatial expression of proposed candidate genes and testing their functional role using reverse genetic approaches.

A case study on plant vigour and soil enhancement in dynamic agroforestry systems with cocoa in Côte d'Ivoire

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More than half of the world's cocoa supply is grown in unsustainable monocultures in Côte d'Ivoire and Ghana. This comes with major disadvantages as it leads to large-scale losses of tropical forests, a decrease in biodiversity, degraded soils, high prevalence of pests and diseases, as well as vulnerability to climate change and subsequently unsustainable livelihoods of the farmers.

Dynamic agroforestry systems (DAFS) seem promising in combating many of these negative effects through higher biodiversity, increased nutrient cycling and diversification of cash crops.

We assessed a four-year-old DAFS with cocoa (*Theobroma cacao*) in Côte d'Ivoire in terms of soil fertility enhancement and checked whether the system created conditions suitable for cocoa growing. Sampling included climatic factors, soil parameters, biodiversity indices, as well as cocoa growth and vigour.

Elucidating beta-amylase 1 regulation for ABA-dependent starch degradation upon osmotic stress

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Under osmotic stress, the degradation of the storage starch sustains the production of soluble sugars acting as osmoprotectants and limiting the detrimental effects of osmotic stress. *Arabidopsis thaliana* α-amylase 1 (BAM1) is a key enzyme involved in the degradation of leaf starch in the light under osmotic stress. Transcriptional regulation of BAM1 by abscisic acid (ABA) uncovers an intimate cross-link between stress-induced ABA signaling cascade and starch metabolism.

In silico analysis revealed the presence of ABA Responsive Element (ABRE) in the BAM1 promoter of several dicotyledons, suggesting the conservation of ABA-mediated regulation of BAM1 among crops of economic importance such as tomato.

Our current and future investigations aim to corroborate the ABA-dependent starch degradation mechanism as a powerful target for the improvement of stress tolerance.

