26th ICSPR Prague 2022

20. - 24. June 2022

International Conference on Sexual Plant Reproduction

WELCOME

Dear Colleagues and Friends,

We are delighted to welcome you to the **26th International Conference on Sexual Plant Reproduction (ICSPR)** that will take place from June 20-24, 2022, at Hotel Pyramida in Prague (Czech Republic).

During the past two years of the COVID-19 pandemic, many workshops and conferences were cancelled or took place as virtual meetings. We have learned how to organize such events technically by using Zoom, Webex, Teams or other platforms, but despite some advantages, we also experienced a large number of disadvantages. We are especially missing the important individual scientific discussions, inspirations and exchange of ideas that take place after talks, during coffee breaks, lunch/dinner, poster sessions, and during meeting excursions. We also need an environment that fosters possibilities to initiate collaborations, make new friends, and to maintain long-standing friendships. We therefore decided to organize the 26th ICSPR exclusively in presence.

Unfortunately, this excludes participation of scientist from countries who followed the wrong COVID strategies. We are also aware that there is a war of aggression in Europe, thus it is difficult for some colleagues to attend, and some scientists are uncomfortable to travel by plane. Thus, despite these and other problems in difficult times, we are very happy to welcome you and more than 330 further scientists in the beautiful and historic city of Prague, the capital city of the Czech Republic, which is also called the “Golden City” or the “City of the Hundred Towers”.

You will find a very exciting program with topics ranging from flowering, gametogenesis, pollen tube growth and fertilization mechanisms to seed/fruit development that includes also ecological and epigenetic aspects of plant reproduction as well as the application of the knowledge generated. We hope you will enjoy the talks, poster session, discussions, and networking during the 26th ICSPR.

Thank you very much for your participation.

David Honys and Thomas Dresselhaus
(on behalf of the whole organizing team)

26th International Conference on Sexual Plant Reproduction

20. – 24. June 2022

Pyramida Hotel, Prague, Czech Republic

TABLE OF CONTENT

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VENUE

The 26th International Conference on Sexual Plant Reproduction will take place in the **OREA Hotel Pyramida Praha** (Prague, Czech Republic).

Address: **OREA Hotel Pyramida Praha**
Bělohorská 24,
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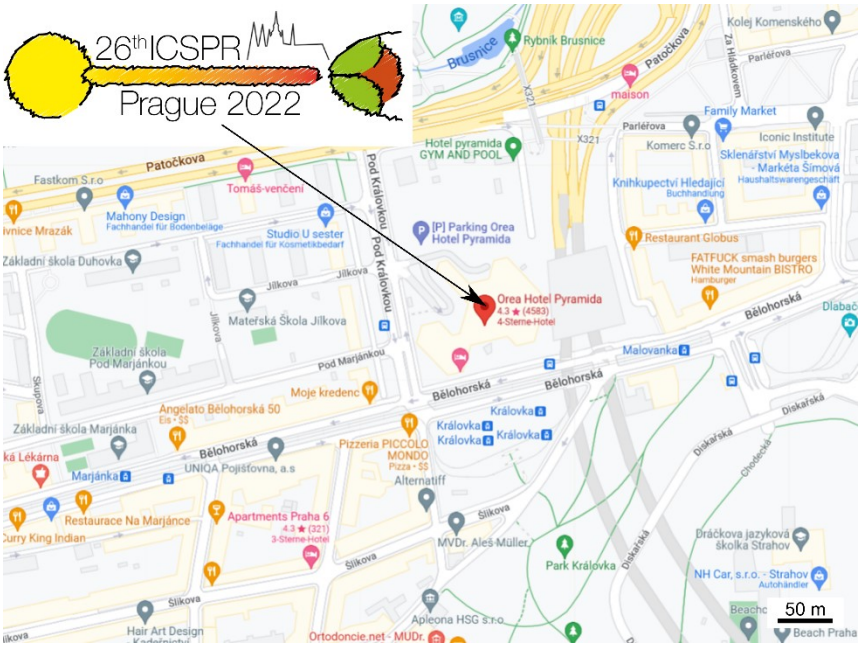
Hotel



WIFI

Free of charge WIFI is available through "**Pyramida**" (no registration required).

Arrival

By plane:

The closest airport to the conference venue in Pyramida Hotel is Václav Havel airport Prague. From the airport, you can travel by several means, public transport, taxi, Uber or Bolt.

Public transport from airport - information

The easiest route to get to the conference is taking the bus number 191 from the airport to Vypich. There, change to the tram, line 22 (direction Nádraží Hostivař) or line 25 (direction Lehovec) and go to the station Malovanka, which is next to the hotel. This journey should take 40 - 45 min.

The ticket (costing 40 CZK; which equals approx. to 1.60 €) can be purchased in the ticket machine at the airport that accepts both cash and cards. The ticket is valid inside whole Prague city limits (zone P) in metro, buses, trams and regional trains and you can change as many times as you wish within its time limit of 90 min. After getting on the first bus or tram or when entering the metro area, you have to validate your ticket.

There is a taxi service available at the airport. However, the reputation of Prague taxi drivers is not particularly high and although the situation is getting better, there are still not infrequent reports of overpriced journeys. As an advice and starting point, insisting on setting on the meter and not negotiating the price in advance might help.

Alternatively, web-operated Uber and Bolt services may be used. They are fairly similar, usually cheaper and reliable with the options to pay cash or by card. For both, iPhone and Android apps are available. To get to the Pyramida hotel, put in the address “Bělohorská 24, Praha”.

By train:

Public transport from train station - information

There are several train stations in Prague but the international trains usually stop in Prague Main Station (Praha hlavní nádraží) in the city center. From there, you can continue by public transport or taxi/Uber/Bolt to the hotel.

The easiest way by the public transport is taking the metro C line (direction Letňany) to the station Vltavská, and there change to the tram, line 25 (direction Bílá Hora) and leave the tram at Malovanka station. This should take around 30 min.

By bus:

The international bus connections usually terminate in the Florenc Bus Terminal in the city center. From there, you can continue by public transport or taxi/Uber/Bolt to the hotel.

Public transport from bus station - information

The easiest way by the public transport is taking the metro C line (direction Letňany) to the station Vltavská, and there change to the tram, line 25 (direction Bílá Hora) and leave it at Malovanka station. This should take around 25 min.

SAFETY RULES

As of 9th April 2022, the protective measures regarding the conditions of entry into the Czech Republic in relation to the epidemic of covid-19 have been suspended. Entry into the Czech Republic is no longer subject to any special epidemiological conditions to prevent the spread of the disease. The entry-ban for foreigners from third-countries and the obligation to prove infection-free status have been lifted.

COVID portal Czech Republic

In any case, please consult the possible specific rules in the countries from which you are travelling.

There are also no specific covid-related measures or duties applied at the moment. Wearing face masks is obligatory only in hospitals and other medical facilities. However, wearing face masks is recommended in public transport and other public indoor areas.

CODE of CONDUCT

The ICSPR Organizing Team is committed to providing a safe and productive meeting environment that fosters open dialogue and the exchange of scientific ideas, promotes equal opportunities and treatment for all participants, and is free of harassment and discrimination. All participants are expected to uphold standards of scientific integrity and professional ethics, treat others with respect and consideration, follow venue rules, and alert members of the organizing team of any dangerous situations or anyone in distress. The organizing team prohibits any form of discrimination, harassment, sexual or otherwise. These should be reported immediately to any member of the organizing team. All complaints will be treated seriously and responded to promptly.

To maintain and foster an environment at the meeting where colleagues feel comfortable and are encouraged to share unpublished data, recording, photographing, or tweeting (or sharing on other social media) of all data, results, hypotheses, conclusions, or any other aspects of a talk or poster are not permitted without the expressed consent of the authors. Also, please refrain from citing abstracts in bibliographies. The information in this abstract book shall be treated as personal communication and shall be cited only with expressed consent of the authors.

The policies herein apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the 26th International Conference on Sexual Plant Reproduction and related events.

SPONSORS and SUPPORTERS

The conference is organized under the auspices of **prof. Eva Zažímalová, the President of the Czech Academy of Sciences**.

The conference is organized under the auspices of **MUDr. Zdeněk Hřib, the Mayor of Prague**.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PROGRAMME

Monday June 20

10:00-13:00	*Registration*
13:00-14:00	*Lunch*
14:00-14:30	**Conference Opening**
14:30-18:00	**Session 1: Flowering and Flower Organ Development** Chair: Lucia Colombo, *Universita degli Studi di Milano*, *Italy*
14:30-15:00	**Keynote Talk 1** **Development and evolution of petal nanoscale ridges that scatter light and influence animal behaviour** Beverley J. Glover, *University of Cambridge, UK*
15:00-15:20	Abstract Talk 1.1 **Control of flowering time and yield by winter bud dormancy in oilseed rape** Steven Penfield, *John Innes Centre, Norwich, UK*
15:20-15:40	Abstract Talk 1.2 **Florogenesis and juvenile phase transition in *Cannabis sativa* plants** Ben Spitzer-Rimon, *ARO - Volcani Institute, Rishon LeZion, Israel*
15:40-16:00	Abstract Talk 1.3 **Unraveling flowering development in the smallest angiosperm** Cristian Mateo-Elizalde, *Cold Spring Harbor Laboratory, New York, NY, USA*
16:00-16:40	*Coffee Break*

16:40-17:00	Abstract Talk 1.4 **The beta-subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of *Arabidopsis thaliana*** Jan Fíla, *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
17:00-17:20	Abstract Talk 1.5 **SCI1 is expressed at the *Nicotiana tabacum* floral meristem and is a direct target of key flower development transcription factors** Maria Helena S. Goldman, *University of Sao Paulo, Brazil*
17:20-17:40	Abstract Talk 1.6 **ARGONAUTE-mediated RNA silencing in anther development** Reina Komiya, *Science and Technology Graduate University, Okinawa, Japan*
17:40-18:00	Abstract Talk 1.7 **Sexual dimorphism, male biasness and ambophily in *Zanthoxylum armatum*; traits for reproductive efficiency** Renu Sharma, *University of Jammu, India*

Welcome Drink
(18:00-20:00)
Hotel Pyramida

Tuesday June 21

9:00-12:50	**Session 2: Gametogenesis and Meiosis** Chair: Ueli Grossniklaus, *University of Zurich, Switzerland*
9:00-9:30	**Keynote Talk 2** **What limits meiotic crossovers?** Raphael Mercier, *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
9:30-9:50	Abstract Talk 2.1 **Structural maintenance of chromosomes SMC5/6 complex is necessary for meiotic chromosome reduction in *Arabidopsis*** Aleš Pečinka, *Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic*
9:50-10:10	Abstract Talk 2.2 **H3K9 demethylases are required for male meiosis in *Arabidopsis thaliana*** Hua Jiang, *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
10:10-10:30	Abstract Talk 2.3 **Dissection of meiotic recombination and genomic architecture of holocentric plants with repeat-based centromeres** Meng Zhang, *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
10:30-11:10	*Coffee Break*
11:10-11:30	Abstract Talk 2.4 **ZIP4: stabilization of wheat as a polyploid and its impact on breeding** Azahara C. Martin, *John Innes Centre, Norwich, UK*

11:30-11:50	Abstract Talk 2.5 **Members of the ELMOD protein family specify formation of distinct aperture domains on the *Arabidopsis* pollen surface** Anna Dobritsa, *Ohio State University, Columbus, OH, USA*
11:50-12:10	Abstract Talk 2.6 **Multi-omics approach to describe gene expression dynamics in developing pollen of *Arabidopsis thaliana*** Božena Klodová, *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
12:10-12:30	Abstract Talk 2.7 **HvTDF1 gene reveals a conserved role in controlling anther tapetum development in dicot and monocot** Zoe Wilson, *University of Nottingham, UK*
12:30-12:50	Abstract Talk 2.8 **The VACUOLAR SORTING PROTEIN 13 (VPS13) affects female germline establishment and progression by acting on small RNA pathway** Mara Cucinotta, *Universita degli Studi di Milano, Italy*
12:50-14:00	*Lunch*
14:00-17:50	**Session 3: Pollen-Pistil Interactions and Pollen Tube Growth** Chair: Ravi Palanivelu, *University of Arizona*, *Tucson, AZ, USA*
14:00-14:30	**Keynote Talk 3** **Death is life – programmed cell death in compatible and incompatible pollen pistil interactions** Moritz Nowack, *VIB and Ghent University, Ghent, Belgium*

14:30-14:50	Abstract Talk 3.1 **Two subgroups of *Arabidopsis* receptor-like kinases regulate intra- and inter-species pollen-pistil interactions** Daphne R. Goring, *University of Toronto, Canada*
14:50-15:10	Abstract Talk 3.2 **Engineered *Arabidopsis* pollen establishes a role of ATP depletion and cytosolic acidification in *Papaver* self-incompatibility** Ludi Wang, *Aberystwyth University, UK*
15:10-15:30	Abstract Talk 3.3 **Flavonols take the heat out of heat stress to protect pollen from elevated ROS** Joëlle K. Mühlemann, *limate Resilient Crop Production lab, KU Leuven, Department of Biosystems, Division of Crop Biotechnics, Belgium*
15:30-16:10	*Coffee Break*
16:10-16:30	Abstract Talk 3.4 **Transcriptome reprogramming in the *Arabidopsis* male germline during the progamic phase** Jörg D. Becker, *Instituto Gulbenkian de Ciencia, Oeiras, Portugal*
16:30-16:50	Abstract Talk 3.5 **Integration of ion dynamics into a membrane potential gradient in pollen tubes** Jose A Feijó, *University of Maryland, College Park, MA, USA*
16:50-17:10	Abstract Talk 3.6 **Molecular basis of pollen germination and tube growth in rice** Ki-Hong Jung, *Kyung Hee University, Yongin, Republic of Korea*

17:10-17:30	Abstract Talk 3.7 **Keeping growth in check – regulation of polar signaling in pollen of *Arabidopsis thaliana*** Philipp Denninger, *Technical University of Munich, Germany*
17:30-17:50	Abstract Talk 3.8 **Enhanced pollen tube integrity was selected during breeding of tomato varieties that set fruit at elevated temperature** Sorel Ouonkap Yimga, *Brown University, Providence, RI, USA*
17:50-20:00	**Poster Session 1**

Wednesday June 22

9:00-12:10	**Session 4: Fertilization Mechanisms** Chair: Stefanie Sprunck, *University of Regensburg, Regensburg, Germany*
9:00-9:30	**Keynote Talk 4** **Novel pathways controlling sperm nuclear migration during flowering plant fertilization** Tomokazu Kawashima, *University of Kentucky, Lexington, KY, USA*
9:30-9:50	Abstract Talk 4.1 **RNA binding proteins at the nexus of pollen tube guidance** Said Hafidh, *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
9:50-10:10	Abstract Talk 4.2 **The JAGGER GPI-anchor defines the protein subcellular localization. Does it mediate JAGGER function?** Sílvia Coimbra, *Universidade do Porto, Portugal*
10:10-10:30	Abstract Talk 4.3 **Redox interplay of ROS-level dynamics and glutathione metabolism upon gamete fusion and subsequent zygotic development in rice** Kasidit Rattanawong, *Tokyo Metropolitan University, Japan*
10:30-11:10	*Coffee Break*
11:10-11:30	Abstract Talk 4.4 **“Cells-in-a-cell”: Which roles for the endo-plasma membrane that wraps the sperm cells?** Thomas Widiez, *Université de Lyon, France*

11:30-11:50	Abstract Talk 4.5 **Fertilization initiates seed nutrition by degradation of callose deposition at the phloem end** Ryushiro Kasahara, *Nagoya University, Japan*
11:50-12:10	Abstract Talk 4.6 **ECS1 and ECS2 suppress the formation of haploid plants by promoting double fertilization** Thomas Nakel, *University of Bremen, Germany*
12:10-13:00	*Lunch*

Excursion
Prague City Centre
(13:00-15:00)

15:00-18:00	**Session 5: Apomixis, Evolution and Ecology** Chair: Viktor Žárský, *Charles University*, *Prague, Czech Republic*
15:00-15:30	**Keynote Talk 5** **From single cells to flowers: Gene-regulatory mechanisms controlling organ specification in *Arabidopsis* flowers** Kerstin Kaufmann, *Humboldt-University, Berlin, Germany*
15:30-15:50	Abstract Talk 5.1 **Transcriptome analysis of sexual and apomictic *Boechera* leads to identification of the RNA helicase GAM as crucial regulator for gametogenesis** Anja Schmidt, *Heidelberg University, Germany*
15:50-16:10	Abstract Talk 5.2 **Development and evolution in male gametogenesis** David Twell, *University of Leicester, UK*

16:10-16:30	Abstract Talk 5.3 **Genomic and ecological differentiation in a South American grass** Diego Hojsgaard, *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
16:30-17:00	*Coffee Break*
17:00-17:20	Abstract Talk 5.4 **Pollen number is regulated by REDUCED POLLEN NUMBER1 encoding ribosome assembly factor and is characterized by an enriched selection signal among traits related to selfing syndrome in *Arabidopsis thaliana*** Kentaro K. Shimizu, *University of Zurich, Zurich, Switzerland*
17:20-17:40	Abstract Talk 5.5 **Pre-zygotic mate selection in *Nicotiana attenuata*** Patrycja Baraniecka, *Max Planck Institute for Chemical Ecology, Jena, Germany*
17:40-18:00	Abstract Talk 5.6 **Phylogenetic and expression analysis of CENH3 and APOLLO genes in sexual and apomictic *Boechera* species** Vladimir Brukhin, *Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg, Russia*
18:00-20:00	**Poster Session 2**

Thursday June 23

9:00-12:50	**Session 6: Embryogenesis, Seed and Fruit Development** Chair: David Honys, *Czech Academy of Sciences, Prague, Czech Republic*
9:00-9:30	**Keynote Talk 6** **Apoplastic modifications in plant reproductive development: The (w)hole story** Gwyneth Ingram, *Université de Lyon, France*
9:30-9:50	Abstract Talk 6.1 **Embryonic elimination and post-meiotic drive of chromosomes – different sites of the same coin?** Andreas Houben, *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
9:50-10:10	Abstract Talk 6.2 **Setting up the stage for analyzing parental-dosage-dependent effects on barley grain development** Anna Nowicka, *Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic*
10:10-10:30	Abstract Talk 6.3 **High temperatures impact on early seed development and embryo morphogenesis in *Arabidopsis thaliana*** Juan Francisco Sánchez López, *CEITEC Masaryk University, Brno, Czech Republic*
10:30-11:10	*Coffee Break*
11:10-11:30	Abstract Talk 6.4 **Do *VIM* genes have a role in embryo and endosperm development?** Karina Orozco Natividad, *Centro de Investigación y de Estudios Avanzados (Cinvestav), Irapuato, México*

11:30-11:50	Abstract Talk 6.5 **Distinct parental signals polarize the *Arabidopsis* zygote to initiate the embryonic patterning process** Martin Bayer, *Max Planck Institute for Biology, Tübingen, Germany*
11:50-12:10	Abstract Talk 6.6 **Time to sleep or to germinate? A case of legumes seed dormancy** Petr Smýkal, *Palacký University in Olomouc, Czech Republic*
12:10-12:30	Abstract Talk 6.7 **Seed coat-derived brassinosteroids non-cell autonomously regulate endosperm development** Rita B. Lima, *University of Potsdam, Germany*
12:30-12:50	Abstract Talk 6.8 **Small RNA functions in plant embryos** Michael Nodine, *Wageningen University, The Netherlands*
12:50-14:00	*Lunch*
14:00-17:10	**Session 7: Epigenetic Mechanisms** Chair: Thomas Dresselhaus, *University of Regensburg*, *Regensburg, Germany*
14:00-14:30	**Keynote Talk 7** **Regulation and function of endosperm cellularization** Claudia Köhler, *Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany*
14:30-14:50	Abstract Talk 7.1 **Active DNA demethylation in pollen counteracts heterochromatic silencing** Daniel Bouyer, *École Normale Supérieure de Lyon, France*

14:50-15:10	Abstract Talk 7.2 **Temperature stress impairs centromere structure and segregation of meiotic chromosomes in *Arabidopsis*** Karel Říha, *CEITEC Masaryk University, Brno, Czech Republic*
15:10-15:30	Abstract Talk 7.3 **RNA directed DNA methylation impacts seed development in the obligate outcrosser *Capsella grandiflora*** Mark A Beilstein, *University of Arizona, Tucson, AZ, USA*
15:30-16:10	*Coffee Break*
16:10-16:30	Abstract Talk 7.4 **DNA methylation and genetic imprinting in water lily (*Nymphaea*) seeds: implications for endosperm and seed evolution** Rebecca A. Povilus, *Whitehead Institute, Cambridge MA, USA*
16:30-16:50	Abstract Talk 7.5 **Homology-based regulation of pollen-side dominance hierarchy between small RNAs and their targets in *Brassicaceae*.** Risa Kobayashi, *Nara Institute of Science and Technology, Japan*
16:50-17:10	Abstract Talk 7.6 **Exploring the cellular basis of organ curvature using 3D digital ovules** Kay Schneitz, *Technical University of Munich, Freising, Germany*
17:10-18:00	**General Assembly**

Conference Dinner
(19:00-21:00)
City of Prague Mayor's Residency
Mariánské náměstí 1/98, Praha 1 – Staré Město

Friday June 24

9:00-9:40	**Special Keynote Talk** **Membrane receptor kinase signaling proteins in plant development** Michael Hothorn, *University of Geneva, Switzerland*
9:40-11:50	**Session 8: Applications in Plant Breeding** Chair: Karina van der Linde, *University of Regensburg, Germany*
9:40-10:10	**Keynote Talk 8** **Exploitation of uniparental genome elimination for accelerated plant breeding and genetics** Ravi Maruthachalam, *Indian Institute of Science Education and Research, Vithura, Kerala, India*
10:10-10:50	*Coffee Break*
10:50-11:10	Abstract Talk 8.1 **Pollen and ovule quality analysis for plant reproduction** Iris Heidmann, *Acepo, Enkhuizen, The Netherlands*
11:10-11:30	Abstract Talk 8.2 **Genome-wide association analysis of wild and domesticated barley identifies hitherto unknown domestication loci as well as new breeding targets for important yield traits** Jesper Harholt, *Carlsberg Research Laboratory, Copenhagen, Denmark*
11:30-11:50	Abstract Talk 8.3 **Generation of haploidy inducers in barley by targeted mutagenesis** Jochen Kumlehn, *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*
11:50-12:10	**Meeting Closure**
12:10-13:10	*Lunch*

ABSTRACTS

KEYNOTE TALKS

KT1 Development and evolution of petal nanoscale ridges that scatter light and influence animal behaviour

Beverley J. Glover[1], Chiara Airoldi[1], Jordan Ferria[1], Carlos Lugo Velez[1,2], Edwige Moyroud[2]

[1]Department of Plant Sciences, University of Cambridge, Cambridge, UK
[2]Sainsbury Laboratory Cambridge University, Cambridge, UK

Pattern formation is key to the development of all organisms. Mechanical instabilities associated with stress development during growth have been proposed as a patterning mechanism of surface morphologies across plants and animals. We have been studying the development of nanoscale ridges on the petals of *Hibiscus trionum*. In this talk I will summarise recent work demonstrating the function of these ridges in the generation of structural colour and a subsequent effect on pollinator foraging efficiency. I will introduce the molecular, genetic and chemical approaches we are taking to understand how mechanical instability and growth-induced stress regulate the formation of these ridges. I will also discuss the evolutionary context of petal nanoscale ridge development.

KT2 What limits meiotic crossovers?

Raphaël Mercier

Department of Chromosome Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Meiotic crossovers shuffle parental genetic information, providing novel combinations of alleles on which selection can act. However, meiotic crossovers are relatively rare, typically one to three per chromosome. Intriguingly, crossover numbers differ between males and females in many species, and this correlates with differences in the length of the meiotic chromosome axes. Perhaps even more intriguing, when multiple crossovers occur on a single chromosome they tend to be distant from each other, a phenomenon called interference whose mechanisms have been a matter of debate for over a century. Using the model plant *Arabidopsis thaliana*, we revealed several mechanisms that limit meiotic crossovers. Mutation of the corresponding genes led to a spectacular increase in genome-wide recombination, showing that crossovers are naturally constrained well below their possible maximum. Our and previous results support a unifying emerging model that accounts for crossover numbers, heterochiasmy, and crossover interference.

KT3 Death is life – Programmed cell death in compatible and incompatible pollen pistil interactions

Moritz K. Nowack

Department of Plant Systems Biology, VIB, and Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium

Programmed cell death (PCD) is a basic principle of plant development. Precise temporal and spatial control of PCD is important for plant growth, and central to successful plant reproduction. Despite the undisputed importance of PCD for plant reproduction, but we are only beginning to understand the molecular genetics regulation of reproductive PCD.

We are studying PCD processes taking place during plant reproduction, focusing on Arabidopsis (*Arabidopsis thaliana*), maize (*Zea mays*), and poppy (*Papaver rhoeas*) as model systems. In the context of compatible pollen-pistil interactions in *Arabidopsis* and maize, we are investigating PCD processes that terminate the fertile window in senescing non-pollinated flowers. Regarding incompatible pollen-pistil interactions, we are studying the Papaver-type self-incompatibility leading to the rejection of “self-pollen” by PCD.

I will give an overview of our recent investigations in reproductive PCD, and highlight challenges and opportunities in this research field."

KT4 Novel pathways controlling sperm nuclear migration during flowering plant fertilization

Tomokazu Kawashima, Mohammad F. Ali, Umma Fatema

Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA

Fertilization involves dynamic cellular processes including gamete nuclear migration. The cytoskeleton generates the mechanical force required to move the gamete nuclei for karyogamy. In most animals, microtubules, organized by centrosomes, play the primary role in gamete nuclear migration. By contrast, flowering plants have lost the genes fundamental to centrosome formation and have evolved actin filament (F-actin) based sperm nuclear migration systems. However, besides the involvement of a reproductive cell-specific Rac/Rop small GTPase (ROP8 in *Arabidopsis thaliana* central cell), the molecular mechanism of how F-actin controls fertilization is largely unknown. Using confocal microscopy live-cell imaging with a combination of pharmacological and genetic approaches, we identified new factors involved in F-actin dynamics and sperm nuclear migration in *Arabidopsis*. Our data suggest that female reproductive cells control F-actin formation and movement through novel reproductive cell-specific pathways. Interestingly, genes controlling gamete nuclear migration differ between the egg and central cells. I will discuss our recent findings about F-actin regulators in double fertilization.

KT5 From single cells to flowers: Gene-regulatory mechanisms controlling organ specification in *Arabidopsis* flowers

Kerstin Kaufmann

Institute of Biology, Humboldt-Universität zu Berlin, Berlin, Germany

Developmental switches and cellular differentiation in plants require coordinated changes in the activities of thousands of genes. These are mediated by concerted activities of transcription factors that integrate growth, patterning and physiological status of cells. How these activities are linked at the molecular level is still poorly understood. Using flower development as a model system, dynamic transcription factor activities and their target networks can now be mapped at the level of individual cells and cell types. By combining single cell transcriptomics and spatial reconstruction of floral meristems in 3D, we can trace gene activities in floral stem cells at unprecedented resolution.

KT6 Apoplastic modifications in plant reproductive development: The (w)hole story

Gwyneth Ingram

Laboratoire Reproduction et Développement des Plantes, ENS de Lyon, CNRS, INRAE, Université de Lyon, Lyon, France

Angiosperm reproductive structures (anthers and ovules/seeds) are complex assemblies containing highly specialized, metabolically diverse, and in some cases genetically distinct compartments. Their successful development depends both on strict inter-tissue coordination, and upon selectively gated inter-tissue communication, particularly at the metabolic level. For these two requirements to be met, dynamic, extensive, and precise remodelling of tissue interfaces, affecting both symplastic (direct cytoplasm-cytoplasm) and apoplastic (involving diffusion through the extracellular matrix) connectivity, is a prerequisite. I will concentrate on apoplastic modifications occurring between key compartments in the developing seeds and anthers of *Arabidopsis thaliana*. I aim to illustrate how related peptide-mediated inter-tissue dialogues are used in both systems to ensure the timely deposition of intact apoplastic filters (barriers). I will present recent unpublished work on the functional and compositional characterisation of a novel apoplastic filter (barrier) present within the maternal tissues of the developing anther.

KT7 Regulation and function of endosperm cellularization

Nicolas Butel[1,2], W. Xu[1], Juan Santos-González[1], Claudia Köhler[1,2]

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The endosperm is developmental innovation of angiosperms that sustains and supports embryo growth. It is a mostly triploid tissue that develops after fusion of a haploid sperm cell with a predominantly diploid central cell. In *Arabidopsis thaliana*, like in most angiosperms, endosperm development occurs in two phases. In the initial phase, endosperm proliferation is uncoupled from cellularization, resulting in the formation of a coenocyte. Then, at a defined timepoint, a wave of cellularization starts from the micropylar region to reach the chalazal endosperm. At the end of the process, most of the endosperm is cellularized and nuclear divisions cease. The timing of the second phase is critical for seed development, for reasons that remain unknown. Thus, delayed cellularization like in response to interploidy and interspecies hybridizations results in embryo arrest and seed abortion.

Auxin was previously shown to play a critical role in determining the timing of endosperm cellularization. Auxin biosynthesis is initiated from the paternal genome and responsible for the first nuclear divisions of the endosperm. Auxin levels cease at the time of cellularization, while conversely, endosperm cellularization failure correlates with increased auxin levels. We identified a family of auxin related factors triggering endosperm cellularization. These factors are specifically expressed from the maternal genome and are active during a sharp developmental time window corresponding to cellularization initiation. In contrast to auxin, increased expression of these genes induces early cellularization. We propose that these maternal auxin related factors act antagonistically to the paternal auxin pathway, explaining the distinct effects of maternal and paternal genomes on timing the transition from endosperm nuclear proliferation to cellularization.

SKT Membrane receptor kinase signaling proteins in plant development

Michael Hothorn

Plant Structural Biology Laboratory, University of Geneva, Geneva, Switzerland

Plants have evolved a unique family of plant membrane receptor kinases that control different aspects of plant growth and development, form the first layer of the plant innate immune system and regulate symbiotic interactions. I will present molecular studies that reveal how different plant receptor kinases specifically sense different small molecule and peptide ligands, how they become activated by shape-complementary co-receptor kinases and how these signaling complexes can be negatively regulated by receptor-like pseudokinases. I will discuss different structural biology and quantitative biochemical methods to match and characterizes novel receptor – ligand pairs and protein engineering strategies to switch on and off receptor kinase signaling pathways in planta. Finally, I will highlight recent challenges in deciphering the cytoplasmic signaling cascades of plant receptor kinases in mechanistic detail.

KT8 Exploitation of uniparental genome elimination for acclerated plant breeding and genetics

Ravi Maruthachalam

School of Biology, Indian Institute of Science Education and Research(IISER), Thiruvananthapuram (IISER TVM), Maruthamala PO, Vithura, Kerala, India

Uniparental genome elimination (UGE) is a process wherein one of the parental set of chromosomes in the zygote is selectively lost during embryonic mitotic divisions resulting in a haploid embryo and plant. These in planta haploids can be exploited as a breeding tool for the rapid generation of inbred lines used in hybrid cultivar development. UGE is observed as one of the genetic consequences of artificial distant hybridization crosses in several plant species, the classical example being the bulbosum method of *in vivo* haploid production in cultivated barley. However, the molecular basis behind the UGE remained elusive for its successful exploitation in other crop species. Working in the plant model, *Arabidopsis thaliana* we have shown that by simple manipulation of the centromere-specific histone H3(CENH3) protein, it is possible to emulate the genetic consequences of distant crosses in an intraspecific cross paving way for the engineering of UGE in crops of agronomic importance. In this talk, I will touch upon how we can manipulate CENH3 to engineer an in vivo haploid inducing(HI) strain and how this strain can be used as a haploid genetics toolbox for accelerated plant breeding and genetics with demonstrated examples in literature.

ABSTRACT

TALKS SELECTED FROM ABSTRACTS

AT1.1 Control of flowering time and yield by winter bud dormancy in oilseed rape

Steven Penfield, Xiang Lu, Carmel M. O'Neill, Samuel Warner, Xiaochao Chen, Rachel Wells

Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, UK

In winter annuals flowering time is well known to require a vernalisation, with the floral transition promoted by lengthening photoperiods and increasing temperatures in spring. However, we and others have shown that in many winter annuals vernalisation actually takes place in autumn, with floral development timed to occur during mid-winter. In European winter oilseed rape (WOSR) reductions in winter chilling are associated with low yields, suggesting that chilling continues to affect plant reproductive development after vernalisation and the floral transition are complete. Understanding the underlying biology is important for maintaining yield resilience during the increasing frequency of warm winters warm associated with climate change. Here we use simulated growing seasons and direct warming in the field to reveal how flowering time and yield are affected by winter chilling in WOSR. Mysteriously, we show that delaying vernalisation and the floral transition by one month only has a trivial affect on flowering time. Furthermore we find that there is a second winter chilling response that promotes flowering by preventing induction of bud dormancy. Warming flower buds causes a delay to bud development and inhibition of the cell cycle, coupled to activation of a conserved BRANCHED1-dependent gene expression programme known to be associated with inhibition of bud outgrowth across diverse species and ABA accumulation. We show that Brassica napus exhibits wide genetic variation in bud dormancy responses to temperature which correlates with crop type and allelic variation of FLC genes which are responsive to winter temperatures, but not sensitive to the autumn chilling that permits vernalisation. It is well known that in temperate perennial crops failure to break bud dormancy results in reproductive abnormalities that affect yields. Here we provide direct evidence that a similar process can occur in winter arable crops if chilling is incomplete.

AT1.2 Florogenesis and juvenile phase transition in Cannabis sativa plants

Hannan Alter, Shai Duchin, Hadas Shafran-Tomer, Reut Peer, Nirit Bernstein, Moshe Flaishman, Rina Kamenetsky-Goldstein, Ben Spitzer-Rimon

Agricultural Research Organization - Volcani Institute, Rishon LeZion, Israel.

Cannabis has been cultivated worldwide for years as an industrial and medicinal crop. Despite the importance of the Cannabis flowering process, in-depth research of its flowering biology is significantly limited. Until recently, adult Cannabis plants were considered obligatory short-day (SD) plants since long-day (LD) conditions contribute to so-called "vegetative growth," whereas the inflorescences develop following exposure to a photoperiod less than 12h. We revisited this assumption and revealed that solitary flowers are developing in each of the leaf axil of mature plants grown under LD conditions. These findings indicate that under these conditions, Cannabis plants are reproductive and not vegetative. Analysis of seedlings during phase transition demonstrated that juvenile seedlings are indeed the vegetative stage of Cannabis, and the establishment of the first pair of flowers at the 7th node is the sole transition to the reproductive phase during the Cannabis life cycle. This transition that occurs under constitutive LD conditions is accompanied by downregulation in the transcription of flowering repressors, in parallel to upregulation in flowering integrator and flower meristem identity genes. These results corroborate the findings that flower induction and initiation are not regulated by day length, but more likely by internal signals. Nevertheless, we show that under SD conditions, dramatic architectural changes that included intense branching and reduction in node size lead to the development of typically condensed inflorescence. This is most likely a result of reduced levels of auxin and gibberellins analyzed in shoot apexes of plants grown under SD photoperiod. The involvement of gibberellins was further demonstrated by the ability of exogenous application under SD conditions to postpone inflorescence development. An understanding of the genetic mechanism governing flowering and inflorescences development will lay the foundations for genetic, biotechnological, and physiological applications to maximize plant productivity and uniformity in medical Cannabis.

AT1.3 Unraveling flowering development in the smallest angiosperm

Cristian Mateo-Elizalde, Evan Ernst and Rob Martienssen

Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA

Duckweeds (Lemnaceae) are a family of aquatic plants that is widely distributed throughout the world. Prior to the establishment of *Arabidopsis* as the preponderant model plant, duckweed was a widely used system for basic plant biology (Hilman, 1963; Baldi et al., 1991) due to its simple morphology, rapid growth rate and easy handling in the laboratory. The advent of the genetic era during the 1990's left Lemna behind from a scientific point of view, since many clones barely produce flowers either nature or in laboratory conditions, making genetics very challenging. But in recent years, new genetic and genomic technologies have opened the door to studying these clonal aquatic macrophytes again in the context of other plant species. The number of scientific papers on Lemna has raised exponentially in the last 20 years and we are now in what many authors has defined as "The return of the Lemnaceae" (Acosta et al., 2021). Because of their predominant clonal growth habit, sexual reproduction is still one of the least studied aspects in this plant family. Recently, the plant hormone, salicylic acid, a floral inducer in duckweed, has been used for transcriptomic and physiological studies (Fouronjian et al., 2021), along with the identification of key flowering regulatory genes such as LgFT1 and LaFTL1 (Fu et al., 2020; Yoshida et al., 2021). However, the mechanism of SA induced flowering, and how is it connected to flowering in nature, is still unknown. Here, we present transcriptomes from duckweed pistils, anthers, seeds and cotyledons in one duckweed short-day species, *L. aequinoctialis*, as well as the use of salicylic acid to promote flower development in *L. gibba* and *W. australiana*. Our study will contribute not only to the further characterization of duckweed sexual reproduction, but also to the understanding on how SA promotes flowering in controlled conditions.

AT1.4 The beta-subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of *Arabidopsis thaliana*

Jan Fíla[1*], Božena Klodová[1,2*], David Potěšil[3], Miloslav Juříček[4], Petr Šesták[1,2], Zbyněk Zdráhal[3,5], David Honys[1,2]

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**both authors contributed equally*

Angiosperm flower development together with male gametophyte development represent important processes of plant reproduction, which are controlled by a common activity of plethora genes. In *Arabidopsis thaliana* genome, there are five genes encoding the NACβ-subunit, and two genes encoding the NACβ-subunit. The double homozygous mutants of both *NACβ* genes were acquired by a conventional cross of two available T-DNA insertion lines. These double homozygous mutants showed several phenotypic traits different from the Columbia-0 wild type plants, such as delayed development, lower chlorophyll content in leaves, abnormal number of flower organs, and abnormally short siliques that carried a lower number of seeds. Both NACβ genes were characterized in more detail – the phenotype of the double homozygous mutant was complemented by a functional *NACβ* copy. Then, both *NACβ* genes were localized to nuclei and cytoplasm and their promoters were active in many organs (leaves, cauline leaves, flowers, pollen grains, and siliques together with seeds). Since flowers were the most affected organs by *nacβ* mutation, the flower buds' transcriptome was identified by RNA sequencing, and their proteome by gel-free approach. The differential expression analyses of transcriptomic and proteomic datasets suggest the involvement of NACβ subunits in stress responses, male gametophyte development, and photosynthesis.

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AT1.5 *SCI1* is expressed at the *Nicotiana tabacum* floral meristem and is a direct target of key flower development transcription factors

Cruz, J.O.[1,2]; San Martin, J.A.B.[1]; Lubini, G.[1,2]; Strini, E.J.[1,2]; Sobral, R.[3], Pinoti, V.F.[1,2]; Ferreira, P.B.[1,2]; Thomé, V[1,2]; Quiapim, AC[1]; Dornelas, M.C.[4]; Pranchevicius, M.C.S.[5]; Madueno, F.[6]; Costa, M.M.R.[3]; Goldman, MHS[1]

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We previously showed that SCI1 (Stigma/style Cell-cycle Inhibitor 1) controls cell proliferation in the pistil of tobacco and *Arabidopsis*. The pistil is derived from the last floral meristematic cells and we decided to investigate when *SCI1* starts to be expressed. *In situ* hybridization experiments have shown that *SCI1* is expressed since floral meristem specification and in all floral organ primordia of tobacco. Its expression is higher in the floral meristem and the organs being specified, and then it decreases from outside to inside whorls when the organs are differentiating. SCI1prom::SCI1-GFP transgenic plants reproduce *SCI1* endogenous tissue-specific expression and developmental regulation. Shoot and root meristems of transgenic plants show no *SCI1-GFP* expression. *In silico* analyses with PlantRegMap identified *cis*-regulatory elements for LEAFY (NtLFY), AINTEGUMENTA (NtANT), AGAMOUS (NAG1) and WUSCHEL (NtWUS) transcription factors (FTs) in the *SCI1* genomic sequence. Yeast one-hybrid assays demonstrated that the NtANT, NAG1 and NtWUS interact with the *SCI1* promoter sequence. Electrophoresis mobility shift assay confirmed the direct binding of NAG1 and NtWUS to *SCI1* promoter and the luciferase activity assay demonstrated that NAG1 is able to activate *SCI1* expression, while NtWUS could not do so. Additional experiments are underway to investigate the regulation by NtLFY and NtANT.

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AT1.6 ARGONAUTE-mediated RNA silencing in anther development

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Reproductive-specific small RNAs are vital regulators of germline development in eukaryotes, by interacting with Argonaute (AGO) proteins. During plant reproduction, numerous phased small interference RNAs (phasiRNAs) that are derived from more than 1000 types of long non-coding RNAs, are produced. However, the roles of reproductive phasiRNAs remain largely unknown.

We have successfully generated various mutant rice about phasiRNA production by genome editing. These mutants have shown defects of anther development with the reduction of phasiRNAs, suggesting that phasiRNAs are crucial for reproduction in rice. In this conference, I will introduce the phasiRNA biogenesis and spatial regulation of ARGONAUTE-mediated RNA silencing in anther development.

AT1.7 Sexual dimorphism, male biasness and ambophily in *Zanthoxylum armatum*; traits for reproductive efficiency!

Renu Sharma and Namrata Sharma

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Among dioecious flowering plants, males and females often have diverse resource allocation strategies, which are reflected in their life-history traits as well as reproductive traits. This is also defined as sexual dimorphism. The strategies of resource allocation and the habitat of these species also pose an impact on their pollination mechanism and reproductive success. *Zanthoxylum armatum* DC. (Rutaceae), an indigenous tropical shrub to a small tree species is commonly known as Indian Prickly ash with wide economic and medicinal value. Due to overexploitation of natural populations in India, the species is rapidly declining and according to International Union for Conservation of Nature, it has been assigned the status as 'Vulnerable'. To manage the population of the species in wild and establishing commercial plantations, detailed knowledge on phenology, sex ratio, reproductive traits and pollination is required which is lacking for the species. Thus, the present study was conducted in natural populations of *Z. armatum* growing in Union territory of Jammu and Kashmir, India. The study revealed that the species exhibits sexual dimorphism both in vegetative and reproductive traits such as, higher number of shoots, more stature, branches and flowers in male plants than female plants. Another important outcome of the study is the finding of a prevalence of male biased sex ratio in natural populations of species, where a survey based on the total of 106 plants revealed an average sex ratio (>:+) of 1.5:1. The floral features correspond to a combination of anemophily and entomophily and the results established the occurence of ambophily in *Z. armatum*. The present study emphasizes the importance of sexual dimorphism, male biasness and ambophily on the reproductive output of the species.

Funding: We acknowledge the Head, Department of Botany, University of Jammu for providing the necessary laboratory facilities.

AT2.1 Structural maintenance of chromosomes SMC5/6 complex is necessary for meiotic chromosome reduction in *Arabidopsis*

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Meiosis is a critical stage of plant sexual reproduction. Its key events include controlled induction of DNA double-strand breaks, exchange of segments between homologous chromosomes, and production of haploid spores. We will show that *Arabidopsis thaliana* Structural maintenance of chromosomes 5/6 (SMC5/6) complex is essential for normal progression of meiosis and healthy offspring. Initially, we found that the SMC5/6 complex mutants produce about 15-20% paternally-induced triploid plants. The analysis of whole male reproductive development revealed a frequent migration of all chromosomes to one nuclear pole during meiosis, resulting in about 30% unreduced microspores. Fertilization with diploid pollen resulted in seeds containing a triploid embryo and tetraploid endosperm, representing in excess of the paternal genome, problems with endosperm cellularization, and frequent seed abortion. However, some of the aberrant seeds survived and gave rise to the aforementioned triploid offspring. In conclusion, we show a novel role of the SMC5/6 complex in the maintenance of gametophytic ploidy in *Arabidopsis*.

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AT2.2 H3K9 demethylases are required for male meiosis in *Arabidopsis thaliana*

Jinping Cheng, Hua Jiang

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Dimethylation of histone H3 lysine 9 (H3K9me2), a crucial modification for heterochromatin formation and transcriptional silencing, is essential for proper prophase progression in mammals. H3K9me2 is usually found at transposable elements and repetitive sequences but is absent from the bodies of protein-coding genes. Here we will show that the *Arabidopsis thaliana* H3K9 demethylase IBM1 regulates crossover formation and chromosome segregation by protecting thousands of protein-coding genes from ectopic H3K9me2, including genes essential for prophase progression. In addition to removing H3K9me2, IBM1 interacts with multiple cohesin complex cofactors. The mutants with compromised cohesin complex cofactors shared similar transcriptional alterations with ibm1, including meiosis-essential genes, yet without affecting H3K9me2 levels. Hence, cohesin complex cofactors, together with IBM1, regulate male meiosis and gene expression independently of H3K9 demethylation. These findings uncover a novel role of H3K9me2 demethylation in meiosis and a new function of H3K9 demethylases and cohesin cofactors in transcriptional regulation.

AT2.3 Dissection of meiotic recombination and genomic architecture of holocentric plants with repeat-based centromeres

André Marques, Meng Zhang

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Meiotic recombination across chromosomes is limited to certain hotspots that are typically distal from centromeres, i.e., crossover (CO) formation is suppressed around centromeric regions. Nevertheless, considering the fact that holocentric chromosomes are characterized by the interspersed centromere-like regions along the entire chromosome, whether recombination of holocentric species is also depleted at centromeric units would be an interesting question to address. Hence we constructed the first recombination map of repeat-based holocentric species *Rhychospora*, i.e. it possesses centromeric repeats analogous to monocentromeres. To quantify the proximity of CO events to holocentromeres. On the one hand, the whole-genome DNA sequencing of selfed F1 plants of *Rhynchospora tenuis* (2n=4) was used to determine their genotypes. No obvious crossovers were observed suggesting that *R. tenuis* is achiasmatic which was consistent with our previous cytological observations. The following CO identification through scRNA-seq of pollens also supported the achiasmatic property of *R. tenuis* and showed biased meiotic segregation for chromosomes with different genotypes. On the other hand, CO events were identified in another holocentric plant *R. breviuscula* (2n=10) after genotyping its pollens with scRNA-sequencing. A further inspection of the distance of CO events to Tyba arrays, the centromeric repeat units specific in *Rhynchospora*, showed neither overlaps between them nor obvious evidence of tendency off from Tyba arrays. Furthermore, to investigate the impacts of holocentromere on the 3D organization of chromosomes and genomes, DNase-based Hi-C of *Rhynchospora* were sequenced and used to generate Hi-C maps. *Rhynchospora* showed, however, significantly fewer inter-chromosomal interactions compared to monocentric plants, such as *Juncus effusus* (a monocentric species close to holocentric plant), *Arabidopsis thaliana*, and *Oryza sativa*. More species will be integrated into this comparison.

AT2.4 ZIP4: stabilization of wheat as a polyploid and its impact on breeding

Azahara C. Martín, Abdul Kader Alabdullah, Graham Moore

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Meiosis is one of the biggest challenges facing a new polyploid. Multiple related chromosomes must be discriminated, ensuring regular chromosome segregation and fertility by allowing only homologous chromosomes to recombine. For 60 years, a locus named *Ph1* (Pairing homoeologous 1), arising on chromosome 5B during polyploidisation, has been considered responsible for stabilising the wheat genome during meiosis, by preventing crossover between related (homoeologous) chromosomes. A 59.3 Mb deletion mutant (*ph1b*) has been used in breeding to allow recombination between wheat and its wild relatives. We have now identified the major meiotic gene ZIP4, as the gene inside the *Ph1* locus responsible for wheat stabilisation.

On wheat polyploidisation, *ZIP4* duplicated from chromosome 3B onto 5B and diverged (*ZIP4-B2*). This duplicated gene performs two key meiotic functions: promotion of faithful chromosome pairing-synapsis, and suppression of related chromosome crossover. We obtained a CRISPR *zip4-B2* mutant with loss of both functions. This mutation yielded 50% fewer grains, confirming the critical role of *ZIP4-B2* in wheat fertility. Next, we generated a novel 'separation of function' *zip4-B2* mutant named *zip4-ph1d*, with loss of the crossover suppression phenotype but retained ability to promote correct pairing-synapsis. Remarkably, this *zip4-ph1d* mutant maintained chromosome stability and preserved grain number. Thus, contrary to accepted wisdom, the key event stabilising polyploid wheat is promotion of correct pairing-synapsis, rather than suppression of crossover between related chromosomes. We recommend the use of this new *zip4-ph1d* mutant in wheat breeding strategies to induce crossover between related chromosomes, rather than using the previously used *ph1* mutants.

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AT2.5 Members of the ELMOD protein family specify formation of distinct aperture domains on the *Arabidopsis* pollen surface

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Pollen apertures, the characteristic gaps in pollen wall exine, have emerged as a model for studying the formation of distinct plasma membrane domains. In each species, aperture number, position, and morphology are typically fixed; across species they vary widely. During pollen development certain plasma membrane domains attract specific proteins and lipids and become protected from exine deposition, developing into apertures. However, how sites for aperture domains are selected is unknown. Through a forward genetic screen in *Arabidopsis*, we have uncovered members of the ancient ENGULFMENT AND CELL MOTILITY DOMAIN (ELMOD) protein family as important regulators of aperture site selection. Although playing important roles in animals, ELMOD proteins have not been previously studied in plants. We have now found that two members of this family, MACARON (MCR) and ELMOD_A, act upstream of the previously discovered aperture proteins and their expression levels influence how many aperture domains will form on the surface of developing pollen grains. A third ELMOD family member, ELMOD_E, can interfere with MCR and ELMOD_A activities, changing aperture morphology and positions and creating new aperture patterns. Our findings reveal key players controlling early steps in aperture domain formation, identify residues important for their function, and open new avenues for investigating how diversity of aperture patterns in nature is achieved.

AT2.6 Multi -omics approach to describe gene expression dynamics in developing pollen of *Arabidopsis thaliana*

Božena Klodová[1,2], David Potěšil[3,4], Lenka Steinbachová[1], Christos Michailidis[1], Dieter Hackenberg[1], Zbyněk Zdráhal[3,4], Jörg D. Becker[6,7], David Twell[5], David Honys[2]

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Angiosperm sexual reproduction depends on successful delivery of the two male gametes to female ovules. During the male gametophyte development, gene expression is strictly regulated and undergo dynamics changes to ensure correct developmental progression, pollen tube germination and rapid growth through the pistil and eventual double fertilisation. Transcriptomic analyses of developing pollen have been addressed in several species. In this study, by integrating various -omics approaches, we addressed the gene expression dynamics in four stages of microgametogenesis in two *Arabidopsis* accessions (Col-0 and Ler-0) to provide complex regulatory map on protein, transcripts and isoform level. We compared original published pollen microarray-based platform data to demonstrate superiority of RNA-seq approach but also high reproducibility of the two methods. The analysis identified additional regulatory mechanisms including several thousands of long non-coding RNAs with potential role in pollen development previously undocumented. Also stood out where differential isoforms events and translation dynamics throughout the developmental stages and across accessions. In summary this work summarises the dynamics in gene expression and main regulatory trends in two accessions of *Arabidopsis thaliana*.

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AT2.7 *HvTDF1* gene reveals a conserved role in controlling anther tapetum development in dicot and monocot

Miaoyuan Hua[1,2], Wenzhe Yin[1]*, José Fernández[1], Jie Zong[2], Guangwei Xing[2], Shuya Shi[1], Zoe A Wilson1†Miaoyuan Hua, Wenzhe Yin, José Fernández, Jie Zong, Guangwei Xing, Denise Mclean, Shuya Shi, Zoe A Wilson[1]

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Barley (*Hordeum vulgare*) a member of grasses species, is one of the major temperate cereal crops grown globally. Understanding and being able to modify crop fertility, particularly pollen development, is critical for hybrid breeding, yield increases and resilience of yield. Several conserved and distinct anther genes have been identified in Arabidopsis and rice as critical for pollen development; we have used these as a starting point to understand barley anther development by translating this knowledge into barley. The tapetum, the innermost somatic cell layer in anther locule, directly communicates with the developing gametophytic cells, providing essential nutrients, critical components for their development and maturity. The anther tapetum plays a key role in pollen wall development, and subsequent programmed degeneration serves to release pollen wall materials for the developing pollen grains. Studies have shown in *Arabidopsis* that this is controlled through a regulatory genetic pathway, DYT1-TDF1-AMS-MS188-MS1. TAPETAL DEVELOPMENT and FUNCTION1 (TDF1) is an R2R3 MYB family transcription factor, which plays an essential role in anther tapetal cell development in Arabidopsis and rice. Here, we characterized the barley orthologous HvTDF1 by a reverse genetic approach. The spatial and temporal analysis of *HvTDF1* expression pattern shows it has the similar expression pattern in barley anthers and is important in the tapetal cell layer and PMC development. *HvTDF1* could recover *Attdf1* mutant fertility in *Arabidopsis*, which suggests a conserved role for TDF1 genes in tapetum development in monocot and dicots. We have used a modified Dual-luciferase assay and transcriptomic analysis to dissect the role that HvTDF1 is playing in barley pollen development. The role of TDF1 and the tapetum regulatory network in barley required for functional pollen development will be presented.

AT2.8 The VACUOLAR SORTING PROTEIN 13 (VPS13) affects female germline establishment and progression by acting on small RNA pathway.

Mara Cucinotta[1], Rosanna Petrella[1], Alessandro Ruiu[1], Peter J. Van Dijk[2], Diana Rigola[2], Rik Op den Camp[2], Lucia Colombo[1]

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Germline specification is a crucial step in plant sexual reproduction. In the nucellus of the ovule one of the sub-epidermal cells differentiates into the megaspore mother cell (MMC), that undergoes meiosis to form four spores. The three most apical spores degenerate, while the remaining one, the functional megaspore (FM), enters megagametogenesis to ultimately form the mature female gametophyte. Emerging data suggest that small RNAs play an important role in megasporogenesis. Here we reported a novel role for VACUOLAR SORTING PROTEIN 13 (VPS13) in ovule development and in the establishment and progression of the female germline. Using different marker lines, we showed that lack or alteration of *VPS13* expression affects MMC and FM identity and the correct progression of megagametogenesis. The regulatory function of VPS13 appears to be linked to the small RNA pathway. In particular, we observed that in *vps13* mutant the production of mature mir390 is impaired, leading to a miss-regulation of *AUXIN RESPONSE FACTOR 3* (*ARF3*), which encode for an important regulator of MMC identity. Our findings reveal the role of VPS13 in the regulation of female germline specification in *Arabidopsis* and pave the way for a better understanding of the small RNAs involved in this process.

AT3.1 Two subgroups of *Arabidopsis* receptor-like kinases regulate intra- and inter-species pollen-pistil interactions.

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In *Arabidopsis*, the regulation of pollen-pistil interactions begins rapidly after pollen grains have landed on the stigmatic papillae at the top of the pistil. As the pollen grains are in a desiccated state for dispersal, the first post-pollination step is for the pollen grains to hydrate. The water for hydration comes from the stigma but is only released when the pollen grain is recognized as compatible. Following hydration and germination, a pollen tube emerges and penetrates the stigmatic surface to begin its journey down the reproductive tract to an ovule for fertilization. In *Arabidopsis*, many signalling players have been identified that regulate the later stages of these pollen-pistil interactions (e.g. ovular pollen tube guidance and reception), but little is known about the regulators of these interactions in the preceding stages. We have identified two groups of *Arabidopsis* receptor-like kinases that play an essential role in the upper pistil for compatible pollen hydration and pollen tube growth. Multiple knockout mutants were generated and combined for these receptor kinase genes, and the mutant pistils were pollinated with wild-type pollen to evaluate the effect of these loss of functions mutations on pollen-pistil interactions. Several phenotypes were observed including reduced wild-type pollen hydration and reduced wild-type pollen tube travel distances. Furthermore, using pollen from related Brassicaceae species, we also discovered that these receptor kinase genes play a role in forming a reproductive barrier in the pistil to prevent interspecies pollen tube growth. Thus, these novel *Arabidopsis* receptor-like kinases play a dual role in the female pistil to promote compatible pollen and block closely related pollen.

AT3.2 Engineered *Arabidopsis* pollen establishes a role of ATP depletion and cytosolic acidification in *Papaver* self-incompatibility

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The self-incompatibility (SI) system in poppy (*Papaver rhoeas*) is mediated by the interaction of two S-determinants: a pollen-expressed plasma membrane-localised protein (PrpS) and a stigma-expressed secreted protein (PrsS). Interaction of cognate PrpS–PrsS triggers a signalling network, causing rapid growth arrest and programmed cell death (PCD) in incompatible pollen. It has been demonstrated that challenging transgenic *Arabidopsis thaliana* pollen expressing *PrpS* with cognate recombinant *Papaver* PrsS proteins triggers remarkably similar cellular events to those observed in incompatible *Papaver* pollen, including alterations to the actin cytoskeleton, cytosolic acidification and PCD. Combined with genetically encoded fluorescent probes and multiparameter live-cell imaging, using transgenic Arabidopsis pollen expressing *PrpS* has allowed us to study SI-induced cellular alterations in much more depth and with genetic tools that were not possible in *Papave*r. Here we present the first live-cell observations, with detailed spatio-temporal dynamics of the actin remodelling during the SI response in pollen tubes expressing Lifeact-mRuby. Our recent work identifies for the first time that SI triggers a rapid and significant ATP depletion in pollen tubes. Artificial depletion of ATP triggered cytosolic acidification and formation of actin aggregates. Our data shows that pH itself can trigger actin filament breakdown and aggregation in vitro and reveals a threshold [pH]cyt of 5.8 for actin foci formation in vivo. These studies uncovered a link between the cellular energy status, cytosolic acidification, and alterations to the actin cytoskeleton, indicating that cellular ATP levels and cytosolic pH both play a pivotal role in mediating *Papaver* SI in pollen tubes. Our study establishes the foundation for new opportunities to elucidate key mechanisms involved in SI and provides novel insights in the components that play crucial roles in plant cells under these extreme, but physiologically relevant circumstances.

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AT3.3 Flavonols take the heat out of heat stress to protect pollen from elevated ROS

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Plant reproduction requires long-distance growth of a pollen tube to fertilize the female gametophyte and this growth is extremely sensitive to high temperature stress. Reactive oxygen species (ROS) are known to be important regulators of pollen tube function, however, mechanisms responsible for modulating ROS signaling in response to elevated temperature are not understood. We examined the anthocyanin reduced (are) tomato mutant, which has reduced synthesis of flavonols - specialized metabolites with potent *in vitro* reactive oxygen species (ROS) scavenging properties. This *flavonoid 3-hydroxylase* (*f3h*) mutant had impaired pollen formation, viability, and tube growth, resulting in reduced seed set. It was also hypersensitive to the effects of heat on pollen viability and tube growth. Consistent with the role of flavonols as ROS scavengers, are had elevated levels of ROS. Inhibition of ROS synthesis or scavenging of excess ROS with an antioxidant reversed the are phenotypes. Interestingly, heat stress-induced overaccumulation of ROS in pollen was amplified in are, but absent in cultivars with thermotolerant reproduction, indicating that ROS are an integral component of the heat stress response. Using *F3H* overexpression lines, we found that flavonol overaccumulation in these lines resulted in higher pollen viability than in the thermosensitive wildtype VF36 during heat stress. Supplementation of the pollen germination medium with the flavonol quercetin also prevented high temperature effects on pollen tube growth in VF36. Taken together, our results reveal that flavonol metabolites regulate plant sexual reproduction at normal and elevated temperatures by maintaining ROS homeostasis. We are profiling the transcriptional responses to high temperature in pollen from reproductively thermosensitive and -tolerant tomato cultivars to provide insight into the molecular events that convey thermotolerance. We have identified pollen-specific genes that putatively control ROS synthesis or scavenging and are testing their function in thermotolerance.

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AT3.4 Transcriptome reprogramming in the *Arabidopsis* male germline during the progamic phase

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A pollen tube's journey through the transmitting tissues of the pistil leads to changes in its gene expression profile. To which extent these changes occur in the vegetative cell (pollen tube) or in the sperm cells it transports has not been addressed so far. This question is of particular importance since sperm cells are believed to acquire competence for fertilization during pollen-pistil interactions. Here, we have compared the transcriptomes of Arabidopsis thaliana sperm cells and vegetative nuclei isolated from mature pollen grains and from pollen tubes grown semi in-vivo. We used an optimized semi in-vivo system and a fluorescent marker line to isolate GFP labelled sperm cells and RFP labelled vegetative nuclei by FACS, followed by RNA-seq. Moreover, to determine how many genes are elicited in the male gametes and the vegetative nuclei exclusively in the presence of female cues, we also analyzed sperm cells and vegetative nuclei from pollen tubes grown in vitro. Our data indicate that sperm cells undergo extensive transcriptomic changes during pollen tube growth, some of which are elicited exclusively when they are passively transported within the pistil, revealing hitherto unidentified transcripts that may be important for sperm maturation and gamete fusion. Similarly, vegetative nuclei undergo even more extensive transcriptome reprogramming during pollen tube growth, mainly through the upregulation of genes associated with pollen tube growth and vesicle mediated transport. Interestingly, comparison with published ATAC-seq data shows that chromatin at the promoters of genes up-regulated in sperm during pollen tube growth is already open in sperm of mature pollen grains, suggesting pre-configured promoter accessibility. Many of these genes were also found to be targets of H3K27Me3. Our study provides the most comprehensive overview of transcriptome dynamics of Arabidopsis sperm cells and vegetative nuclei during pollen tube growth to date.

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AT3.5 Integration of ion dynamics into a membrane potential gradient in pollen tubes

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Pollen tubes (PT) are excellent models for studying processes involved in apical growth and cell polarity. Their growth relies on a choreography of extracellular ion fluxes and intracellular ion gradients of Cl^-, Ca^{2+}, H^+ and K^+ that create a unique electrochemical environment at the tip. These dynamics are relevant for chemotropic sensing of external clues. Given the magnitude of the ion dynamics, the pattern of extracellular fluxes and intracellular ion concentrations, we hypothesized that the electric potential differences between the tip and the shank are compatible with a standing membrane potential gradient (MPG) focused at the tip. Moreover, we posit that the consequence of a standing electric field in the PT's tip establishes cell polarity, which agrees with mounting evidence of electrotactic-like phenomena in PTs and other systems. Expression of a genetically-encoded K^+ probe in PTs revealed a standing negative gradient from 250 mM at the tip to 450 mM at the shank, hinting at an efflux of K^+ at the tip. Assuming that membrane potential is Nernstian to $[K^+]$cyt, this gradient should contribute to a depolarized tip. To test the existence of a MPG, we used two membrane potential dyes with opposite fluorescence kinetics that revealed a 15 µm long MPG. The voltage-dyes were calibrated using depolarizing $[K^+]$out to assess their dynamic range and the tip MPG was estimated to be about 30 mV depolarized compared to the shank, compatible with [K+]cyt. Further, we tested ion-channel mutants showing severe male-fertility phenotypes, either because PTs growth is impaired, or because chemotropism is defective. Mutants showed a departure in membrane potential compared to WT and the MPG is partially dissipated. The correlation of the MPG with apical growth and the bioelectrical state with chemotropism opens important questions in our understanding of the upstream processes determining PTs polarity, growth and chemo-sensing pathways.

AT3.6 Molecular basis of pollen germination and tube growth in rice

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Rice is the major staple cereal food and a model crop plant which is important for studying agro-nomically valuable traits. Although knowledge on the regulation of pollen germination and interaction between gametes has advanced rapidly in model plant species such as *Arabidopsis*, the molecular mechanism determining pollen germination and tube growth in rice for male-female interaction remain largely unknown. Unlike *Arabidopsis* with many ovules in one carpel, the carpel of rice only contains one ovule, therefore gametic defect of rice frequently is not able to result in homozygous mutants, which makes more difficult for functional study. Previously, we have found 627 late-pollen specific genes by genome-wide identification in rice two subspecies, *japonica* and *indica*. Functional characterization of these late pollen-specific genes will help elucidate molecular processes required for pollen germination and pollen tube growth in rice. To reduce efforts to screen all the genes' function, we have analyzed the distorted segregation of heterozygous plants from 216 lines of T-DNA indexed mutants and estimate functional dominancy of the target genes using CAFRI-Rice database. Subsequently, we found the 19 candidate genes playing key roles in male gene transmission. To perform detailed functional study, we create homozygous mutant lines for the key candidate genes at T0 generation using CRISPR/Cas9 system. In addition, we generate gene editing mutants for multiple targets which have functional redundancy revealed by normal segregation ratio in the T-DNA insertional lines. Thus, we expect that our novel mutant resources will be applied for crop improvement through enhanced pollination or hybrid breeding.

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AT3.7 Keeping growth in check – regulation of polar signaling in pollen of *A. thaliana*

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Polar signaling and growth are required for most cellular and developmental processes. Pollen germination is a crucial step in fertilization of flowering plants, during which dormant pollen grains establish a de novo polar growth domain at the contact site with the floral tissue. This leads to the formation of a pollen tube that grows into the floral tissues towards the female gametophyte. In order to allow only compatible pollen to germinate on the stigma or to prevent premature germination of pollen tubes, pollen germination needs to be tightly regulated.

We investigate the role of RECEPTOR-LIKE KINASES (RLKs) and RhoGTPase OF PLANTS (ROP) signaling to understand how a new polar growth domain is established to form a pollen tube and how this process is kept in check to prevent premature pollen germination. We identified activators of ROP signaling, ROP GUANIN NUCLEOTIDE EXCHANGE FACTORS (ROPGEFs), which specifically activate pollen germination. Mutants of these ROPGEFs have a reduced pollen germination efficiency and live cell imaging revealed a transient accumulation of these ROPGEFs at the germination site shortly before pollen germination. Additionally, we identified an RLK and a ROPGEF-regulating cytoplasmic kinase that inhibit pollen germination. Overexpression of either protein completely abolished pollen germination, while mutants prematurely germinate in moist conditions.

In summary, our data shows that pollen germination is activated by a specific subgroup of ROPGEFs and that multiple proteins negatively regulate the activation of ROP signaling to prevent premature pollen germination.

AT3.8 Enhanced pollen tube integrity was selected during breeding of tomato varieties that set fruit at elevated temperature.

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Increasing temperature extremes during reproduction threaten crop yields and food stability. Grain and fruit crops depend on the ability of pollen tubes to deliver sperm to female gametes for fertilization and this critical and temperature-sensitive phase of the reproductive process could limit productivity under heat stress. Cultivated tomato (*Solanum lycopersicum*) is a useful model because fruit production requires successful pollen tube growth and tomato varieties have been bred that continue to produce fruit at temperatures that limit production in other varieties. We are using tomato as a model to test the hypothesis that breeding for enhanced fruit set at high temperature selected variants that enhance pollen tube performance under temperature stress. We developed a live imaging system that allows us to quantify several parameters of pollen performance while pollen grains germinate and elongate in-vitro at either control (28°) or heat stress (34°) temperatures. We built a python-based tool (TubeTracker) that automates quantitative analysis of pollen germination, tube extension, and integrity. We conducted a comprehensive analysis of pollen performance for two standard and four improved varieties and found that temperature stress affects at least one parameter of pollen performance in all varieties analyzed. Stinkingly, pollen tube rupture was a common response to temperature stress in all varieties. However, three of the four varieties bred for fruit set at higher temperatures, showed significant reductions in pollen tube rupture under heat stress. These findings indicate that selection for enhanced pollen tube integrity occurred during the process of breeding thermotolerant fruit production. Analysis of the pollen tube transcriptome points to shared and unique responses to heat stress across these varieties and our focus now is on identifying the molecular basis for enhanced pollen tube integrity by analyzing variants in gene sequence and expression for pathways known to regulate pollen tube rupture.

AT4.1 RNA binding proteins at the nexus of pollen tube guidance

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Cellular fate of messenger RNAs (mRNA) is predetermined by cis-acting elements that are platforms for RNA binding proteins (RBPs). RBPs act immediately co-transcriptionally through close association with RNA PolII C-terminal domain before mRNA nuclear export and as late as stalling ribosome scanning during active translation. RBPs define RNA storage fate, half-life duration, active translation or RNA turnover. Here, we summarize our recent findings of two RBPs with direct impact on pollen tube guidance. LA and related proteins (LARPs) share La motif (LAM), a structured RNA-binding domain, and an RNA recognition motif (RRM1) immediately after the LAM that together forms a bipartite RNA-binding unit called the La-module. In dry pollen, LARP6C binds to transcripts encoding proteins that function in lipid synthesis and homeostasis, vesicular trafficking, and polarized cell growth in cytoplasmic granules that also contain the poly(A) binding protein. Loss of LARP6C negatively affects the quantities and distribution of storage lipids, as well as vesicular trafficking. Using a reporter mRNA, we show that LARP6C shifts from a repressor function in pollen to an activator of translation in pollen tube. This hint on the LARP6C likely role on mRNA repression and storage during pollen maturation. Another, Pre-mRNA PROCESSING factor 8, PRP8A and PRP8B, regulate splicing of 3% of ovule-expressed genes as well as self-splicing. Mutant *prp8a prp8b* fail in pollen tube attraction and ovule targeting suggesting a regulatory function in both gametophytes. This work is an ongoing initiative on the reinforced mechanisms of RBPs on mRNA fate associated with polar proteins on cell-elongation and cell-cell communication for successful fertilization.

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AT4.2 The JAGGER GPI-anchor defines the protein subcellular localization. Does it mediate JAGGER function?

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In angiosperms, pollen tube reception by the embryo sac implies a series of cell-cell interactions between the male and female gametophytes and with the female sporophyte. Fertilization in flowering plants involves the delivery of two sperm cells by the pollen tube to the embryo sac, forming the seed embryo and endosperm. Polytubey, the simultaneous penetration of ovules by more than one pollen tube is usually avoided, preventing polyspermy. How plant ovules regulate the rejection of extra tubes after successful fertilization is not well understood. Arabinogalactan proteins (AGPs) are highly glycosylated proteins involved in several steps of the reproductive process. Particularly, JAGGER (AGP4) is an important molecule necessary to prevent polytubey in Arabidopsis thaliana. JAGGER is a glycosylphosphatidylinositol (GPI)-anchored protein, a class of cell surface proteins that may participate in signal transduction pathways, mediating cell-to-cell signalling. Our results pursue the importance of the JAGGER GPI-anchor in the protein subcellular localization and the putative impact on polytubey block. For this, JAGGER was fused to citrine yellow fluorescent protein (JAGGER-YFP) and several JAGGER-YFP deletions variants of the GPI-anchor signal were produced. The functional characterization of these mutants is discussed.

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AT4.3 Redox interplay of ROS-level dynamics and glutathione metabolism upon gamete fusion and subsequent zygotic development in rice

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Despite their well-known cytotoxicity, reactive oxygen species (ROS) have recently been widely accepted as potent signaling molecules that regulate many physiological processes in plants. However, ROS-mediated signaling mechanism in early developing zygotes is still not yet well understood. Therefore, dynamics of ROS level and their functions were analyzed in this study using rice zygotes produced by in vitro fertilization. Diphenyleneiodonium (DPI), an inhibitor for ROS production, was found to fully inhibit the first cleavage of the rice zygotes, despite the occurrence of karyogamy, indicating the important role of ROS in promoting zygotic development. After gamete fusion, overall intracellular ROS levels gradually dropped toward the minimum extent during early development of zygotes and, thereafter, increased again in late-stage zygotes. Although total intracellular superoxide (O_2^-) levels showed no significant difference between egg cells and fertilized zygotes, the levels of mitochondrial O_2^- were abruptly diminished immediately after fertilization, indicating that entry of a sperm cell into an egg cell can quickly trigger the conversion of O_2^- into hydrogen peroxide (H_2O_2), specifically in mitochondria, to possibly activate the rapid physiological response via H_2O_2-mediated signaling mechanism. In addition, the rebound of high O_2^- level was estimated to be important for proper development of globular-like embryo. Moreover, the crucial role of glutathione metabolism in early zygotic development was shown by the blockage of glutathione synthesis using buthionine sulfoximine (BSO), which arrested zygotic development at two-cell embryo stage. Not only the upregulation and nuclear localization of glutathione peroxidase 1 (GPX1) were observed in zygotes, we also found that the inhibition of GPXs by mercaptosuccinic acid delayed and inhibited zygotic cleavage in a concentration-dependent manner. This suggests an interplay of H_2O_2 production and glutathione metabolism in the redox signaling mechanism during zygotic development in rice.

AT4.4 "Cells-in-a-cell": Which roles for the endo-plasma membrane that wraps the sperm cells?

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Accurate seed development relies on double fertilization that requires two separate and parallel fusion events between male and female gametes. On the male side, sperm cells are immobile and pollen ensures their transportation to the female gametes. Sexually mature pollen encloses two sperm cells in a unique "cells within a cell" structure. Indeed, within the large pollen vegetative cell the smaller sperm cells are tightly wrapped by an endo-plasma membrane (endo-PM) that originates from the vegetative cell. We document differences in the properties of the vegetative cell plasma membrane and this endo-PM, and decipher the mechanisms that confer attachment of a protein, the phospholipase NOT-LIKE-DAD (NLD, also known as MATL or ZmPLA1), specifically to this unique endo-PM. We demonstrate that contrary to earlier reports NLD is not localized inside but outside of the sperm cells, on the poorly characterized endo-PM. This finding triggers new questions of the instructive role of the vegetative cell in reproduction, knowing that the absence of NLD leads to genomic instability within the sperm cells and later on to maternal haploid embryo induction. Using cell biology tools, pharmacological and targeted mutagenesis strategies, we were able to show the implication of both lipid anchors and electrostatic interaction between membrane and NLD to target this non-transmembrane protein specifically to the endo-PM. Furthermore, the use of genetically encoded lipid sensors revealed a particular phospholipid signature of the endo-PM. After pollen tube burst the endo-PM locates at the apical region of the egg apparatus, the place of sperm cell discharge, and certainly helps to deliver the sperm cells. These results highlight the importance of this still enigmatic interfacial endo-PM for proper plant reproduction and gamete formation.

AT4.5 Fertilization initiates seed nutrition by degradation of callose deposition at the phloem end

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Seed formation is not only crucial for plant life but also for human life as nutrients from seeds are critical for human development. We identified a functional domain required for normal seed formation, which is located at the chalazal end of the ovule and is the phloem end also called phloem unloading region. The region has a unique structure supporting its transport function but is blocked by the deposition of callose. Callose deposits were removed after central cell fertilization (open state), allowing outer chemical substances to be transported to the seed. However, if fertilization fails, callose deposition will persist (closed state), preventing the phloem end from transporting chemicals into the ovule. As regulators of callose removal, CDE1 and CDE2 were identified. The *cde1* mutant shows the phloem end in the closed state and produces smaller seeds in response to incomplete callose degradation, indicating that the phloem end regulat es substance flow via callose deposition/degradation. In contrast, the OECDE1 overexpression line produced larger seeds than the wild-type in response to continuous callose degradation. The final form of the phloem end provides an explanation for why plants need fertilization to produce seeds.

AT4.6 ECS1 and ECS2 suppress the formation of haploid plants by promoting double fertilization

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In flowering plants, the number of pollen tubes that provide sperm cells to the female gametes is restricted by a pollen tube block. This safeguard mechanism is only activated after successful fertilization of both female gametes and involves the disintegration of pollen tube attracting synergid cells. It was previously reported that the endopeptidase ECS1 and ECS2, which are secreted by fertilized egg cells, prevent the attraction of supernumerary pollen tubes by cleaving the pollen tube attractant LURE1. Here we report on an earlier defect in *ecs1 ecs2* mutants that is manifested by fertility defects of either egg or central cell. The defect is accompanied by a delay in synergid disintegration providing an alternative explanation for the extra pollen tubes observed in the double mutant. These results are corroborated by our finding that *ecs1 ecs2* plants segregate haploid plants.

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AT5.1 Transcriptome analysis of sexual and apomictic *Boechera* leads to identification of the RNA helicase GAM as crucial regulator for gametogenesis

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Plant reproduction is of great importance for animal and human nutrition. To form seeds, in higher plants sexual and asexual reproduction (apomixis) have evolved. The evolutionary advantage of the co-existence of both reproductive modes and the gene regulatory control underlying apomixis are to date not fully understood.
Resembling crucial features of animal germlines, the importance of RNA helicases for plant reproduction has previously been proposed. RNA helicases are evolutionary ancient and functionally involved in basically any aspect of RNA metabolism, including splicing control, translational regulation and ribosome assembly. However, to date, only little is known about their roles during plant reproductive development.
To gain further insights into the spatial and temporal expression of RNA helicases during germline development, we applied transcriptional profiling of reproductive tissues from sexual and apomictic accessions of the genus *Boechera*, a close relative of *Arabidopsis*. This confirmed previous findings in *Arabidopsis* of an enriched expression of RNA helicases during germline formation. Furthermore, we identified a few genes of this family to be differentially regulated in sexual as compared to apomictic *Boechera* at a later developmental stage. This includes *GAM*, which is a unique gene in *Arabidopsis*, but represented by one ortholog and two paralogs in *Boechera*. Interestingly, phylogenetic analysis of sequence variants from a total of 24 different *Boechera* accessions revealed, that allelic variants of one paralog form closer groups dependent on the reproductive mode. This designates *GAM* as a potential candidate for apomixis. Investigations on the likely functional diversification of the genetic variants are ongoing. Nevertheless, we could already demonstrate that *GAM* is crucial for reproductive development in Arabidopsis, as lines carrying mutant alleles showed severe defects during male and female gametogenesis. Further analysis suggested an association of *GAM* to ribosome biogenesis. Therefore, our work identified *GAM* novel player, which is crucial for plant reproduction.

AT5.2 Development and evolution in male gametogenesis

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Recent studies have advanced our understanding of the development and evolution of the male germ cell lineage produced by the gametophyte generation of land plants. A central outstanding question in angiosperms is how asymmetric division of the microspore is linked to the specification of the de novo generated male germline. Studies of *Arabidopsis* mutants that disturb microspore division reveal that initiation of germ cell fate depends upon cell polarity, but not on the isolation of germ cell chromatin, supporting the notion of male 'germ-plasm'. Once the male germline is segregated, the differentiation of sperm cells depends on the MYB transcription factor DUO1 and its target genes which act during and after fertilisation. Male gametogenesis also depends upon on bHLH transcription factors, some of which are conserved in bryophytes along with DUO1. Conserved targets of DUO1 include the DAZ1 class of C2H2 zinc finger proteins that interact with the co-repressor TOPLESS, highlighting an emerging role for repressive mechanisms in male gametogenesis. Our initial efforts to uncover the scale and specificity of the DUO1-DAZ1 regulatory network have linked large-scale changes in gene expression with deregulated chromatin pathways in mutant germ cells. Recent and ongoing phylogenetic studies of the DUO1-DAZ1 regulatory module in diverse land plants are also providing new perspectives on the evolution of pathways controlling germline morphogenesis and gamete differentiation.

AT5.3 Genomic and ecological differentiation in a South American grass

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Reproductive syndromes and ploidy shifts play major roles in plant evolution. The establishment of new polyploids and the emergence of barriers to random gene flow drastically influence species' evolutionary trajectories. Independently evolved polyploids will be subjected to different biotic and abiotic constraints as well as different amounts of gene flow from co-occurring lineages. Previously, we found that sexual diploids and apomictic tetraploids of the grass species *Paspalum intermedium Munro* are ecologically differentiated along a latitudinal gradient in South America. To better understand polyploid evolution, we use genomic (Genotyping-by-sequencing), environmental (Ecological Niche Modelling) and reproductive data from 22 *P. intermedium* populations to assess population-level genetic variation and its correlation to environmental and reproductive variables. Diploids are genetically less variable than tetraploids (based on >180,000 informative SNPs). Neighbor-Net analysis shows reticulation consistent with a narrow diploid gene pool from which tetraploids arise. STRUCTURE analysis shows three consistent K values that relate to genetic and ecological attributes of *P. intermedium*. While K = 2 defines two groups each relating to a ploidy state, either diploid or tetraploid individuals, K = 5 and 8 also segregate polyploids into genetic groups due to lineage ancestry and geographic locality. Principal component and minimum spanning network analyses based on genetic data support a narrow cluster of diploid sexuals and more diverse tetraploid lineages differentiated by region (North, Centre, South) and cytotype co-occurrence (sympatry, parapatry, allopatry). Reproductive mode ratios (i.e., sexual and apomictic) correlate to environmental variables and levels of clonal diversity within populations in marginal areas. Overall, our analyses provide insights into early mechanisms of speciation highlighting patterns of intraspecific geographic genetic differentiation within and between cytotypes and under distinct gene flow conditions.

AT5.4 Pollen number is regulated by REDUCED POLLEN NUMBER1 encoding ribosome assembly factor and is characterized by an enriched selection signal among traits related to selfing syndrome in *Arabidopsis thaliana*

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Pollen number is highly variable within and between species, and is studied from the viewpoints of evolution and breeding. Reduced pollen number is a hallmark of selfing syndrome, which is observed in a predominantly selfing *Arabidopsis thaliana*. Two contrasting theories were proposed: maladaptation due to inbreeding depression, or adaptive evolution to reduce cost of pollen production. However, little was known about the molecular basis of this quantitative trait to test them using molecular tools. We previously conducted genome-wide association studies (GWAS) on pollen number, and isolated REDUCED POLLEN NUMBER1 (RDP1) gene encoding a homolog of yeast ribosome assembly factor Mrt4p (mRNA turnover4). To study biological processes in which RDP1 is involved, we conducted the RNA-seq analysis of the flower bud tissue of genome-editing frameshift mutant rdp1-3. Genes responsible for ribosomal large subunit assembly were enriched among upregulated genes, supporting the hypothesis that ribosome biogenesis is disturbed in the mutant. Pollen-development genes such as ABORTED MICROSPORES and its downstream genes were downregulated. To examine selection, we compared the enrichment of selection signals on the Arabidopsis GWAS peaks of traits related to selfing syndrome including pollen number, ovule number and floral organ sizes as well as of previously reported 107 diverse traits. Pollen number showed the strongest enrichment followed by ovule number. This suggested that the evolution of pollen number was adaptive. Taken together, our result indicated a specialized function of ribosomes in pollen development through RDP1, which harbors natural variants under selection.

AT5.5 Pre-zygotic mate selection in *Nicotiana attenuata*.

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Nicotiana attenuata is a self-compatible species in which every cross is accepted and self-incompatibility is not observed. However, in the genetically diverse native populations over 30% of seeds are derived from opportunistic outcrossing events and certain pollen donors are consistently selected in mixed pollinations. A crucial role of the genes homologous to *Solanaceae* SI factors and the post pollination ethylene burst (PPEB) was previously described, but the knowledge of the exact mechanism, regulation and ecological function of this polyandrous mate selection remain unknown. To identify genetic loci and uncover novel elements potentially involved in the control of mate choice, we took advantage of the fact that PPEB is an accurate predictor of pre-zygotic mate selection and seed paternity. We applied QTL mapping to the PPEB data obtained after pollination of each member of 26-parent MAGIC population with standardized pollen donor. To further test the role of selected candidate genes, we performed mixed pollination experiments using the RNAi transgenic lines and equal mixture of 14 pollen donors. We employed various analytical and molecular biology approaches to explore the role of previously described NaS-like-RNases and ethylene signaling in this process. We set up a seed bank experiment and offspring fitness tests to address the ecological function of the mate selection and explored whether this process is adaptive. In summary, the results discussed here not only provide new insights into pre-zygotic mate selection in *N. attenuata*, but also broaden our understanding of the sexual reproduction system of Solanaceae and plants in general.

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AT5.6 Phylogenetic and expression analysis of *CENH3* and *APOLLO* genes in sexual and apomictic *Boechera* species

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So far *APOLLO* gene encoding aspartate glutamate aspartate aspartate histidine exonuclease is one of the very few described genes associated with apomixis in *Boechera* species. The centromere-specific histone H3 variant encoded by *CENH3* gene is essential for cell division. Mutations in *CENH3* disrupt chromosome segregation during mitosis and meiosis since the attachment of spindle microtubules to a mutated form of the *CENH3* histone fails. We studied *in silico* characteristic of *APOLLO* and *CENH3* genes, which may affect apomixis. Study expression levels of *APOLLO* and *CENH3* transcripts was performed in gynoecium/siliques of the natural diploid apomictic and sexual *Boechera* species at the stages of meiosis and before and after fertilization. The *APOLLO* gene has several polymorphic alleles associated with sexual and apomictic reproduction in the *Boechera* genera. Expression of the *APOLLO* apo-allele during meiosis was upregulated in gynoecium of apomict *B. divaricarpa* downregulating after meiosis. In sexual *B. stricta gynoecium* and siliques *APOLLO* apo-allele did not express. Expression of the *APOLLO* sex-allele during and after meiosis in gynoecium of sexual plants was several times higher than that in apomictic gynoecium. At the meiotic stage, the expression level of *CENH3* in the gynoecium of apomicts was two times lower than that of the sexual *Boechera*, decreasing in both species after meiosis and keep remaining very low in siliques of both species for several days after artificial pollination. We also discuss polymorphism and phylogeny of the *APOLLO* and *CENH3* genes. The results obtained may indicate to a role of the *CENH3* and *APOLLO* genes in the development of apomixis in species of the genus *Boechera*.

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AT6.1 Embryonic elimination and post-meiotic drive of chromosomes – different sites of the same coin?

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Not necessarily all cells of an organism contain the same genome. Some eukaryotes exhibit dramatic differences between cells of different organs, resulting from programmed elimination of chromosomes or their fragments. Here, we present a detailed analysis of programmed B chromosome elimination in plants. Using goatgrass *Aegilops speltoides* as a model, we demonstrate that the elimination of B chromosomes is a strictly controlled and highly efficient root-specific process. At the onset of embryo differentiation B chromosomes undergo elimination in proto-root cells. Independent of centromere activity, B chromosomes demonstrate nondisjunction of chromatids and anaphase lagging, leading to micronucleation. Chromatin structure and DNA replication differ between micronuclei and primary nuclei and degradation of micronucleated DNA is the final step of B chromosome elimination. This process might allow root tissues to survive the detrimental expression, or overexpression of B chromosome-located root-specific genes with paralogs located on standard chromosomes. Comparing the cellular process of post-meiotic B chromosome drive in *Ae. speltoides* and rye with the process of B elimination reveals striking similarities. B chromosomes are often preferentially inherited, deviating from Mendelian segregation. In both processes nondisjunction of Bs occurs despite centromere activity and centromere-tubulin interaction. Spindle symmetry differs between the two processes: during the first pollen mitosis an asymmetric cell division occurs whereas the spindle in roots is symmetrical. As a consequence, in roots, lagging B chromosomes form micronuclei and undergo elimination. In contrast, due to the asymmetric geometry of the spindle at first pollen mitosis, the inclusion of the lagging joint B chromatids into the generative nucleus takes place and chromosome accumulation occurs. Differential RNAseq analysis revealed candidate genes controlling the processes of chromosome drive and chromosome elimination.

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AT6.2 Setting up the stage for analyzing parental-dosage-dependent effects on barley grain development

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Cereal grains represent a specific type of seeds where the largest part is formed by endosperm, a specialized triploid tissue for embryo protection and nourishment. The endosperm combines both parental genomes in an unusual ratio of two maternal and one paternal copies (2m:1p), and this ratio is crucial for normal seed development. As a model to study parental-dosage-dependent grain development in cereals, we use cultivated barley (Hordeum vulgare subsp. vulgare) and its wild progenitor (*H. vulgare subsp. spontaneum*). We developed and characterized barley synthetic autotetraploid lines, and performed inter-ploidy reciprocal crosses. We found that the viability of inter-ploidy hybrid seeds was impaired in both directions of hybridization but in different ways. Pollination of 2x mothers with pollen from 4x fathers resulted in failed endosperm cellularization, whereas the endosperm of 4x mothers pollinated by 2x fathers hybrids cellularized precociously. This finding sets a new long-term direction for our study to understand the molecular mechanism of 'parental conflict' in intra- and interspecific hybridization in cereals.

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AT6.3 High temperatures impact on early seed development and embryo morphogenesis in *Arabidopsis thaliana*

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In the last decades, we have witnessed the worldwide effects of climate change. The average global temperature and frequency of extreme temperatures increased year after year. The yield production of temperate crops has decreased due to high temperatures and drought and is expected to reduce even more in the upcoming years. In plants, the reproductive phase is a developmental stage sensitive to high temperature with heat-sensitive processes such as pollen development. The response to high temperature involves transcription factors, such as phytochrome interacting factors or heat shock factors, chaperone proteins, and phytohormones production, creating a complex response with different levels of regulation. However, most of the available data focus on heat shock and pollen development, while information regarding long-term adaptation is scarce, especially related to ovule and embryo development. We have performed a complete phenotyping analysis of the reproductive phase of wild-type *Arabidopsis thaliana* plants and two temperature-sensitive mutants, *hsbp-II* and *hot1-3*, with different high-temperature scenarios. Also, we analyzed the expression profile of genes regulating the heat response in pistils and siliques of *Arabidopsis thaliana*. Furthermore, with the assistance of Labdeers s.r.o., we studied the yield and quality of seeds produced in high-temperature conditions. Our results provide new insight into long-term adaptation to high temperatures during the reproductive phase of plants. We observed that pollen, but especially ovule development, are negatively affected by high temperatures. Embryo development is accelerated by high temperatures, resulting in morphological abnormalities similar to defects in various auxin-related mutants. We showed that abnormal embryos have a different auxin signaling pattern when compared with WT-like embryos. The expression of some auxin biosynthetic genes is also altered. The reduction of fertility is translated to smaller seed production and a worsened quality of those seeds.

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AT6.4 Do *VIM* genes have a role in embryo and endosperm development?

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"DNA methylation and histone modifications are known regulators of gene expression. In mammals, DNMT1 is the main enzyme responsible for DNA methylation maintenance. DNMT1 is recruited to hemi-methylated DNA by its cofactor UHRF1, which also recognizes silent histone marks and recruits effectors for chromatin silencing, providing a connection between DNA methylation and histone modification. In plants, DNMT1 and UHRF1 homologues are called MET and VIM, respectively. Of the six *VIM* genes in Arabidopsis, *VIM1*, *VIM2* and *VIM3* are functionally redundant and the triple mutant presents alterations in chromatin modifications and DNA methylation, suggesting a similar role to DNMT1 in mammals. The role of *VIM* genes during seed development has not been described.

In this work, we are testing whether *VIM* genes have a significant role during embryogenesis, and whether they are preferentially expressed from the paternal allele. An analysis of public transcriptome data showed that in the embryo all *VIM* genes but *VIM5* are expressed from 14 hap to 3 dap, while in the endosperm all *VIM* genes were expressed at 4 and 6 dap. 4 out of 6 *VIM* genes had paternally biased expression at one or more timepoints. To validate preferential paternal expression and tissue-specific expression patterns, I am currently generating reporter lines for each of the *VIM* genes. I am also examining seeds from individual vim mutant lines for abnormal phenotypes, and so far have found four consistent embryo phenotypes for 4 different *VIM* genes. I am now generating triple and quadruple mutants to further dissect the role of *VIM* genes during seed development."

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AT6.5 Distinct parental signals polarize the *Arabidopsis* zygote to initiate the embryonic patterning process

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Plant embryogenesis is initiated by an asymmetric cell division of the zygote. In *Arabidopsis thaliana*, the cellular identity of the resulting daughter cells is controlled by a MAP kinase signaling-cascade including the MAP3K YODA (YDA). Polar activation of YDA suppresses embryo formation in the basal daughter cell and ultimately promotes suspensor formation. In *Brassicaceae*, the YODA pathway can be activated by distinct parental contributions: This includes canonical receptor-mediated signaling by a maternally provided receptor complex including ERECTA and BRASSINOSTEROID SIGNALING KINASE1 (BSK1) and BSK2. In addition, the paternally provided pseudokinase SHORT SUSPENSOR (SSP/BSK12), a *Brassicaceae*-specific member of the BSK family, activates YODA in a receptor-independent fashion. We present new data on the impact of polar YODA activation on early embryonic patterning and shed light on the mechanism and evolution of distinct modes of YDA activation in the zygote on a molecular and structural level. We furthermore discuss possible benefits of different modes of YDA activation and their distinct parent-of-origin effects.

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AT6.6 Time to sleep or to germinate? A case of legumes seed dormancy

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Timing of seed germination is one of the key steps in plant life. It determines when plants enter natural or agricultural ecosystems. Plants have evolved various mechanisms to control the entry of the quiescent seed protecting embryo into vulnerable environment. Understanding of the genetic basis of local adaptation has relevance to climate change, crop production as well as understanding of the speciation. Along with other traits, seed dormancy has been removed during domestication. We have used a comparative anatomy, metabolomics and transcriptome profiling of pea seed coats in order to identify changes and genes associated with loss of seed dormancy in relation to domestication. In parallel, we tested adaptation to environmental conditions influencing dormancy release and the timing of legume seed germination, using wild pea (*Pisum sp*.) with relevance to crop and *Medicago truncatula* models. Level of *Medicago* seed dormancy correlated with increased aridity, suggesting that plastic responses to external stimuli provide seeds with strong bet-hedging capacity and the potential to cope with high levels of environmental heterogeneity. Similarly, in pea, dormant accessions were found in the environment with higher annual temperature, smaller temperature variation, seasonality and milder winter. Genome-wide association analysis of sequenced *Medicago* lines identified candidate genes associated with dormancy release related to secondary metabolites synthesis, hormone regulation and modification of the cell wall. Analysis of chemical composition of pea seed coat using mass spectrometry identified differences in the profile of proanthocyanidins, glycosylated flavonoids and fatty acids, related to impermeability for water. RNA sequencing identified several dozen differentially expressed genes between dormant and non-dormant pea seeds, and genome wide approach applied to RIL mapping population yielded candidate loci

regions. This analysis has been recently extended to chickpea and lentil crops and has therefore applicability to other economically important legume species.

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AT6.7 Seed coat-derived Brassinosteroids non-cell autonomously regulate endosperm development

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In spermatophytes, the establishment of a new plant generation relies on the successful formation of seeds. The seed is formed by the embryo and endosperm, which are the direct products of the double fertilization event, and by the surrounding seed coat. Besides their distinct roles within the seed, these tissues must communicate with each other in order to ensure synchronized development throughout seed formation. The hormone auxin was shown to play a crucial role in the communication between endosperm and seed coat. While maternal auxin biosynthesis genes are repressed by the POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) in the central cell, upon fertilization auxin is produced through the expression of imprinted paternal genes. This leads to central cell replication and consequently, endosperm formation. In addition, auxin is transported to the integuments triggering seed coat development by lifting the repressive effect of the PRC2 in these tissues. Interestingly, this does not seem to be a single way communication, since genetic evidence from our group and others indicates that the seed coat also influences endosperm development, because the endosperm proliferation rate is dependent on seed coat expansion and fate acquisition. These observations suggest that besides auxin, there are other signaling mechanisms from the seed coat to the endosperm that promote and sustain its growth. In this project, we explore Brassinosteroids (BR) as one of these signaling mechanisms. Our data reveals that mutants deficient for BR have altered endosperm development. Furthermore, we show that BR generate a signal in the seed coat, which in turn regulates endosperm development. This signifies that endosperm and seed coat communicate non-cell autonomously in bi-directional manner.

AT6.8 Small RNA functions in plant embryos

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The basic body plan and epigenetic landscapes of plants are re-established during early embryogenesis. We use *Arabidopsis thaliana* embryos as a model system to understand how small noncoding RNAs regulate cellular and epigenetic differentiation early in plant life. In my talk, I will highlight how embryonic microRNAs and small interfering RNAs help establish the body plan and epigenetic marks in the new generation, respectively. Additionally, I will discuss how small interfering RNAs help balance genome defense with growth, as well as how a single embryonic microRNA prevents the ectopic and persistent methylation of thousands of protein-coding genes.

AT7.1 Active DNA demethylation in pollen counteracts heterochromatic silencing

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Active DNA demethylation is carried out by DNA glycosylases in plants and is most dynamic during reproduction. This pathway is essential for seed development on the maternal side and we have recently shown that the demethylases DEMETER and REPRESSOR OF SILENCING 1 are also required to activate genes important for pollen function in Arabidopsis. Several of these genes are heavily methylated throughout the vegetative state, thereby resembling heterochromatin. Indeed, these loci are controlled by constitutive heterochromatic pathways and as a consequence, impairing this repressive chromatin regulation is able to restore male fertility in DNA demethylation deficient pollen. We will discuss these results in the scope of molecular evolution observed for genes implicated in reproductive development.

AT7.2 Temperature stress impairs centromere structure and segregation of meiotic chromosomes in *Arabidopsis*

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It has been estimated that temperature changes caused by global warming may decrease yield of staple crops by 20% by 2030 under a high greenhouse gas emission scenario. Temperature stress is known to have severe effects on plant growth, physiology and reproduction. Plants exposed to increased temperature have reduced fertility due to decreased pollen viability, which is accompanied by altered chromosome segregation. However, molecular causes underlying this phenomenon are not well understood. We have identified an *Arabidopsis* mutant with reduced level of centromeric histone (*cehh3-4*) that formed smaller centromeres and kinetochores. While cenh3-4 plants are viable and fertile under standard conditions, we noticed reduced fertility at a moderately increased temperature. Live imaging of meiosis showed that at 26°C, *cenh3-4* mutants exhibit chromosome mis-segregation, formation of micronuclei and altered dynamic of meiotic progression compared to wild type. This data indicate that impaired centromere structure sensitizes plants to higher temperature. To assess whether high temperature affects Arabidopsis centromeres, we studied chromosome segregation and centromere structure in wild type plants exposed to heat stress. We found that plants grown at 30°C have reduced amount of centromeric histone and of the kinetochore protein BMF1 at meiotic and mitotic chromosomes. Intriguingly, whereas progression through mitosis was not affected, we observed prolonged spindle assembly checkpoint and chromosome segregation defects in meiosis I. This was accompanied by a lower yield of viable pollen and reduced fertility. Our work suggests that centromeres and kinetochores may represent the Achille heel of plants in adaptation to increasing temperature and further elucidation of this phenomenon may unravel strategies for breeding heat-resilient crops.

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AT7.3 RNA directed DNA methylation impacts seed development in the obligate outcrosser *Capsella grandiflora*

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DNA methylation during seed development is partially controlled by the small RNA-directed DNA methylation (RdDM) pathway. In RdDM, small interfering (si)RNAs, which are abundant in gametophytes and developing seeds, are produced by processing transcripts from RNA Polymerase IV. These siRNAs interact with Pol V transcripts via complementary base-pairing, thereby targeting methylation machinery to proximal regions of the genome, a process critical for proper expression of imprinted genes. *Arabidopsis thaliana RdDM* mutants show no obvious reproductive defects; however, *Brassica rapa RdDM* mutants show a significant reduction in seed set and seed weight. Two major differences between *A. thaliana* and *B. rapa* are breeding system and ploidy, suggesting that RdDM may be more critical in *B. rapa* because methylation is mediating conflicts between maternal and paternal genomes (imprinting) and/or because it mediates conflict among subgenomes. To test these hypotheses, we generated CRISPR-induced *RdDM* mutants in the self-compatible species *Capsella rubella* and its self-incompatible sister-species, *C. grandiflora*, as well as in the self-compatible polyploid species *Camelina sativa*. We observed reproductive defects in all three species, but *C. rubella* and *C. sativa* had more moderate defects when compared to the drastic reduction in seed set and seed weight observed in *C. grandiflora RdDM* mutants. To determine the molecular changes correlated with this phenotype, we sequenced small RNAs in reproductive and vegetative tissues in both species. The recent speciation of *C. rubella* from a *C. grandiflora* progenitor, the associated reduction in the effect of *RdDM* mutations on seed development in *C. rubella*, along with our small RNA sequencing results for both species, permit new insights into the genomic regions imprinted via RdDM that are relevant to the transition from outcrosser to inbreeder.

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AT7.4 DNA methylation and genetic imprinting in water lily (*Nymphaea*) seeds: implications for endosperm and seed evolution

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Endosperm is the product of a second fertilization event that occurs at the inception of flowering plant seeds and is an important mediator of developmental and nutritional dynamics between an embryo and its mother. The biparental nature of endosperm is a key feature of this seed component, as the ratio of maternal and paternal genomes has been widely shown to impact endosperm development - and seed development as a whole. This parental genome dosage sensitivity has been tied to epigenetic modifications (such as DNA methylation) and imprinted gene expression (when allele expression depends on which parent it is inherited from). The mechanistic basis for parental genome dosage sensitivity has only been examined in systems with triploid endosperm (2:1 maternal:paternal genome ratio), although endosperm ploidy and parental genome dosage show remarkable variation across flowering plants. In order to provide a new perspective on the evolution of endosperm parental genome dosage sensitivity, we examined genetic/epigenetic aspects of endosperm development in *Nymphaea* - which is part of a lineage that is sister to nearly all other flowering plants. Furthermore, water lily endosperm is diploid (1:1 maternal:paternal genome ratio) and yet has been previously shown to exhibit parental genome dosage sensitivity. We find some water lily-specific patterns in endosperm DNA methylation, while genome-wide DNA hypomethylation appears to be a deeply conserved endosperm trait. We also find that a set of genes are genetically imprinted in water lily endosperm, including transcription factors and terpene synthases. Together, our results indicate that epigenetic modification and imprinted gene expression, while deeply conserved, have likely been evolving in concert with endosperm ploidy and function.

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AT7.5 Homology-based regulation of pollen-side dominance hierarchy between small RNAs and their targets in *Brassicaceae*.

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Brassicaceae plants have the sporophytic self-incompatibility (SSI) to avoid self-fertilization and maintain genetic diversity within populations. The S-haplotype-specific interaction between pollen and pistil determinants, *SP11* and *SRK*, respectively, induces the SSI response. Strict co-dominance of *SP11*s causes limitations on compatible mates. We previously reported that the small RNA (sRNA) from the dominant allele induces DNA methylation at the cis-element of the recessive *SP11* promoter region and suppresses its expression in *Brassica rapa*. Also, we proposed a model wherein a homology-based interaction between sRNAs and their targets regulates the linear dominance hierarchy of *SP11* (S44>S60>S40>S29); however, whether the underlying mechanism regulating complex dominance hierarchy is conserved among *Brassicaceae* was unclear. We identified two sRNAs and their targets that can explain *A. lyrata's* linear dominance hierarchy (S39, S20, S50)>(S13, S16)>(S18, S14)>(S1). We tested our homology-based model to explain *A. lyrata's* linear dominance hierarchy by creating transgenic lines. Our results suggest that *A. lyrata's* one is also controlled by a homology-based interaction between sRNAs and their targets. We further analyzed the mechanism regulating *B. rapa's* pollen dominance to find another target sequence of sRNA. The recessive *SP11* specifically contains three times repetitive sequence (*REP*) homologous to dominant sRNAs at the 5' upstream region. We introduced base substitution into recessive sRNA to be homologous to the dominant SP11. From our preliminary results, the mutated sRNA induced de novo DNA methylation against dominant *SP11* at both *REP* and the cis-element and strongly suppressed the *SP11* expression. These results suggest that *REP* is also regulated in a homology-based manner. Our study suggests that *B. rapa* and *A. lyrata* share a common underlying mechanism regulating pollen-side dominance hierarchy. We will present a model on the *REP* function for strong suppression of *SP11*s, creating the species-specific mechanism.

Funding: JST Support for Pioneering Research Initiated by the Next Generation program and NAIST Touch Stone program; Foundation for NARA INSTITUTE of SCIENCE and TECHNOLOGY

AT7.6 Exploring the cellular basis of organ curvature using 3D digital ovules

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How complex organ shape emerges *in vivo* is a fundamental question in biology. In this study we focus on the cellular basis underlying the curved shape of the ovule, the major female reproductive organ of higher plants. We ask the question what 3D cellular patterns underly the curvature in the ovule of the model system Arabidopsis thaliana. To address this topic, we have developed tools to study the 3D morphogenesis of ovules with cellular resolution. Briefly, we perform 3D-imaging of fixed, cleared, and stained ovules. The cell walls are stained with SR2200 and nuclei with TO-PRO-3 iodide. The technique enables deep imaging and thus the 3D digital representation of the cellular architecture even in interior regions. After deep imaging, we proceed to 3D cell segmentation using the machine-learning-based PlantSeg pipeline, followed by cell-type labeling, and quantitative analyses using MorphoGraphX software (morphgraphx.org). Here, we present the results of tissue-specific and organ-wide 3D spatial quantitative analyses of cellular patterns contributing to ovule curvature in *Arabidopsis*. To address ovule curvature further we investigate the cellular architecture of ovules in plant species that exhibit differences in ovule curvature. The comparative analysis revealed exciting insights into the cellular basis of ovule curvature. We show the specific roles of different tissues, including their cell numbers, volumes, growth rates, and their overall organization in shaping ovule curvature. In summary, we have gained qualitative and quantitative insights into the cellular growth patterns that underlie ovule curvature.

Funding: This work was funded by the German Research Council (DFG) through grants FOR2581 (TP7) to KS.

AT8.1 Pollen and ovule quality analysis for plant reproduction

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Pollen and ovule quality are equally important for plant breeders, fruit, and seed producers. While pollen quality analysis either through viability or germination assays is common practice prior to pollinations, ovule quality is never assessed. Only the combined quality of both pollen and ovules reflected by the amount of fruits or seeds per plant is measured at the end of the production process, thus weeks or months after the initial pollination.

The first experiments to analyse pollen quality using an impedance flow cytometer (IFC) were performed ten years ago. Since then this technique has developed into species-independent, fast, and reliable method to analyse pollen quality which was adopted by all major breeding companies as well as seed producers to optimize their processes.

In practice, however, yield variations observed in seed production can't be explained by pollen analysis alone. To gain insight into the limitations on the female side attempts were taken to analyse ovule quality by IFC, too.

This presentation will show how IFC can act as a selection or diagnostic tool under simulated stress conditions along with the first data on IFC-based ovule analysis.

AT8.2 Genome-wide association analysis of wild and domesticated barley identifies hitherto unknown domestication loci as well as new breeding targets for important yield traits.

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The domestication of barley can be narrowed down to two events, which took place in the area known as the fertile crescent several thousand years ago. Mutations in *BRTL1/2* and *VRS1* led to a non-brittle six rowed barley, enabling easy harvesting as well as increased yield. However, since these pivotal events changed human history, substantial breeding efforts have led to the modern barley varieties used today. The causative genetical traits are to some extend known, but a large proportion remains elusive. Here we show that the probability score of *Teosinte branched 1 INTC*-a allele can be used as a guideline to detect known and novel loci. *Teosinte branched 1 protein a* allele is known to be associated with partial filling of lateral spikelet and hence a genomic trait likely selected for by prehistoric farmers. Four GWAS data sets were generated from exome sequencing data recently published and a normalized -log(P) value of *INTC-a* allele was used as a guide for filtering between datasets. This approach enabled direct re-discovery of specific SNPs in known flowering time associated *PDD-H1* and *CEN1*, as well as novel SNPs in both known yield loci and novel putative yield loci. Examples of identified uncharacterized SNPs that we can link to an observable phenotype are found in AP2, that affect the important lax phenotype yield trait and gives lax spikes, and in CDL1, a kinase presumably involved in grain specific brassinosteroid signal transduction that leads to increases in grain weight. Our results demonstrate that utilizing known SNPs as guideline to filter GWAS data may enable discovery of genomic traits, usable in breeding efforts, which otherwise remain elusive with conventional analysis methods. Furthermore, this study highlights several key genomic traits likely responsible for the early development of modern barley cultivars used today.

Funding: This work was supported by The Innovation Fund Denmark (IFD) and The Carlsberg Foundation.

AT8.3 Generation of haploidy inducers in barley by targeted mutagenesis

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The use of doubled haploid (DH) lines is one of the most effective biotechnological measures in modern plant breeding. Each DH line is a genetically unique result of meiotic recombination, while being entirely true-breeding. That is, once selected in the breeding process, useful recombinants can be identically reproduced. In barley, DH lines can be efficiently obtained via microspore-derived plant regeneration. However, this principle is genotype-dependent to some extent. An alternative means to produce DH lines is to employ haploidy-inducing lines as paternal parents. Among the progeny resulting from such crosses, maternal haploids can be found that have lost the paternal genome during early embryogenesis. The phenomenon of uniparental genome elimination was reported to occur in mutants including those for Centromeric histone 3 (CenH3), Phospholipase (PLA1, PLD3) as well as DUF 679 membrane protein (DMP) genes of various species. Whereas a previous approach to engineer the barley *CenH3* alpha and beta genes resulted in only very low efficiency of haploid formation, primary barley mutants carrying Cas9-triggered mutations in *PLA1* produced about 6% haploid progeny upon pollination of wild-type plants. The haploidy-inducing capacity of homozygous *pla1 M2* mutants was then validated by pollination of various barley accessions. In a further approach, we are employing cas9/gRNA-transgenic *pla1* mutant barley to deliver these transgenes and their respective products from sperm cells to the zygote via fertilization. Any maternal parents of choice can thus be subjected to genome editing, while the transgene-carrying paternal genome is lost during embryo formation. The site-directed mutations obtained are instantaneously rendered homozygous via colchicine-induced whole genome duplication. This latter concept holds great promise for barley genome editing with considerably reduced genotype dependency.

ABSTRACTS

POSTERS

P1 On the development of iridescent cuticular patterns on the petals of *Hibiscus trionum*

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In flowering plants, sexual reproduction usually involves a third partner to transport pollen from one plant to another. Flowers make an amazing use of pigments, shape and smell in order to lure animals to carry their precious pollen. Some plants have evolved other tricks to stand out from other flowers: *Hibiscus trionum* displays nano-scaled ridges at the surface of its petal that are capable of interfering with light and specifically reflect ultra-violet and blue light, two wave lengths visible to pollinators [1]. In this work we study the chemistry and physical mechanisms underpinning the production of such cuticular patterns, as well as the molecular pathways that regulate the development of such structural colours at the surface of *Hibiscus trionum* petals. Transcriptomic analyses, liquid extraction surface analyses coupled with mass-spectrometry and a mutant screen have been performed on *Hibiscus trionum* so as to unravel the development of such structures.

[1] Moyroud et al, 2017. Nature

P2 Cellular-resolution imaging of auxin and cytokinin signaling of the shoot apical meristem in rice

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Flowering is a developmental transition where vegetative shoot apical meristem (SAM) turns into the inflorescence meristem (IM). The IM initiates formation of primary branch meristems (PBMs), and PBMs produce secondary branch meristems (SBMs): These branch meristems finally converted into floral meristems, and the balance of branch-flower conversion regulates inflorescence architecture. In rice these processes are regulated by florigen FT/Hd3a protein, which is produced in leaves and transported into the SAM. Florigen promotes flowering, and it affects inflorescence architecture by precocious conversion of inflorescence branch meristems to floral meristem. In addition to florigen, Proper development of IM, PBMs and SBMs require complex interactions of plant hormones such as auxin and cytokinin. Auxin accumulation decides the site of lateral organs in the SAM. Mutation of auxin biosynthetic enzyme gene causes abnormal phenotypes, such as small panicles [1]. Loss of function of cytokinin inactivating enzyme OsCKX2/Gn1a causes gain of cytokinin contents, resulting in increasing numbers of branch meristems [2]. Although auxin and cytokinin play a role in inflorescence architecture, it is not revealed that distribution of them and their interaction with florigen.

In this study, we reveal that the distribution of auxin and cytokinin signalling in the meristem of rice using auxin response reporter DR5 and cytokinin response reporter TCSv2.

We generated TCSv2:tdTomato transgenic rice plants and observed TCSv2 signal. It was detected in the sub-epidermal layer of the SAM, then TCSv2 signal expands to outer cell layers when the SAM turns to the IM. Comparing spatio-temporal distribution of TCSv2 signal with Hd3a-GFP, we revealed these two signals occupy complementary regions of IM. Our functional analysis revealed that Hd3a reduces, but cytokinin increases the number of inflorescence branches. These results suggest that antagonistic interaction of cytokinin and florigen regulates inflorescence development in rice.

[1] Yoshikawa et al, 2014. Plant J.
[2] Ashikari et al., 2005. Science

P3 Sympetaly in the mimosoid clade (*Leguminosae, Caesalpinioideae*): an asterid characteristic in a rosid group

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The new classification proposed for Leguminosae includes, among the main modifications, the inclusion of the subfamily traditionally known as Mimosoideae in the circumscription of the subfamily Caesalpinioideae. Mimosoideae is no longer accepted as a subfamily and is now informally treated as the mimosoid clade. One of this group's most special flower conditions is the sympetalous corolla. This study aims to examine whether the petal union leading to sympetaly in the mimosoid clade is widely spread and whether there is variation in type and extent. For this purpose, floral buds and flowers of 16 species of 13 genera were collected, fixed, and processed for analysis in light and scanning electron microscopy. Most species studied display pentamerous, sympetalous with free lobes corolla. The petal primordia are individualized and appear simultaneously in the floral meristem. Petals remain free at the beginning of the intermediate stage of development. Subsequently, the petals curve and approach each other, and their edges touch. Epidermal papillae are found on the petals apical edge interconnecting the lobes, which enclose the inner organs in the floral bud. Four different types were found and classified as follows: I. Sympetaly 1 - petals truly united in the basal portion and coherent in the median and apical portion (*Abarema cochliacarpos*, *Inga laurina*, *Inga vera*, *Pithecelobium dulce*, *Samanea saman*); II. Sympetaly 2 - petals fully united along their entire length (*Inga edulis*); III. Pseudosympetaly 1- free petals at the base and intertwined by papillae in the middle and apical portion (*Anadenanthera colubrina*, *Leucaena leucocephala*, *Plathymenia reticulata*); IV. Pseudosympetaly 2 - petals intertwined by papillae throughout (*Adenanthera pavonina*, *Entada acaciifolia*, *Mimosa artemisiana*, *Pentaclethra macroloba*, *Piptadenia gonoacantha*, *Stryphnodendron polyphyllum*, *Tetrapleura tetraptera*). The sympetaly occurs by postgenital union, which may be by tissue union or intertwining of epidermal papillae.

P4 The evolution and development of nectar spurs

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Nectar spurs are tubular organs derived from sepals and petals, which typically contain a floral reward. Spurs confer specificity on the interaction between a spurred flower and its pollinators, allowing only pollinators with specialised feeding organs to access the floral reward contained within the spur. Despite playing a key role in floral evolution and ecology in thousands of angiosperm species, few spurred systems have been studied in depth and little is known about how nectar spurs form.

This poster summarises ongoing attempts to establish the non-model spurred genus *Linaria* as a suitable system for studying spur development. We have developed a protocol for the stable transformation of *L. vulgaris* and have demonstrated the efficacy of virus-induced gene silencing (VIGS) in this system. An F2 population segregating for spur length has been studied and will form the basis of a bulked segregant analysis (BSA) to map the genetic loci underpinning spur length. Image recognition software has been created to extract key parameters, including nectar spur length, from photos of *Linaria* flowers, allowing floral variation in the F2 population to be accurately characterised.

P5 Functional analysis of *FLOWERING LOCUS T*-like (*FTL*) genes from *Chenopodium ficifolium* and virus induced gene silencing (VIGS) in *Chenopodium*

Oushadee A.J. Abeyawardana, Claudia Belz, Tomáš Moravec, David Gutierrez-Larruscain, Helena Štorchová

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We studied floral induction in *Chenopodium ficifolium*, a diploid relative of the important crop *Chenopodium quinoa*. We identified the homologs of the crucial genes responsible for the control of flowering in this species. The *CfFTL1* gene is the ortholog of the floral activators *BvFT2* in sugar beet. The *CfFTL2-1* and *CfFTL2-2* are the orthologs of the floral inhibitor *BvFT1* in sugar beet. The switch in function from the activation to inhibition of flowering often occurs in *FT* genes, functional evidence is therefore necessary to prove gene function. We performed the overexpression of *CfFTL* genes in *Arabidopsis thaliana* and demonstrated the prominent acceleration of flowering in the *CfFTL1* overexpressing lineages. However, *Arabidopsis* is phylogenetically distant from *Chenopodium*, the regulatory proteins may not function in *Arabidopsis* in the same way as in *Chenopodium*. As *Chenopodium* species is recalcitrant to the transformation by *Agrobacterium*, we adopted virus induced gene silencing (VIGS) to inhibit the selected genes in *C. ficifolium* and *C. quinoa*. We utilized the marker gene *PHYTOENE DESATURASE* (*PDS*) involved in chlorophyll synthesis to follow successful infection and gene silencing. At first, we transformed *Nicotiana benthamiana* by *Agrobacterium* with plasmid constructs carrying cDNA of the ALSV virus and marker gene, then we used tobacco leaf extract containing recombinant ALSV virus to infect *C. ficifolium* and *C. quinoa*. We observed PDS gene silencing visible as yellowish sectors of leaves in both species. We confirmed the presence of ALSV RNAs and the decrease of *PDS* transcripts by RT qPCR. We are now continuing VIGS experiments with flowering related genes in *C. ficifolium* and *C. quinoa*.

P6 The transcriptomic study of the flowering related genes in *Chenopodium ficifolium* ecotype 283

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We investigated the control of flowering in *Chenopodium ficifolium*, a diploid relative of the important crop *Chenopodium quinoa*, staple food in the Andes. The ecotype 283 of *C. ficifolium* exhibits accelerated flowering under long days, without concomitant activation of *FLOWERING LOCUS T* like (*FTL*) genes. We followed the growth of this ecotype under long days (18 h light) and short days (6 h light). RNAseq data in 8 – 15 day-old seedlings at six time points in the course of floral induction were analyzed and the reads mapped against the newly constructed reference transcriptome from *C. ficifolium*, ecotype 283. The assembly was made with Trinity v2.0.6 followed by the application of CDhit v4.6.8 and Evigene, eliminating most duplicates and reducing the number of transcripts to a final of 41,294. Clusters of differentially expressed genes (DEG) were constructed with the focus on the DEGs with gradually increasing expression under long days, but not under short days. Besides many genes associated with photosynthesis, sugar metabolism, growth and reaction to light, we identified several genes, which might have been involved in floral induction. The *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*) and *SPL5* orthologs were induced under longs days and may have participated in floral induction in *C. ficifolium*, ecotype 283. The function of the selected flowering-related genes is now tested by their overexpression in *Arabidopsis thaliana*. *Chenopodium* species are recalcitrant to transformation by *Agrobacterium*, but we are now trying to use virus induced gene silencing to provide the evidence about gene function.

P7 A comparative approach to floral ontogeny in Melastomataceae

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Despite the efforts of the last decade, Melastomataceae remains an under-studied family from a floral ontogenetic perspective. We currently have some information about only 80 of the more than 5,000 species described in the family. The floral diversity of Melastomataceae is stunning and clearly expressed in sepal and stamen structure and in the position of the ovary. Thus, our study deals with comparative floral development of Melastomataceae species, that are effective in order to understand these variations because they reveal the often-enigmatic origin of the structures. The diverse calyx structure originates from variations in the degree of union between the sepals. The contort corolla aestivation, widespread in the family, is influenced by the floral architecture. Stamen size and shape depend on the space available in the floral bud after the growth of the perigynous hypanthium that may cause the delay in stamen emergence and flexion. Dimorphic stamens originate from differences in their developmental time and position. Prolonged connectives and most of their appendages are formed late during floral development. Ontogeny also explains the decrease or increase in organ number. The intercalary meristems can promote the formation of a hypanthium associated with the gynoecium, and their extension is responsible for the gradual variation in ovary position. These meristems also act on the development of a perigynous hypanthium. Thus, intercalary meristems play an important role for floral diversification in Melastomataceae. The data obtained currently revealed some robust development patterns, such as contorted dextrorse aestivation of the corolla, flexion of the stamens in the floral bud, and the late emergence of the stamens. These features are frequent in other Myrtales, although they are much more conserved in this family.

P8 KIL1 terminates fertility in maize by controlling silk senescence

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Plant flowers have a functional life span during which pollination and fertilization occur to ensure seed and fruit development. Once flower senescence sets in the potential to set seed or fruit is irrevocably lost. In maize, silk strands are the elongated floral stigmas that emerge from the husk-enveloped inflorescence to intercept airborne pollen. Here we show KIRA1-LIKE1 (KIL1), an ortholog of the *Arabidopsis* NAC transcription factor KIR1, promotes senescence and programmed cell death in the silk strand base, ending the ovary's accessibility for fertilization. Loss of *KIL1* function extends silk receptivity and thus strongly increases kernel yield following late pollination. This offers new opportunities for possibly improving yield stability in cereal crops. Moreover, despite diverging flower morphologies, and substantial evolutionary distance between *Arabidopsis* and maize, our data indicate remarkably similar principles in terminating floral receptivity by programmed cell death whose modulation has a potential to be widely used in agriculture.

P9 The homolog of HANABA TARANU floral transcription factor exhibits sex-specific expression in *Silene latifolia*

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Silene latifolia is a dioecious plant model possessing evolutionarily young sex chromosomes X and Y. The sex determination pathway of this plant is of great interest, but specific genes on the Y remain to be indentified. A GATA transcription factor playing a role in the flower development was found using chemical genetics in S. latifolia. The homologous gene in *Arabidopsis* is called *Hanaba Taranu* and plays a role in flower development by forming and maintaining the boundaries between floral organ primordia. We confirmed that this gene is sex-linked in *Silene* and the X and Y alleles exhibit different expression levels. Our research consists of a genomic screen, a functional study using transgenic Silene plants and most importantly the analysis of expression levels and localization in various flower development stages.

P10 Factors influencing the disc/ray floret ratio in *Chrysanthemum morifolium*

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Chrysanthemum has a composite flower, an inflorescence structure where many flowers/florets mimic a single flower. These florets include the showy female ray florets and hermaphroditic disc florets. A variance in the ratio between these floret types influences the appearance of the ornamental flower, but also its total seed set. In this project, we investigated how this ratio is determined. We identified two mutants with an altered disc/ray floret ratio compared to their respected 'normal' varieties and performed transcriptomic analysis during key developmental stages. Data analysis identified 275 genes that were similarly differentially expressed in both mutants. The list of differentially expressed genes contained interesting transcription factors in addition to a high abundance of genes related to Brassinosteroid (BR) signalling. We performed a functional analysis with seven genes and showed that downregulation of an *Arabidopsis PROTODERMAN FACTOR 2* (*PDF2*) homolog in *Chrysanthemum* could increase the number of disc florets. This was also the case when *DWARF1*, an early BR biosynthesis gene, was downregulated, thereby reducing BR levels. Exogenous application of Brassinazole, a BR inhibitor, had a similar effect. All these findings are in accordance with the differential expression analysis, strongly suggesting that PDF2 and BR have important functions in controlling disc/ray floret ratio in *Chrysanthemum*.

P11 Validation of the role of vernalization (*Vrn*) genes for the switch to generative development of winter cereals

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The reduction of generation time is of great advantage for cereal breeding. While the duration of generative development can already be reduced considerably under so-called Speed Breeding conditions, minimizing the period required to vernalize winter cereals poses a further challenge. The competence of shoot apical meristems of winter cereals for ear formation is typically acquired under short-day conditions lasting about two months in combination with temperatures just above freezing. In barley and wheat, this need for vernalization is regulated at the molecular level by various constellations of the factors VERNALISATION 1 (VRN1), VRN2/ZCCT1, VRN3/FT1 and VRN4. A central role is played by the signal molecule VRN3 that is transported from leaves to shoot apex as ultimate outcome of the complex regulatory process to trigger the switch towards generative development. VRN2 is a direct repressor of the *Vrn3* gene. The aim of this study is to find out what consequences manipulations of these factors lead to. The role of VRN2 will be determined by target sequence-specific mutagenesis using RNA-directed Cas9 endonuclease. Two gene-specific target motifs were selected, a gRNA/cas9 construct created and then used to genetically transform winter barley. Sixteen regenerated plants proved to be mutated by deep-sequencing of PCR amplicons derived from target region. Another approach aims to induce ear formation by overexpressing the so-called florigen VRN3. For this purpose, transcription of recombinant *Vrn3* is regulated by maize *UBIQUITIN 1* promoter, which, in contrast to the native *Vrn3* promoter, is expected to ensure the activity of the transgene even in presence of the suppressor VRN2. Current experiments are conducted to reveal whether either *VRN2* loss-of-function or ectopically expressed *VRN3* do suffice for the generative development to be initiated in winter barley in the absence of short day and low-temperature conditions.

P12 Spliceosomal core component GAMETOPHYTIC FACTOR 1 (GFA1) as a key photoperiodic switch?

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Day-length perception and interpretation are central mechanisms to choose the ideal flowering time point. We provide evidence that the core spliceosomal component GAMETOPHYTIC FACTOR 1 (GFA1) constitutes a key photoperiodic switch, which enables flowering under long-day (LD) conditions. We have previously shown that GFA1 is essential for reproductive success, as homozyous mutants cannot be recovered. In order to investigate the role of GFA1 in sporophytic tissue, we generated *gfa1* hypomorphic (*gfa1hyp*) plants, which fail to initiate flowering in LD. A comprehensive transcriptome survey suggests, that GFA1 mediates pre-mRNA splicing in a substrate specific manner. We, in addition, present the identification and characterization of GFA1 targets and discuss the implication of our results for flowering time control.

P13 Multi omics of tassel development in maize (*Zea mays*)

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Maize (*Zea mays*) male inflorescent development progresses through a series of meristems until finally anthers, in which pollen matures, are formed. First, the inflorescence meristem (IM) initiates branch meristems (BM) with an indeterminate fate and spikelet pair meristems (SPM). SPMs subsequently divide into two spikelet meristems (SM). Each of those produce two glumes as well as the upper floral meristem (FM). Then the SM adapts FM fate. Florets contain four concentric whorls that are formed sequentially from the FM. First the palea and the lemma are initiated, followed by the lodicule, then three stamens, and finally three carpels. In maize tassels carpels abort while the stamens differentiate into a supporting filament and the terminal anther. Within each anther 4 lobes are formed, in which the central germline cells are surrounded four distinct somatic cell layers. So far only a few factors have been identified that control establishment and termination of the different meristems as well as stamen initiation.

To determine RNA, protein, and small RNA changes during tassel ontogeny, RNA-sequencing, mass spectrometry, and small RNA-sequencing data were collected at 4 stages of early tassel development (0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm). At the 0.5 cm and 1.0 cm stages tassels lack stamens, the 1.5 cm stage has stamens including anther primordia, and the 2.0 cm stage has anthers as large as 0.1 mm, prior to germinal specification within lobes. These data are the foundation to define meristem- and anther-specific genes for further functional study.

P14 Identification of molecular mechanism regulating petal spot development in sexually deceptive South African daisy *Gorteria diffusa*

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Gorteria diffusa (*G. diffusa*), a daisy endemic to northwest South Africa belongs to the Asteraceae family. *G. diffusa* exhibits a very distinct raised dark petal spot at the base of the subset of its ray florets. This dark petal spot appears to mimic small bee flies (*Megapalpus capensis*), which pollinate *G. diffusa*. *G. diffusa* is self-incompatible and relies fully on *Megapalpus* for successful pollination. Field investigations showed that the dark spots induce mating behavior in male flies, the only known example of pollination by sexual deception outside the Orchidaceae. The *G. diffusa* petal spot is a three-dimensional elaboration of the petal epidermis that is rich in anthocyanin pigment and composed of three specialized cell types: smooth white highlight cells, heavily pigmented flat interior cells, and dark multicellular papillae. These specialized cell types must differentiate in the correct positions with respect to one another on the ligule, a structure formed of multiple fused petals. It is known that if these cell types are not organized in an integrated way with respect to each other, sexual deception does not occur. In this work with the help of laser capture microdissection and RNA seq, we aim to understand how petal spot development is coordinated between the petals forming a ligule.

P15 Novel role of auxin conjugation in tomato flower development

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We applied a combination of hormone-metabolites and transcriptome profiling to reveal auxin components regulating tomato reproductive organ development. For hormonal profiling physiologically active hormones, as well as their precursors and catabolites, were purified and fractionated by solid-phase extraction (SPE) and detected by UPLC-ESI-MS/MS. The measurements were conducted at different stages of flower (3, 5, 7, and 10 mm of flower bud length, and at anthesis) development. Different flower (sepals, petals, stamen, pollen, carpels) organs were examined.

Hormonal analysis revealed a gradual decrease in free IAA concentration together with the accumulation of its oxidized and conjugated forms in each flower organ, especially in stamens. Notably, a dramatic increase in IAA-methyl-ester content was observed in mature pollen. Co-expression analysis highlighted genes putatively involved in auxin conjugation processes, including several *GRETHEN-HAGEN 3* (*SlGH3*) and *IAA-METHYLTRANSFERASE* (*SlIAMT*) genes. *In vitro* analysis of enzymes' substrate preference confirmed the ability of GH3s to bind IAA to a wide range of amino acids. Transiently overexpressed candidate genes decreased free IAA level in *N. benthamiana* leaves and elevated the levels of the corresponding conjugates. *Slgh3-15* knock-out lines exhibited a dramatic decrease in pollen viability and germination coupled with increased IAA in maturating stamen, leading to the development of parthenocarpic fruits. A similar aberrant phenotype was observed in *iamt1* mutants. In contrast, *Slgh3-2* knock-out plants did not show this severe phenotype. Finaly, label-free shotgun proteomics revealed low abundance of SlGH3-2 protein in flower organ suggesting posttranscriptional “fine-tuning” of auxin conjugation during flower development.

Thus, IAA–amino acid conjugation and methylation play a pivotal role in the regulation of free hormone content and therefore may contribute to normal flower development, especially to stamen morphogenesis and pollen development thus determining the plant fertility in general.

P16 Quantitative analysis of cellular growth patterns in integument morphogenesis and ovule curvature in *Arabidopsis thaliana*

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How the Gestalt of a plant tissue is generated remains an open question in plant developmental biology. Ovule curvature represents a unique phenomenon in plant tissue morphogenesis and invites interesting questions regarding the cellular, molecular, and mechanical mechanisms involved in the formation of this characteristic shape. The ovule of *Arabidopsis thaliana* represents an excellent model system to address this question. At maturity, the ovule exhibits a distinctive curved shape such that the micropyle is placed next to the base of the funiculus, resulting in a 180° bending (anatropy). *A. thaliana* carries bitegmic ovules as the chalaza originates two integuments, the precursors of the seed coat. It has been postulated that differential growth of the outer integument contributes to curvature. Comparative evolutionary studies suggested that the presence of an outer integument is crucial for anatropy in bitegmic ovules. Moreover, genetics provided additional evidence on the central role of the outer integument, through the identification of mutants carrying ovules with a defect in outer integument development. We have performed a quantitative analysis of the cellular growth patterns of the integuments in wild-type and mutants at different developmental stages. Our pipeline consisted of 3D image acquisition of fixed samples at cellular resolution followed by image processing and analysis in MorphographX. The results indicate that the outer layer of the outer integument is a central regulator of ovule curvature. Furthermore, they validate our quantitative approach as a promising strategy to explore the cellular mechanisms underlying ovule curvature.

P17 Molecular dissection of floral proximal-distal patterning in *Torenia fournieri*

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The three-dimensional shape of a flower is integrated by morphogenesis along different independent floral axes. Differentiation along the floral radial and dorsal-ventral axes have been well studied; however, it is still obscure how a flower patterns its proximal-distal axis. The corolla of a *Torenia fournieri* flower differentiates into distinct regions along the proximal-distal axis, including a distal lobe, an intermediate tube that consists of an upper and a lower part based on distinct pigmentation, and a proximal corolla neck. We reported an *ALOG* (*Arabidopsis LSH1* and *Oryza G1*) gene, *TfALOG3*, which was expressed in the epidermis of the proximal neck, and the *TfALOG3* loss-of-function mutants failed to develop into a corolla neck. Further biochemical approaches unraveled a BOP (BLADE-ON-PETIOLE) factor, TfBOP2, which physically interacted with TfALOG3. *TfBOP2* was specifically expressed in the proximal corolla as well as the fusion region of the intermediate tube. CRISPR knockout mutants of *TfBOP2* not only lost the proximal corolla neck, but also showed defects in the fusion margin of the corolla tube. We recently characterized another mutant from the background of *TfALOG3* mutant, which formed neck-free flowers with an extremely shortened lower part in the intermediate tube. Our findings demonstrate that, a flower patterns its proximal-distal axis by recruiting ALOG-BOP factors, and *T. fournieri* provides an ideal model to study floral proximal-distal patterning.

P18 Night break aborts floral induction in *Chenopodium rubrum*

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The seedlings of *Chenopodium rubrum* may be induced to flowering by a single period of 12 hour-darkness at age of six days, the flower buds are visible after two weeks. Flowering is induced by the activation of the floral inducer *FLOWERING LOCUS T Like 1* (*CrFTL1*), which peaks about six hours after light-on. When the plantlets are illuminated by red light for 15 minutes in the middle of the dark period, *CrFTL1* is inhibited and floral induction does not occur. The accurate timing of floral induction and its abortion makes possible to analyze the upstream signals possibly regulating *CrFTL1* and other factors, which may participate in floral induction in *C. rubrum*. We sampled RNA in six time points after the night break and after light-on, and performed IlluminaHiSeq sequencing. We mapped the reads against the reference transcriptome of *C. rubrum* and estimated differentially expressed genes (DEGs) between night break-treated and control plants (with uninterrupted dark period) at each time points. We identified several candidate genes for the triggers of *CrFTL1* activation. Besides several *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes, three *B-BOX DOMAIN PROTEIN* (*BBX*) genes including *BBX19*, known to affect flowering in sugar beet, were downregulated after night break. Long non-coding RNA was highly significantly upregulated 0.5 h and 1 h after the end of night break, which may suggest its function as a negative regulator of flowering. We plan to analyze the function of selected genes by virus induced gene silencing.

P19 Role and mechanisms of linker histone (H1) eviction during *Arabidopsis sporogenesis*

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Our research aims to elucidate chromatin dynamics principles underlying cellular reprogramming during developmental or physiological transitions. My project focuses on the role of linker histones during the somatic-to-reproductive cell fate transition which leads to germline differentiation. We previously found that the differentiation of female spore mother cells (SMC) is accompanied by large-scale chromatin reprogramming including chromatin decondensation and loss of the key epigenetic mark H3K27me3 [1]. These events are preceded by the eviction of linker histones (H1), key components of chromatin structure and composition [2]. Specifically, the goal of my project is to address the role and mechanisms of H1 dynamics during female sporogenesis, focusing on the ubiquitin & proteasome-mediated degradation pathway. We engineered inducible mutants to downregulate specific components of the ubiquitination pathway in the SMC, and others to ectopically express *H1* mutant variants modified at potentially target sites of Ub.

[1] She et al, 2013. Development
[2] Rutowicz et al, 2019. Genome Biol.

P20 Floral induction in *Chenopodium ficifolium*, the close relative of *Chenopodium quinoa*

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Chenopodium ficifolium is a close diploid relative of the tetraploid crop *Chenopodium quinoa*. It has a short juvenile phase and may be induced to flowering at seedling stage. Two ecotypes differing in the photoperiodic response, exist in *C. ficifolium*. The short-day ecotype flowers earlier under short days, which is accompanied by the strong upregulation of *FLOWERING LOCUS T like 1* (*FTL1*), the ortholog of the sugar beet floral activator *BvFT2*. In contrast, long-day *C. ficifolium* flowers without the concomitant induction of *CfFTL1*. *C. ficifolium* also contains the *CfFTL2-1* gene, which exhibited low expression during floral induction in both *C. ficifolium* ecotypes.

To provide the evidence about the function of the *FTL* genes, we overexpressed them in *Arabidopsis*. The *FTL1* gene accelerated flowering in both the wild type and in *ft* mutants of *Arabidopsis*. Surprisingly, the *CfFTL2-1* gene, which is homologous to the sugar beet floral inhibitor *BvFT2*, activated flowering in *Arabidopsis* even more strongly than *CfFTL1*. It is therefore possible, that the minor increase of the *CfFTL2-1* expression, observed in course of floral induction in long-day *C. ficifolium*, may cause floral induction. Our experiments help to clarify the adaptation of flowering time to the growth in higher latitudes not only in *C. ficifolium*, but also in the promising crop *C. quinoa*. To understand the adaptation to seasonal climate is of utmost importance for the efforts to expand the cultivation of *C. quinoa*.

P21 Floral organ developmental aspects in the common dandelion (*Taraxacum officinale*; Asteraceae): The origin of the pappus and the inferior ovary

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Dandelion belongs to one of the two large, derived crown groups of the Asteraceae, the Cichorioideae, which is characterized by its ligulate flowers. These flowers, called florets in Asteraceae, are placed close together on a common receptacle, the capitulum, a highly compressed racemose inflorescence that itself resembles a flower. The florets in Asteraceae usually contain five fused petals, five epipetalous stamens and two fused carpels; sepals are absent in most Asteraceae, but often few to numerous pappus parts are present instead. It is generally assumed that the pappus parts represent the outer floral whorl where the sepals are usually located, however, there are other theories as well, for example that they are of trichome origin. The ovary in dandelion is inferior, meaning that the inner floral whorl of carpels is formed below the level of attachment of the outer floral whorls. Also, several theories exist on this developmental aspect, one suggesting that the receptacle part on which the outer whorls initiate is lifted up and receptacular tissue is fused to the ovary wall (receptacular theory), and another proposing that the lower parts of the outer floral whorls are fused together and with the ovary wall (appendicular theory). We analyzed the pappus–sepal homology and the origin of the floral organs in dandelions by using micromorphological and floral gene expression analyses. We found evidence (1) that the pappus is homologous to the sepals, and (2) for the receptacular theory regarding the development of the inferior ovary. Results will be presented and discussed.

P22 Proteomic analysis reveals that the wheat *Ms2* gene causes male sterility by regulating nucleic acid synthesis and signaling pathways

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The male-sterile single dominant gene *Ms2* was identified from Taigu genic malesterile wheat and has been used in Chinese wheat breeding for over four decades, and was recently cloned as the first dominant male sterile gene in world. However, the mechanism of male sterility caused by *Ms2* gene is not clearly. Based on the previous works, by proteomics approach, we analyzed the protein components in spikelets of *Ms2* transgenic wheat and the gene expression in *E. coli*, and compared the spikelet proteomes of three different groups of wheat near-isogenic lines in which the *Ms2* and a dwarf gene (*Rht-D1c*) were co-introduced. The Ms2 protein could be detected from *Ms2* gene expressed *E. coli* but not from *Ms2* transgenic wheat. In comparative proteomic analysis, a total of 6010 protein groups were accurately quantified from three groups of wheat near-isogenic lines, and among them, 164 protein groups were identified as the differentially abundant proteins (DAPs, ≥2 fold) between all near-isogenic lines and their recurrent parents. The down-regulated DAPs were mainly associated with protein, DNA, RNA, cell, amino acid metabolism and signaling pathways, and the up-regulated DAPs were mainly enriched in protein, PS, stress, secondary metabolism and major CHO metabolism pathways. The results showed that even though it was very difficult to detected the Ms2 protein in wheat, *Ms2* gene might lead to anther sterility by blocking pathways of nucleic acid synthesis and signaling, and on the other hand, dwarfing gene *Rht-D1c* might enhance the regulation of photosynthesis and stress resistance in wheat. Overall, this work provides a new insight for the understanding of the mechanisms of male sterility caused by *Ms2* gene.

P23 Identification of the *ASG-1* gene family in *Panicum maximum* Jacq.

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Apomixis is a reproductive mode that bypasses meiosis and syngamy to produce seeds genetically identical to the mother. Its use in breeding programs is expected to simplify the development of hybrid cultivars and production of commercial hybrid seeds. This study aimed to isolate aposporous apomixis gene and analyze how the gene(s) will be expressed in the aposporous *Panicum maximum* Jacq. A new classification method using the ovary length as an index was developed to sample differently developmental stages of ovaries and buds in obligate sexual and facultatively apomictic guineagrass. A cDNA library was derived from the ovaries of aposporous accession N68/96-8-o-11 staged at the appearance of aposporous initial cells (AICs) to isolate AIC stage-specific genes. Using differential screening method, four AIC stage-specific cDNA clones were obtained from ten thousands of plaques by Northern blot hybridization. They showed the same start codon and sequences, ranging in lengths from 577 to 1182 bp. *In situ* hybridization of the cDNA clone revealed gene expression specifically in AIC, AIC-derived embryo sacs (ESs), and root tips and meristems of aposporous accession, as well as in the embryo developmental process in both sexual reproduction and apospory. The amino acid sequences and homologies of the four cDNA clones are similar to *ASG-1*, an apomixis-specific gene reported previously in N68/96-8-o-11, indicating that they are the members belonging to the *ASG-1* gene family (*ASG-1*GF). Our results of *ASG-1*GF achievement define a previously unidentified role of *ASG-1* not only in appearance of AIC and AIC-derived embryo sac formation, but also in the organ tissues of apomictic guineagrass. *ASG-1*GF will provide valid information for further isolating of apospory gene from apomictic plants.

P25 Reproductive fitness of *Limonium* experimental hybrids and apomicts from *ex situ* collections (Plumbaginaceae)

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In flowering plants hybridization and polyploidy can be connected to changes in the reproductive system, as illustrated by sea-lavenders (*Limonium* Mill., Plumbaginaceae). Plants show striking flower morphisms like ancillary pollen and stigma heteromorphisms that prevents self and intramorph mating. The goal of this study was to evaluate the reproductive fitness of experimental hybrids and apomicts and to investigate species composition of insect visitors in a semi-closed greenhouse. Progenitor, hybrid and apomictic plants from controlled crosses representing different species, i.e. diploid (*Limonium ovalifolium*, *Limonium nydeggeri*) or tetraploid (*L. binervosum*, *L. dodartii*), and reproductive modes (sexual or apomixis), were selected. The ploidy level, flower phenotype, pollen viability, and seed germination of plants derived from these crosses were evaluated. Censuses of different insect floral visitors were determined. Results showed that offspring plants originated from diploid crosses mostly present similar ploidy level and inflorescence types as parental plants, although some of them had inflorescences characteristic of tetraploid plants. Diploid plants exhibited A or B the pollen-stigma combination, and medium to high pollen viability. While, tetraploid plants derived from heteroploid crosses only showed flower phenotypes like the female progenitors and very low pollen viability. Plants originated from heteroploid crosses produced a higher amount of seeds than plants from homoploid crosses but with higher seed viability than diploid plants. Irrespective to the species and reproductive modes, insects of the orders of Lepidoptera, Hymenoptera, Diptera, Coleoptera and Heteroptera visited scapes and a considerable number had A and/or B morph pollen grains on their bodies, especially two species of moths and ants. The reproductive fitness of plants derived from homoploid or heteroploid crosses was different, although the species composition of species of insects visiting hybrid and apomictic plants was similar.

P26 Functional conservation of the DUO1-DAZ1 germline regulatory module in tomato

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The male germline-specific MYB transcription factor DUO1 POLLEN 1 (DUO1) is a key regulator of male gametogenesis in *Arabidopsis thaliana*. The role of DUO1 in gamete differentiation is shared between angiosperms and bryophytes but important differences exist between these phyla in the target genes under DUO1 control. Further differences include the requirement of DUO1 in *Arabidopsis* for mitotic division of the generative cell to form two sperm cells. Important targets of DUO1 in *Arabidopsis* include a pair of functionally redundant genes (*DUO1-ACTIVATED ZINC FINGER1* (*DAZ1*) and *DAZ2*), which encode repressor proteins with three C2H2 zinc fingers and two ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motifs. This DUO1-DAZ1 regulatory module is conserved in early land plants, but the evolution of the DUO1-DAZ1 module among angiosperms remains unexplored.

We are investigating the functional conservation of the DUO1-DAZ1 regulatory module in tomato (*Solanum lycopersicon*). There are two *DUO1* orthologs (*SlDUO1A* and *SlDUO1B*) and a single *DAZ1* (*SlDAZ1*) ortholog in the tomato genome. Analysis of RNA-seq data has revealed that transcripts for all three orthologues are highly enriched in generative cells and in sperm cells. We have also shown that SlDUO1A and SlDAZ1 can rescue germ cell division and male transmission defects in the corresponding *Arabidopsis* mutants. In current work we have generated knockout alleles of *SlDUO1A*, *SlDUO1B* and *SlDAZ1*. Single *slduo1a* and *slduo1b* mutants are fertile and show normal male transmission, while *sldaz1* knockout alleles fail to transmit through pollen. Further genetic and phenotypic analysis is underway to uncover the cellular and molecular roles of the DUO1-DAZ1 module in tomato male gametogenesis.

P27 Characterization of APOSTART: a candidate gene for apomixis

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Apomixis and sexual reproduction are considered to be developmentally and evolutionarily closely related including sharing many regulatory components. Once apomictic genes initiate embryo development and the initial cell forms and divides, the genes controlling embryo cell formation and patterning are most likely the same as those required for sexual embryo development. Therefore, two strategies were proposed: the identification of genes exclusively expressed in apomictic plants or the activation of genes encoding proteins with a novel initiating function or an altered function, causing some components of apomixis in sexual plants. Following the first strategy we used the cDNA-AFLP technique to isolate in *Poa pratensis* a gene named *APOSTART* (*PpAPO*) that showed a putative function in programmed cell death, predicted to be involved in the nonfunctional megaspore and nucellar cell degeneration events that permit enlargement of maturing embryo sacs. APOSTART protein shares high similarity with two *A. thaliana* genes APOSTART1 (At5g45560) and EDR2 (renamed APOSTART2, At4g19040). To investigate their biological role, we analyzed two independent T-DNA insertional mutant lines, *Atapo1* and *Atapo2* and *apo1/apo2* double homozygous mutants. Subtle phenotypical differences in germination rates, plant growing stages and seed production were found comparing these lines with a control line (Col-0). Moreover, high variability was found between and within mutant lines. In this context a bisulfite sequencing analyses was performed for checking methylation level on the promoter regions of the two genes.

In summary, characterization of T-DNA mutant lines, including methylation analysis and chromosome counts of the double mutants for *AtAPOSTART* genes, will be reported and discussed.

P28 Functional conservation of *DUO1* genes in *Physcomitrella patens*

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Basal land plants such as bryophytes produce motile sperm in contrast to flowering plants that produce non-motile gametes. Different modes of spermatogenesis in the land plant lineage may be associated with diversification in ancestral gene regulatory networks during land plant radiation. This raises the question of the conservation of germline development among land plants and whether sperm cell differentiation mechanisms share a common molecular origin. An established model explains how the *Arabidopsis thaliana* germline-specific MYB transcription factor DUO1 is a key player in male germ cell development. DUO1 binds to the promoters of several target genes that are important in germ cell division and gamete interactions. Although the function of DUO1 is established in flowering plants, its role in basal land plants has only recently been confirmed in Marchantiophyta - one of the three bryophyte divisions. We are investigating the functional conservation of DUO1 in the model moss species *Physcomitrella patens*. Two DUO1 homologs (PpDUO1A and PpDUO1B) exist in P. patens. Expression analysis shows that transcripts of both genes are highly enriched in male reproductive organs (antheridia). The inability of double mutant *Ppduo1ab* mutant plants to form sporophytes highlights the critical role of PpDUO1A and PpDUO1B in *P. patens* fertility. Spermatogenous cell morphology and spermatogenesis are affected in the *Ppduo1ab* double mutant while single mutants are unaffected and fertile. Our data shows that *PpDUO1* genes have an important role in male germ cell differentiation. The lack of locomotary apparatus in a form of flagella has affected the ability of *Ppduo1ab* mutant plants to form a functional spermatogenous cells, preventing fertilization. Our current data clearly show a wider conservation of DUO1 function among bryophytes.

P29 Long term cold stress affects reproduction in apomictic *Boechera formosa* (Brassicaceae)

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Diploid *Boechera* exhibit highly variable modes of seed formation, from obligate sexuality, through varying levels of sexual and facultative apomictic seed formation in individual taxa, populations and plants, to obligate apomixis. Diploid *B. formosa* accessions are recognized as sexual with low level of apomixis or highly apomictic. The embryological data is unknown for most populations. To assess mode of the ovule and anther development in control (grown in room temperature conditions) and cooled (grown at 5 °C) plants of *B. formosa* from Utah, we conducted cyto-embryological analyses using clearing, immunofluorescence and flow cytometry. We found that both diplosporic and aposporic gametophytes occurred in each flower, with a small portion of sexual ones. Male sporogenesis and gametogenesis were highly unstable, resulting in formation of pollen grains with variable morphology, size and nuclei number. Flow cytometry seed screening revealed that control plants mainly produced apomictic seeds with expected 2C:6C embryo to endosperm (Em:en) ratio. However, nearly 10% of the seeds demonstrated variation in the Em:en ratio which indicated an excess of maternal or paternal genome and fertilization of the egg cell. When plants grew continuously under low temperature, they accumulated much more callose in the meiocyte or aposporous initial cells walls, and the walls of their cell derivatives. Interestingly, these plants were characterized by heterochronic ovule development and higher numbers of both meiotic and aposporous embryo sacs. Cold-treated plants suffered much higher pollen degeneration than control plants and, as a consequence, higher levels of seed abortion. These results confirmed that fertilization is required for seed development in this population, and the origin of the “cold tolerant” seeds remains to be determined. Taken together, our data in *B. formosa* show that plant fitness, as measured by efficiency and mode of reproduction, is likely influenced by environmental (here - temperature) fluctuation.

P30 Heat induced meiotic restitution and sexual polyploidisation through natural variation of the *Arabidopsis CYCA1;2/TAM*

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Meiotic restitution of male meiosis under high temperature stress has been described in multiple plant species. This phenomenon may have contributed to ancient and recent whole genome duplication events in the plant lineage. In parallel, multiple genes have been discovered that, when mutated, induce the production of restituted male gametes under normal temperature conditions by affecting meiotic progression or spindle organisation and chromosome segregation (e.g. *CYCA1;2/TAM*, *OSD1*, *atPS1*). In search of a molecular link between temperature stress and meiotic restitution, we explored the natural variation in heat induced meiotic restitution by screening 200 *Arabidopsis* ecotypes at the tetrad stage of pollen development after a 24h at 32°C heat treatment. Our analysis revealed a large phenotypic variation, with some ecotypes being resilient to high temperature and several that produce up to 100% dyads instead of the regular tetrads. F2 mapping analysis of one of the most sensitive accessions and subsequent complementation tests between the highest dyad producing accessions revealed the involvement of *CYCA1;2/TAM* in governing the heat induced phenotype. Analysis of pollen development in these accessions showed that heat stress induced diploid pollen formation that generated triploid offspring. The high incidence of heat sensitive *CYCA1;2/TAM* alleles among *Arabidopsis* accessions points to a prominent role for cell cycle regulation in sexual polyploidisation under conditions of high temperature.

P31 Callose deposition in anthers regulates timely initiation and progression of male meiosis in rice (*Oryza sativa* L.)

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Sexual reproduction is an important step in establishment of the genetic makeup of offspring in all sexually reproducing organisms. Meiosis is an important phase of sexual reproduction cycle which contribute to enormous variation mirrored in the progenies of subsequent generations. In rice, MEL2 (MEIOSIS ARRESTED AT LEPTOTENE2), an RNA recognition motif (RRM) protein functions in faithful transition of spore mother cells from mitosis to meiosis. Several gene transcripts which have significant roles in meiosis progression were co-immunoprecipitated with MEL2. One of MEL2-bound mRNAs encode callose synthase which is responsible for filling anther locules with callose (beta-1,3-glucan) polysaccharide during mitosis-meiosis transition phase. Several reports have well elucidated role of callose at pollen formation stage, however, its importance in meiosis has largely remain unknown till date and thus biological meaning of callose deposition in anther during meiosis onset is extremely limited. To probe significance of callose in plant meiosis, a callose synthase loss-of-function line that showed complete sterility was produced in rice. Callose accumulation in this mutant was found to be greatly reduced at onset of meiosis and early meiosis and such germ cells enter into meiosis early compared to wild type. The precocious meiosis initiation was accompanied by several major defects in condensation and behaviour of meiotic chromosomes, in addition to abnormality in homologous synapses and bouquet structures. Further, there was a great delay in meiosis progression, especially in the early meiosis stages in the mutant anthers. Altogether, these findings strongly imply the essentiality of callose for proper meiosis initiation and progression and reveals a previously unknown association between callose and meiosis in flowering plants. Recent updates on this work will be presented at ICSPR 2022 meeting in Prague.

P32 Molecular mechanisms controlling crossover interference in *Arabidopsis* meiosis

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Meiosis is a specialized cell division that reduces the chromosome number by half, generating haploid spores that are key for sexual reproduction. Together with this ploidy reduction, meiosis also serves to create novel genetic variability in resulting gametes by reshuffling parental alleles via homologous recombination. This process involves tight pairing of homologous chromosomes and reciprocal exchange of genetic information via the establishment of physical links; i.e. crossovers (COs).

In plants, like in other organisms, meiosis exhibits a low number of COs that are non-uniformly distributed across the genome, with most COs occurring at (sub-)telomeric regions. This specific CO landscape reflects a spatio-temporal regulation of meiotic recombination, with multiple mechanisms determining CO designation. One mechanism is CO interference (COI), a phenomenon whereby the establishment of one CO decreases the possibility of a second CO nearby. In most plant species, the majority of COs are subject to COI (class I COs), and their formation is mediated by a specific pathway involving the ZMM proteins. However, despite insights on the molecular factors that promote class I CO formation, little is yet known about the molecular mechanisms that determine their positioning and interference.

In order to gain more insights into the regulatory mechanism(s) determining CO interference, we are performing a dedicated forward genetic screen in *Arabidopsis thaliana* using the pollen FTL-based system. By performing this in a *mus81* background (and thus excluding class II COs, i.e. COs that are not subject to COI), the screen is specifically designed to identify genes that are putatively involved in the homeostasis and positioning of class I COs, and thus also in COI. As COI is an important process in determining genetic variability, results of this research are relevant for both fundamental plant biology and evolution, but also may serve useful for innovative crop breeding.

P33 A forward genetic screen reveals *OSD1* and *CDM1* as regulators of meiotic exit in *Arabidopsis thaliana*

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Meiosis ensures the ploidy maintenance and reproductive success of sexually reproducing species. Miotic chromosome segregation requires extensive reprogramming of the cell division machinery. In angiosperm plants, meiosis followed by mitotic divisions that produce rudimentary haploid gametophytes. Therefore, after completing meiosis, the cell division program has to switch back to mitosis. We are using model plant *Arabidopsis thaliana* male meiosis to study the termination of meiosis and the transition to gametophytic development. We found two genes are essential for the meiotic exit, *SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7* (*SMG7*) and *THREE DIVISION MUTANT1* (*TDM1*). Mutations in these genes lead to continuous meiotic divisions, resulting in sterility. To understand molecular mechanisms governing meiotic exit, we conducted a forward genetic screen to identify suppressor lines that rescue the fertility of *SMG7*-deficient plants. We found mutations in *OMISSION OF SECOND DIVISION1* (*OSD1*) and *CALLOSE DEFECTIVE MICROSPORE1* (*CDM1*), which reverts the *smg7* sterile phenotype. We will present in depth analysis of these genes in meiotic exit, including detailed phenotyping of mutant lines and protein localization studies during meiosis.

P34 The effects of a splicing regulator, Serine/Arginine-rich 45, on *Arabidopsis thaliana* sperm transcriptome

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In flowering plants, pollen grains are the male gametophyte that produces sperms. Studies have shown that there is a transcriptome switch during pollen mitosis II when producing mature pollen grains. *Arabidopsis* Serine/Arginine-rich 45 (SR45) is a splicing regulator that is highly expressed during pollen development. The *sr45-1* null mutant plant exhibits pleiotropic phenotypes throughout the life cycle, including a mild sterility and a reduced seed set. To investigate SR45's effects in reproduction, sperm cells were isolated from both wild-type and the *sr45-1* mutant mature pollen using FACS. The bulk sperm transcriptome was sequenced and compared between the two genotypes. The RNA-seq data analyses suggest that SR45 promoted the expression of genes involved in bioprocesses for pollen tube development and suppressed the expression of genes involved in pollen sperm cell differentiation and mitotic cell cycle phase transition regulation. Sperm markers are used to validate this transcriptome profile. Using the 3D RNA-seq App, we also found 87 genes with differential alternative splicing (DAS) events between the two genotypes. There was little overlap between the differentially expressed genes and DAS genes. However, transcripts that showed different abundance between two genotypes are overrepresented in mRNA splicing and phosphorelay signal transduction systems. This suggests that SR45-dependent AS events may preferentially contribute to the sperm identity in mature pollen grains via maintaining a homeostasis in alternative splicing and components of signal transduction. Some of the DAS events showed significant switches in isoform abundance and potential structural changes in the predicted protein products. Among genes exhibiting isoform switches, *RAD4* and *RAD51* are selected to explore possible isoform-specific functional difference.

P35 Transcriptomics of the *PARTHENOGENESIS* locus in egg apparatus of sexual, apomictic and *PAR*-deleted dandelions.

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The common dandelion (*Taraxacum officinale*; Asteraceae) exists of obligate sexual and apomictic (micro)species, with the apomicts producing clonal seeds spontaneously. Apomixis in dandelion is of the 'gametphytic' type, in which a normal, but unreduced, female gametophyte is formed. The unreduced egg cell develops spontaneously (parthenogenetic) into an embryo, while the endosperm also forms autonomously in dandelion. In combination as well as independently these apomixis elements are of large interest in plant breeding, for instance, to maintain all heterozygosity and epistatic interactions of vigorous F1-hybrids by producing clonal seeds, and to initiate embryogenesis in vitro in (doubled) haploids formation. We focussed on the unraveling of the molecular basis of parthenogenesis via comparative transcriptomics in laser assisted micro-dissected egg cell apparatus (egg cell + synergids) and central cells of sexual, apomictic, and parthenogenesis deleted dandelions. The genes in sequences associated to the parthenogenesis deletion were analyzed for differential expression. Two genes showed unique expression from the apomictic allele, one of which was highly expressed during early gametophyte development, well before cellularization and egg/central cell fate determination, while the other was expressed in the mature egg cell apparatus, conform to expectations for the *PAR* gene. *In situ* hybridization confirmed these spatio-temporal expression patterns, while analysis of the pollen of sexual dandelion confirmed the expression of par. A large, 1.3 kb insertion was found in the promoter region of the *PAR* allele. Based on all results, we propose that PAR proteins are repressors of an unknown gene that suppresses embryo development in the plant egg cell. The recessive par alleles in egg cells of sexual dandelion are silenced, while they are expressed in pollen, resulting in a release of suppression after fertilization. In apomicts, the insertion disturbs the silencing of *PAR*, allowing its transcription, formation of PAR proteins, and embryo development.

P36 Identification and functional proof of the parthenogenesis gene in apomictic dandelions (*Taraxacum officinale*)

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We identified the parthenogenesis (*PAR*) locus in dandelion by a combination of genetic and deletion mapping of Loss of Apomixis phenotypes. The dominant *PAR* gene was singled out by CRISPR-Cas9 targeted deletion of genes within the ~1 Mb locus. The *PAR* gene is a small 513 bp gene with a specific zinc finger domain and a repressor binding EAR motif. Complementation of the *Taraxacum PAR* locus deletion line resulted into restoration of apomixis. When the dandelion *PAR* or *par* gene, driven by an egg cell specific *Arabidopsis* promoter is introduced into sexual lettuce, the egg cells produce embryo-like structures without fertilization. This is functional proof that the *PAR* gene causes parthenogenesis. In comparison to the sexual allele, the dominant *PAR* allele contains a large MITE transposon (1335 bp) in its promoter, 110 bp upstream of the start codon of the CDS. Remarkably, a similar MITE insertion was found in the *PAR* gene of apomictic hawkweed *Pilosella piloselloides*. We hypothesize that the MITE insertion causes parthenogenesis. The insertion site is 27 bp upstream of the insertion site in *Taraxacum*, suggesting independent parallel evolution of apomixis in these two genera. The EAR motif suggests that *PAR* which is highly expressed in the pollen, functions as a repressor of a repressor of embryogenesis.

P37 Influence of nutrient deficiency on meiotic recombination and pollen size variation in rye (*Secale cereale)*

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Meiotic recombination increases allelic diversity via crossover or gene conversion and is therefore a major driver of the evolution of organisms. Studying meiotic recombination allows us to better understand how speciation proceeds and is also valuable for plant breeding for creating new varieties providing food security. Another important mechanism generating novel allelic combinations enabling adaptation to changing environments is cross-pollination. Past studies have shown that meiotic recombination and pollen size are depending on environmental factors and nutrient balance, but the underlying genetic architecture is remaining elusive. The goal of our study is to investigate meiotic recombination and pollen size variation in a heterogeneous population under nutrient deficiency caused by long-term monoculture to understand the genetic architecture underlying these stress response interactions. For this purpose, we will use an outbreeding, wind-pollinating grass species (*Secale cereale*) which has been growing in monoculture with and without fertilizer for 140 years in a historic field trial. We will investigate the meiotic recombination rate variation by genotyping single pollen nuclei of individual plants. This method allows us to directly determine the crossover events in haploid DNA samples derived from diploid heterozygous plants. In order to unravel the genetic architecture of abiotic stress responses of the meiotic recombination we will use GWAS to identify genes involved in that process. Further on, we will study the pollen size variation by flow cytometry which will enable a high-throughput measurement of thousands of pollen grains per plant. Our study will help to extend our knowledge of the molecular and evolutionary mechanisms of allelic diversity generated by recombination in plants.

P38 A microspore-specific gene *SRL1* required for fertility

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To identify microsporogenesis specific functions, we searched the TAIR database and identified candidate genes specifically expressed in the developing male gamete. T-DNA insertions linked with candidate genes were analyzed for reduced fertility and selected for further study when pollen quality was poor. Here we present the result obtained for a candidate locus *SRL1*, encoding a serine rich like protein. The *SRL1* gene was exclusively expressed in anthers during the late stages of flower development. Pollen from plants heterozygous for the T-DNA insertion show reduced fertility and arrest at the early bicellular development stage. In contrast, homozygous plants were fully viable and produced fertile pollen. The predicted SRL1 protein is targeted to the mitochondria, which was confirmed by expressing GFP tagged SRL1 in mature pollen. Future studies aim to unravel the mechanism by which heterozygous plants show the reduced pollen while homozygous plants make normal pollen.

P39 Apospory expressivity modulation by environmental and epigenetic changes in diploid genotypes of *Paspalum rufum*

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In angiosperms, gametophytic apomixis is strongly associated with polyploidy and hybridization. Most apomictic polyploids are facultative, and apomixis and sexuality coexist in the same plant or even in the same ovary, but finally, only apomixis succeeds. At the diploid level, the opposite occurs, and although some genotypes can produce aposporous embryo sacs (AES), the seeds derived from sexuality. We previously observed that some natural diploid genotypes of *Paspalum rufum* could reproduce by apomixis, and also aposopory expressivity could be significantly increased by hybridisation. In this context, here we explore environmental stability and epigenetic variation associated with the trait at the diploid level on *P. rufum*.

Apospory expressivity (%AES) was evaluated by cytoembryological analyses of diploid sibling genotypes of *P. rufum* exposed to two different environments and across the years. DNA methylation levels (at CG, CHG, and CHH contexts) were analysed using a methylation content-sensitive enzyme ddRAD (MCSeEd) strategy. Full spikelets (at pre-meiosis/meiosis and post-meiosis stages) of full sibling diploid *P. rufum* genotypes with differential %AES were compared.

Our analysis showed that the %AES was significantly influenced by different environments but remained stable across the years. Principal component analysis and heatmaps, based on the relative methylation level, discriminated samples with contrasting apospory expressivity. Differential methylated contigs (DMCs) showed 14% of homology to known transcripts of *Paspalum notatum* reproductive transcriptome, and almost all of them were also differentially expressed between apomictic and sexual samples. DMCs showed homologies to genes involved in flower growth, development, and apomixis. Moreover, a high proportion of the DMCs aligned on genomic regions associated with apomixis in *Setaria italica*.

Our results underline the importance of environmental influence in modulating apospory expressivity and identified several stage-specific differential methylated sequences associated with apospory expressivity,

which could guide future functional gene characterisation concerning apomixis success at diploid and tetraploid levels.

P40 Morphological characterization of the meiotic process in sexual, apomictic, and loss-of-diplospory ovules of *Taraxacum officinale* L.

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Taraxacum officinale L., the common dandelion, is a member of the Asteraceae family and it is characterized by sexual diploid and apomictic polyploid genotypes. In sexual diploids, the MMC (megaspore mother cell) undergoes meiosis to produce four haploid megaspores, one of which develops into the haploid embryo sac. This contains the haploid egg cell that will form the diploid embryo after being fertilized by a haploid sperm cell.

Instead, the apomictic polyploids reproduce asexually through a form of gametophytic apomixis – named meiotic diplospory – in which, although the MMC starts the meiotic process, it fails to segregate the chromosomes. This aberrant meiosis produces only two megaspores that are unreduced. One of the two megaspore gives rise to an unreduced embryo sac, which contains an unreduced egg cell that develops into an embryo by parthenogenesis. We have characterized a loss-of-diplospory *LOD* mutant generated by gamma-irradiation from a triploid apomictic plant, thus allowing the identification of the putative locus that controls diplospory in dandelion.

The female gametophytic development of the *LOD* mutant has been compared with the apomictic triploid and the sexual diploid to determine the differences among them.

This will deepen our knowledge on the still unclear process of asexual reproduction and will enable to unveil the mechanisms regulating apomixis. The introduction of apomixis in sexually reproducing plants could have a huge impact on plant breeding, allowing the fixation of valuable traits across subsequent generations regardless the genetic complexity.

P41 Spotting the targets of the aposporous apomixis controller TGS1 in *Paspalum notatum*

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Sexuality and apomixis are interconnected plant reproductive routes possibly behaving as polyphenic traits under the influence of the environment. In *Paspalum notatum*, an apomictic subtropical forage grass, one of their main apomixis controllers is TRIMETHYLGUANOSINE SYNTHASE 1 (TGS1). The silencing of *TGS1* induces a sexuality decline and the emergence of aposporous-like embryo sacs. In animal and plant models, TGS1 was characterized as a multifunctional gene expression regulator involved in the establishment of heterochromatin as well as the modulation of transcription and RNA processing. Our work here was aimed at identifying TGS1 targets in the *Paspalum notatum* floral transcriptome. First, we mined available RNA databases originated from florets of sexual and apomictic plants, which display a contrasting TGS1 representation, to identify differentially expressed mRNA splice variants and miRNAs. The role of TGS1 in the generation of these particular RNA processing products was investigated in antisense *tgs1* sexual lines by using qPCR and *in situ* hybridization. We found that CHLOROPHYLL A-B BINDING PROTEIN 1B-21 (LHC Ib-21, a component of the chloroplast light harvesting complex), QUI-GON JINN (QGJ, encoding a MAP3K previously associated with apomixis) and miR2275 (a meiotic 24-nt phasi-RNAs producer) are direct or indirect TGS1 functional targets. Our results point to a coordinated control of signal transduction and siRNA machineries in the sexuality to apomixis transition.

P42 Impact of terminal heat and drought co-stress during microgametogenesis on development, viability and functionality of wheat pollen.

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Although formation of functional vegetative and generative cells through the first pollen mitosis (PM) and sperms cells by the second PM in species with trinucleate pollen release, development of the inner pollen wall and deposition of starch and protein reserves needed for functional pollen and successful fertilization, all take place during microgametogenesis, very few attempts have been made to reveal the environmental stress sensitivity of this important developmental period. Our previous experiments using wheat genotypes with contrasting stress tolerance subjected to heat and drought (HD) co-stress indicated that reduced functionality of pollen accounted for 66% of the total generative organ and cell-triggered fertility loss, the background of the phenomenon remained still unclear.

The aim of this study was to reveal the effect of isochronal high temperature and total water withdrawal during male gametogenesis on development, structure, ultrastructure and functional characteristics of male reproductive cells and organs and, phenology, morphology and yield components of wheat genotypes with contrasting HD tolerance; prove the tolerance-dependence of antioxidant enzyme synthesis under HD co-stress conditions; demonstrate whether HD co-stress induced symptoms in anthers and pollen cells vary with the genotype and floret position in the spike; understand the link between HD-induced lipid peroxidation, anatomical alterations, functional anomalies and consequent yield loss. We show that genetic variability for the tolerance to HD co-stress applied during microgametogenesis is available for wheat.

The tolerant phenotype was highly correlated with a potential to avoid severe damage of cell membranes and preserve native anatomy and intact ultrastructure of anthers and pollen cells through the maintenance of water content and antioxidant enzyme activities. In the long term,

exploring the mechanisms underlying the HD co-stress tolerance of generative processes will contribute to the development of new genotypes capable of adapting to changing climate.

P43 Revealing the transcriptional activities of pollen expressed bZIP transcription factors.

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bZIP transcription factors (TFs) are evolutionarily conserved TFs found to play a role in energy metabolism, unfolded protein response, senescence, flowering, pollen development, seed maturation, and abiotic stress signaling. These TFs possess a basic DNA-binding domain and a leucine zipper that enables bZIP dimerization. The bZIP family in *Arabidopsis* comprises 78 members, differentiated into 13 groups. Of the 78 members, 9 are expressed in developing pollen and serve as potential candidates for transcriptional regulators of pollen development. We have previously demonstrated the roles bZIP18 and bZIP34 play in pollen development, but have not elaborated on their transcriptional properties. Here, we show which promoters are bound by the pollen-expressed bZIP homo- and heterodimers, and their transactivation properties, as revealed through GUS/NAN assays in *Arabidopsis* protoplasts.

P44 Exploring the dimerization potentials among pollen expressed bZIP transcription factors.

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The regulation of the gene expression is essential for plant growth and differentiation. Transcriptional control is one of the most important means for regulating gene expression, and in plants is especially complex, as evidenced by the significant expansion of their transcription factor (TF) families during evolution. One of the largest groups of TFs in plants is the basic region/leucine zipper (bZIP) family whose members regulate critical processes in development and stress responses. All members of this family contain a bZIP domain which consists of a basic region (BR) followed by a leucine zipper (LZ). The BR carries a nuclear localization signal and interacts with DNA, whlile the LZ mediates bZIP dimerization. Based on the conserved sequence of the BR and other functional motifs outside of the bZIP domain, the *Arabidopsis* bZIPs are sorted in 10 groups (A to I, and S). One of the main characteristics of bZIP TFs is that they act as dimers, however, they are not dimerizing promiscuously, and specific interactions are preferred. This feature represents one major way of producing a large repertoire of regulatory responses. The formation of bZIP homo- or hetero-dimers offers a tremendous combinatorial flexibility to a regulatory system. Here, we expanded on the previously published bZIP dimerization network in pollen, through the addition of additional pollen-expressed bZIP TFs. We used bimolecular fluorescence complementation and yeast two hybrid analyses to demonstrate the dimerization potentials among these pollen-expressed bZIP TFs.

P45 Genetic regulation of *STICKY GENERATIVE CELL* critical for generative cell internalization and differentiation in *Arabidopsis* pollen

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Flowering plants proliferate through double fertilization that occurs between distantly located male and female gametophytes. Some of major challenges rooted from non-motile male gametes and deeply embedded female gametes have been resolved by adopting cell patterning and differentiation of three cells in the male gametophyte. First, undetermined haploid microspore asymmetrically divides to a larger vegetative cell and a smaller generative cell with different fates. Second, the generative cell, the progenitor of two sperm cells, completely internalizes into the vegetative cell to produce a pollen tube that later rapidly delivers the sperm cells toward the female gametophyte for double fertilization. Third, two sperm cells and the vegetative nucleus are physically linked to form the male germ unit. In our previous study to unravel genes critical for the strategic pollen patterning, we identified that *STICKY GENERATIVE CELL* (*SGC*) encoding a highly conserved domain of unknown function 707 (DUF707) is required for the generative cell internalization and differentiation. In *sgc* pollen, mutant generative cells remain at the pollen wall due to the ectopic callose deposition in the nascent generative cell. Interestingly, an earlier study showed that generative cells in double mutants of *BONOBO1* (*BNB1*) and *BNB2*, two evolutionary conserved the VIIIa bHLH transcription factors in *Arabidopsis*, not only fail to specify the male germline cell fate but also remain at the pollen wall. We now try to unravel the genetic relationship between BNB and SGC regarding the generative cell internalization and differentiation. To this end, we carry out comparative analysis of pollen ontogeny in *sgc* and *bnb1bnb2*. We also investigate whether *SGC* expression is dependent to BNBs by analyzing the *SGC* promoter activity in *bnb1bnb2* mutants or in transgenic lines expressing ectopic *BNB*s. We present our progress on genetic regulation of *SGC* critical for pollen patterning and germline differentiation.

P46 Inspecting the seed formation pathways in diploid *Paspalum notatum*

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Paspalum notatum is a forage grass with sexual self-incompatible diploids (2n = 2x = 20) and aposporous-apomictic pseudogamous tetraploids (2n = 4x = 40). Although sexuality and apomixis seem to be isolated by the ploidy level, some diploid genotypes can form aposporous-like embryo sacs in low proportion. On the other hand, triploids have been found in natural diploid populations. In previous works, cytoembryological observations detected that the plant #R1 produced about 1% of its ovaries containing a meiotic sac and an aposporic sac, suggesting some capability for apomictic reproduction. The objective of this work was to explore the reproductive pathways operating in the genotype #R1 after homo (2x x 2x) and interploidy (2x x 4x) crosses. Reexamination of #R1 ovules at anthesis confirmed the presence of aposporous-like embryo sacs. Crosses (and their reciprocal ones) between #R1 with diploid (2x) and tetraploid (4x) co-specific pollen donors were performed to evaluate the seed set and the flow cytometric seed screen method (FCSS) was used to reconstruct the seed formation pathways. Plant #R1 showed a normal seed set in homoploid crosses (2x x 2x), where all seeds derived from sexuality (FCSS histograms showing 2C:3C peaks). Besides, only a few (8) seeds were recovered in interploidy crosses carried on more than 1000 florets when #R1 was used as a female parent (2x x 4x), and no seeds were obtained when #R1 was used as pollen donor (4x x 2x). One seed obtained from 2x x 4x cross showed an FCSS histogram compatible with apomictic reproduction (2C:6C peaks). Results presented in this work suggest that both sexuality and apomixis coexist in plant #R1. Thus, the possible combination of both reproductive strategies at the diploid level can affect the adaptation and evolution of the *P. notatum* agamic complex.

P48 Exogenous application of auxin on young inflorescences affects the reproductive development in tetraploid *Paspalum notatum*

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Paspalum notatum is a subtropical forage grass that combines vegetative propagation, sexuality and apomixis. Tetraploid races (2n=4x=40) reproduced by obligate or facultative aposporous apomixis. Fully sexual tetraploid plants were never found in nature, but were artificially generated. Apomixis in the species is characterized by the formation of unreduced embryo sacs (ESs) from nucellar cells (i.e. apospory initials), the emergence of maternal embryos by parthenogenesis, and the formation of endosperm after fertilization of polar nuclei (pseudogamy). Parthenogenetic embryos are frequently observed within aposporous ESs prior to anthesis. The so-called “auxin test” was traditionally used in grasses for screening of apomictic and parthenogenetic individuals. The objective of this study was to evaluate the effect of external auxin application on young inflorescences of tetraploid fully sexual and apomictic plants of *P. notatum*. Inflorescences of three sexual and seven apomictic individuals were sprayed with an aqueous solution of 2,4-D (80mg/l +Tween 20) 10-to-3 days prior to anthesis. Control inflorescences were treated with distilled water. Auxin treatment was assessed cytoembryologically by scoring the nature (reduced or unreduced) of ESs and proembryos derived parthenogenetically (from egg cells) and somatically (from nucellar cells) in ovules at anthesis. Two sexual plants developed aposporous-like ESs in 15% and 19% ovules. In five out of the seven apomictic plants, the percentages of ovules containing parthenogenetic proembryos increased significantly compared to control plants. In addition, auxin treatment also triggered the formation of proembryos in unpollinated reduced ESs (1-11%) collected from five plants while all mature reduced ESs in control plants contained an egg cell. Finally, we also observed the formation of ectopic somatic embryos (1-2%) from nucellar cells in three apomictic plants. Our results indicate that exogenous auxin treatment induce aposporous-like ESs in sexual genotypes and significantly increase the proportion of parthenogenetic embryos.

P49 Effects of heat stress during pollen development

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Major shifts in the duration and intensity of ambient temperature affects plant development and reproduction. In maize and other cereals, pollen development is especially sensitive to abiotic and biotic stresses [1]. Using the Leaf Collar Method, we are able to track discrete pollen developmental stages allowing us to study their responses to environmental stimuli [2]. To understand how heat stress impacts individual developmental stages during pollen development, we imposed a moderate (35°C/25°C day/night) heat stress treatment on maize plants at the tetrad, unicellular, bicellular and tricellular stages. During the tetrad stage we observed a strong variation in basic metabolic pathways resulting in reduced starch content, decreased enzymatic activity, and thus generating germination-defective pollen, ultimately leading to sterility [3]. Similarly, at the unicellular stage, heat stress strongly affected pollen viability and pollen tube growth resulting in severe sterility and reduced seed set. Unlike early stages, bicellular pollen appeared less sensitive to heat stress. We explored the responses at different pollen developmental stages to heat stress by RNA-seq and identified a set of up- and down-regulated genes including transcriptional regulators with a potential role to mitigate the effect of heat stress during pollen development. To functionally characterize differentially expressed candidate genes, we generated maize CRISPR-Cas9 lines to study their function during pollen development under normal conditions and heat stress. Engineering such candidate genes could potentially help in the future to improve thermal resilience in crop plants.

[1]Begcy and Dresselhaus, 2018. Plant Reproduction
[2]Begcy and Dresselhaus, 2017. Plant Reproduction
[3]Begcy et al, 2019. Plant Physiology

P50 Understanding the molecular mechanism of parthenogenesis in cereals

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Parthenogenesis, meaning “creation by virgin”, is a key component of apomixis (asexual reproduction through seeds) and describes spontaneous embryogenesis from an unfertilized egg cell and thereby generates offspring genetically identical to the mother plant. Investigating parthenogenesis in crop plants thus has high potentials to immediately fix desired traits including heterosis and thus would create great economic values. It can also help to understand how embryogenesis initiation is regulated and how egg cell fate is determined. Nevertheless, the underlying molecular mechanisms of parthenogenesis remain poorly understood. Here, we use the apomictic grass *Tripsacum dactyloides* and maize to address these questions. *Tripsacum* is sexually reproducing as a diploid, but all polyploids display apomixis via parthenogenesis. As such, we collected egg cells from diploid and tetraploid *Tripsacum* lines to compare their gene expression profiles. As *Tripsacum* is the closest wild relative of sexual maize, *Tripsacum*-derived RNA-seq reads were sucessfully mapped to the maize B73 reference genome and further counted. For further studies we selected several promising candidates which are highly expressed in parthenogenetic eggs and which are almost completely silenced in sexual eggs. We will characterize their funtions in activating embryogenesis via creation of egg cell-expressed lines and knock-outs in maize. The ultimate goal of this research is to gain mechanistic understandings of parthenogenesis and embryogenesis initiation in cereals and utilize the knowledge generated to contribute and improve the production of haploid maize lines or clonal seeds.

P51 Telo-box: plausible pollen development regulatory motif

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Pollen grain plays an essential role in angiosperm reproduction and also represents valuable model system for research of cellular processes such as cell division, differentiation, signaling and many others. Since the pollen development is a very complex process, it must be precisely regulated by vast number of mechanisms, which are still being unraveled. These include regulation of gene transcription with the help of regulatory elements. Interestingly, based on our preliminary data, short interstitial telomere motifs (telo-boxes) represent plausible pollen development regulatory element and are often accompanied by site II motifs. Telo-boxes belong among *cis*-regulatory elements and are frequently present within promotor sequences and they can be recognized by proteins with Myb-domain such as telomere repeat-binding factors (TRBs). Moreover, TRB proteins are linked to the epigenetic regulation by the recruitment of Polycomb or PEAT complex, which may also play an important role in the male gametogenesis. Thus, we aim to study cis-regulatory elements present in the promotor regions of genes expressed throughout the pollen development and their interacting protein factors, with the main focus onto the telo-box, TRBs and other telo-box or site II binding proteins.

P52 A chromosome scale assembly of *Eragrostis curvula* genome identify a region linked to apomixis

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Eragrostis curvula is a grass species that is being studied in order to identify the genes and genomic regions involved in apomixis, an asexual mode of reproduction that avoid meiosis (apomeiosis) and fertilization of the egg cell and develops an embryo by parthenogenesis. Transferring apomixis to economically important crops will revolutionize the agriculture as we know it today since it can fix the hybrid vigor for generations. The identification of genomic regions associated to this trait and its regulatory components is central to transfer apomixis to other crops. In this way, we already sequenced a sexual diploid genotype, however, more complex apomictic tetraploids are necessary to perform comparative analyses. The tetraploid genome of the apomictic genotype Don Walter (2n=4x=40, ~1200 Mb) was sequenced using a combination of technologies to obtain a chromosome scale assembly. We used 10x genomic reads that were assembled with supernova software. Then, 20x of nanopore long reads were added with DGB2OLC software. Finally, Omni-C, the latest proximity ligation technology was used for scaffolding. The assembly has 1,171 Mb distributed in 3037 scaffolds. The N50 was 96 Mb and the number of genes was 133,619. The completeness of the genome was assessed using the BUSCO software finding 96,6% of complete genes. Performing a syntenic analysis with the diploid assembly we could get fully covered the 10 basic chromosomes of *E. curvula*. Through this analyses we could find regions that are only present in the apomictic genotype. Interestingly, SNPs markers linked to apomixis and candidates genes previously identified were mapped on a single region, togheter with other genes related to reproduction. Since the genome is collapsed in 10 chromosomes we could not distinguish if this region is present in all the haplotypes. More nanopore reads will be added in order to obtain an haplotype resolved assembly.

P53 Depletion of *TRIMETHYLGUANOSINE SYNTHASE1* in *Arabidopsis* phenocopies components of apomixis

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Some flowering plants reproduce without meiosis or fertilization but retain the gametophytic and sporophytic generations typical of sexual plant life cycles. These reproductive modes, termed gametophytic apomixis, perpetuate for generations the maternal genotype, an appealing outcome for crop breeding. Here, we report the characterization of plant RNA methyltransferases, orthologous to yeast trimethylguanosine synthase1 and whose depletion was associated with apomixis in Paspalum grasses. Phylogenetic analyses indicated that land plant genomes all encode a conserved, specific TGS1 protein. Using reporter constructs in *A. thaliana*, we show that it is expressed in the placenta and ovule primordia except in the L1 cell layer and the nucellus that completely lacked signal; in the functional megaspore and during gametogenesis, and; in the zygote and growing embryos. High proportions of aborted seeds in *tgs1* plants and distortion of segregation against *tgs1* alleles suggested defects in gametogenesis or embryogenesis. Indeed, paternal alleles reactivated early during zygote development and were expressed at least up to 10 DAP, indicating that a wild type paternal allele cannot rescue the depletion of the maternal copy during early embryo develoment. Depletion of *TGS1* in *Arabidopsis* combined with the use of cell-identity markers revealed cell fate alterations during female meiosis, gametogenesis and early seed development, resulting in phenotypes resembling components of apomixis including: formation of more than a single female gametophytic precursor, supernumerary embryo sacs and extra embryos. Our results indicate that plants have evolved specific RNA methyltransferases essential for reproductive fate determination in plants.

P54 Expression and function of ARGONAUTE proteins in the male and female gametophyte of *Arabidopsis thaliana*

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ARGONAUTE (AGO) proteins play a central role in the regulation of gene expression networks, orchestrating the establishment and the maintenance of cell identity throughout the entire life cycle of a eukaryote. First, they act as mediators of gene silencing by binding small noncoding RNAs, directing them to complementary messenger RNA (mRNA) targets to cause translational repression or exonucleolytic mRNA decay of these transcripts. In addition to their role in post-transcriptional regulation of gene expression, AGO effector complexes in plants are also involved in transcriptional gene silencing through epigenetic modification of chromatin via RNA-dependent DNA methylation (RdDM).
The few-celled male and female gametophytes of flowering plants have become versatile model systems to study the molecular mechanisms involved in pattern formation and gamete specification, since all haploid cells of the gametophytic generation arise from a single diploid progenitor cell, the so-called microsporocyte and megaspore mother cell (MMC), respectively. Both spore formation (sporogenesis) and gametophyte development (gametogenesis) involve critical processes of cell specification. We aim to understand the role of AGOs in the establishment of the different cell identities in *Arabidopsis thaliana* male and female gametophytes. To identify those *AGOs* expressed during gametophyte development we analyzed RNAseq data and created a set of promoter-reporter and promoter-reporter-gene constructs for investigating gametophytes of different flower developmental stages by confocal laser-scanning microscopy. In addition, we generated single and higher-order *ago* mutants using CRISPR-Cas9 and examined them with different microscopy techniques for possible morphological differences in the gametophyte and potential effects on reproduction. To identify potential AGO interactors and to address the question of the composition of AGO RNP complexes in the egg cell, we performed yeast two-hybrid and affinity purification experiments in conjunction with mass spectrometry analyses, the results of which we will present.

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P55 An evolutionary approach to optimising synthetic apomixis in cereal crops

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Seed-mediated apomixis evolved as an alternative to the reproductive pathway whereby unreduced cells within the ovule acquire a reproductive fate. A recent breakthrough study showed that male-derived expression of the transcription factor *BBM1* in rice, which triggers the embryonic programme upon fertilisation, can be used to deliver parthenogenesis when ectopically expressed in the oocyte. Feasibility of apomixis in rice was shown by combining the *BBM1* expression in the egg with a mitosis/meiosis substitution construction known as MiMe. However, as MiMe disables both male and female meiosis, it affects both gametophytes, creating polyploids and this 'disarming meiosis' approach also leads to inevitable change to the expected 2:3 zygote:endosperm genome ratio. Thus, whilst we aim to introduce the proof-of-concept rice system into barley, we also seek to contend with its shortcomings: high frequencies of polyploids and sexual offspring. Our goal is to address these weaknesses through targeted dissection of the natural apomictic system in *Eragrostis curvula*. Apomixis in *E. curvula* starts with the formation of the embryo sac from the MMC itself, avoiding meiosis and following directly into two rounds of mitosis, generating ultimately two synergid cells (2n), the egg cell (2n) and the polar nucleus (2n). Only the polar nucleus is fertilised creating the endosperm (3n) which results in the 2:3 embryo-endosperm ratio. Our study aims to gain sufficient molecular understanding of the *E. curvula* system through forward-genetics approaches including single-cell transcriptomics, to replace the meiosis-disabling method with one that circumvents female meiosis in the MMC thereby avoiding problems associated with unreduced male gametes. Reverse genetics is being used to replicate the rice approach and validate *Eragrostis* candidates in barley, whilst further optimisation of the systems is to be achieved through inducing male sterility and using haploid-inducer lines as the male parent.

P56 Exploring how sequence divergence and sex affect meiotic recombination patterning in tomato hybrids.

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Meiosis is a specialized cell division and an essential feature of sexual reproduction. During meiosis, crossovers (CO) between homologous chromosomes, and random segregation, lead to unique genetic combinations in gametes [1]. Meiotic COs are not uniformly distributed and what exactly determines where a CO occurs is still not fully understood. In Arabidopsis, COs mostly occur in gene-rich euchromatin and are associated with AT-rich DNA motifs and lower nucleosome occupancy [1,2,3,4], while heterochromatin rich centromeres and telomeres are depleted of COs. Improving our understanding of CO formation and distribution is crucial for understanding the evolution of sexually reproducing species and relevant to plant breeding as crossovers are an important substrate for developing new varieties [5].

We explore how sequence divergence and sex affect meiotic recombination patterning in tomato intra- and inter-specific hybrids. First, we generated a high-quality reference genome of *S. lycopersicum* cv. Moneyberg-TMV - a recurrent parental line in our study [6]. Next, we selected three further accessions (*S. lycopersicum* cv. Microtom, *S. cheesmaniae* LA1039 and *S. pennellii* LA0716) with increased evolutionary distance and assembled them into chromosome scale genomes using a combination of PacBio HiFi and Omni-C sequencing data. We identified and characterized large and small genomic polymorphisms by comparing these assemblies to our Moneyberg-TMV genome. We generated F1 hybrids between Moneyberg-TMV and the three selected accessions and used these hybrids to generate large male and female backcross populations. Offspring from the six backcross populations has been sequenced to generate sex-specific recombination landscapes for each hybrid. Our findings will provide insight into how sequence divergence and sex affect crossover landscapes and might have direct implications for breeding programs.

[1] Mercier et al, 2015.
[2] Choi et al, 2018.
[3] Blackwell et al, 2020.
[4] Lian et al, 2022.
[5] Wang et al, 2021.
[6] van Rengs et al, 2022.

P57 Severe defect in fertility for tomato class I crossover mutants is suppressed by *recq4* anti-crossover mutant

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Meiotic crossover is a mechanism of exchanging genetic information between homologous chromosome and leads to genetic diversity in gametes and offspring. Two different crossover pathways (class I and class II) are active during meiosis and also non-crossover pathways prevent the formation of meiotic crossover. The class I pathway contributes most meiotic crossover events and requires MLH1 and ZIP4 in diverse species. Here, tomato *mlh1* and *zip4* mutants were generated using CRISPR-Cas9 and showed severe defects in fertility. *Slmlh1* mutants produce an average of 13% viable pollen and occasionally produce seeds by selfing, while the *Slzip4* mutant has only 1.7% of viable pollen and is completely self-sterile. An anti-crossover *Slrecq4* mutant also showed reduced fertility with an average of 33% viable pollen. Despite this, *Slmlh1 Slrecq4* and *Slzip4 Slrecq4* double mutants have increased pollen viability to 32% and 34% respectively when compared to the *Slmlh1* and *Slzip4* single mutants demonstrating that *Slrecq4* can partially suppress the infertility of tomato class I crossover mutants. Further confirmation, using cytology and genomic analysis is underway.

P58 Functional characterization of the *Arabidopsis thaliana* bZIP52 transcription factor

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The basic region leucine zipper transcription factors (bZIP TFs) are evolutionarily conserved TFs in eukaryotes. Members of large bZIPs family in *Arabidopsis thaliana* play roles in many aspects of plant development as well as abiotic stress responses. A few TFs out of the 78 *Arabidopsis thaliana* bZIP TFs, including bZIP52, are expressed also during pollen development. bZIP52 is a close homolog of bZIP18, which was previously shown to be involved in pollen development. Moreover, bZIP52 is able to heterodimerize with bZIP18, along with other pollen-expressed bZIP TFs. Here we present the expression and localization patterns of bZIP52 in both gametophytic and sporophytic tissues. Finally, we show its involvement in the heat stress response.

P59 Rare events in apomicts – what does flow cytometry hide and SSR-seq reveals

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Apomixis is asexual reproduction through seeds, copying maternal genotype into the offspring. In most apomicts, fertilization is necessary for endosperm development (pseudogamy), which serves as a nutritive tissue for the embryo. Thus the pollen donor can affect the quality and vigor of seeds regardless of the mode of reproduction. Unfortunately, it is practically impossible to identify pollen donors in apomictically originated seeds and consequently estimate the paternal effect on apomicts' reproductive success.

Here, up-to-date methods in a unique combination are applied to every seed: (i) crossing between known genotypes, (ii) Flow Cytometric Seed Screen (FCSS) – allowing rapid detection of reproductive mode, and (iii) SSR-sequencing without extensive DNA extraction – allowing genotyping of seeds as small as 1 mm and 100 µg. The approach circumvents possible errors of each method, enables detection of rare events, such as multiple sperms, or determines the genotype of pollen involved in the endosperm development.

The method was applied in three different apomictic genera (Rubus, Potentilla, and Taraxacum). In apomictic Taraxacum with autonomous endosperm development, the offspring was always a copy of the maternal individual lacking any non-maternal alleles. In Rubus and Potentilla, missing or extra alleles occurred in several presumed apomictic seeds. Based on the results, we claim that (i) FCSS does not always reflect the mode of reproduction, (ii) a variable number of cells gives origin to endosperm, and (iii) multiple pollen tubes can reach an ovule and enable genetically different sperms to fertilize the egg cell and the central cell.

P60 A high-efficient *Agrobacterium*-mediated genetic transformation method for maize – a power booster to study crop meiosis

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Genetic transformation of plants is a powerful tool for the functional analysis of plant genes and the understanding of the underlying biological processes. In case of maize, the typically applied procedure severely limits functional studies since it requires elaborated and work-intensive handling that yet results in usually low transformation efficiency. To overcome these limitations, we have developed an improved *Agrobacterium*-mediated transformation protocol optimized for two maize inbred lines, A188 and B104. With our protocol, we have boosted transformation efficiencies compared to previously applied methods: For A188, from 18 % to 177 % (10 experiments, median: 68 %, average: 77 %); for B104, from 20 % to 192 % (8 experiments, median: 121 %, average 102 %), as calculated by the number of regenerated shoots per immature embryos inoculated. As a showcase for our high-efficient protocol, we are developing several genomic reporters and mutant lines via CRISPR/Cas9 system to study the regulation of meiotic events, such as chromosome dynamics and recombination. To complement this approach, we have also successfully established a promoter sequence which provides specific and strong expression only in maize meiocytes. Here, we present our detailed transformation protocol and an overview over the generated tools. We hope that our approach will be helpful for the meiosis and maize community to promote the understanding of molecular and cellular processes.

P61 3D quantitative analysis of ovule primordium architecture and female germ cells precursors formation in maize

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In higher plants, the formation of female gametes is a crucial step in the plant reproductive cycle and determines seed formation, hence participating in crop yields. The plant female germline initiates in the ovule primordium, with the specification of the MMC, the Megaspore Mother Cell, the only cell which will undergo meiosis to produce gametes. However, reproductive cell fate in the early ovule appears flexible. Genetic variants and apomictic species show that somatic cells neighboring the MMC can enter the MMC identity program or even directly produce female gametophytes without meiosis. Moreover, this developmental plasticity is at least partially controlled by ovule tissue growth, as shown recently in *Arabidopsis*. However, we don't know if such model is conserved in grasses ovules, and could be part of the mechanisms explaining the shift from sexual to apomictic reproductive mode in these species. To set up a framework to study ovule morphogenesis and MMC formation in grasses, in 3D and at cellular level, we use maize as a sexual plant model and multiphoton microscopy to monitor step by step ovule primordium development. Such 3D quantitative atlas allows to correlate organ level morphogenetic changes with a first precise cellular description of gradual MMC formation, and will be further used as a reference to explore reproductive fate plasticity in aposporous ovules.

P62 Live cell imaging of meiosis in maize

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Meiosis is a specialized cell division characteristic of eukaryotes and essential for sexual reproduction. During meiosis, one round of DNA replication is followed by two chromosome segregation events which eventually lead to the formation of four daughter cells with half the genetic material of the parental cells and with a new assortment of genetic alleles. Hence, meiosis is key to biological diversity and lies at the heart of plant breeding since new, possibly advantageous, allele combinations can be generated. However, despite of its importance, many crucial steps in meiosis are not well understood. So far, most studies of meiosis in plants have relied on classical genetic analyses and on cytological observations of fixed chromosome spreads, which, despite being informative, can capture the underlying cellular dynamics only to a small degree. Here, we present our set-up of a live cell imaging system for maize meiosis that is based on a previously established protocol in our team to follow meiosis in Arabidopsis. The method relies on the observation of stable transgenic lines producing fluorescently labelled proteins that highlight hallmarks of meiosis and can be used as live-cell imaging markers. We have established a series of reporter lines, e.g., to visualize chromosomes (DSY2) and centromeres (CENH3), to monitor synapsis (ZYP1), and to study microtubule arrays (TUB2). Spikelets from plants carrying the construct of interest are sampled, cultured on medium and imaged over time by confocal laser scanning microscopy. Our first live-cell imaging data utilise a combination of the reporters for DSY2 and TUB2 and set the basis for the establishment of a meiotic landmark system to precisely analyze meiotic progression, temporally dissect meiosis and define a cytological framework of meiosis in a crop model system, such as maize.

P63 A reproductive calendar of apomictic and sexual genotypes of *Eragrostis curvula* (Schrad.) Ness

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Weeping lovegrass (*Eragrostis curvula*) is a perennial forage grass native to southern Africa naturalized in semiarid regions of Argentina that reproduces mainly by apomixis, showing full, facultative and sexual genotypes. The type of apomixis present in *E. curvula* is diplospory, where the megaspore mother cell (MMC) undergoes mitosis instead of meiosis and there is no fertilization of the egg cell, only the polar nucleus is fertilized to form the endosperm. To study the genetic basis of the different components of apomixis in this grass, it is important to be able to recognize and isolate pistils at different developmental stages in order to identify differentially expressed genes. The aim of this work was to develop a detailed and precise reproductive calendar for its use in transcriptomic analysis. The methyl salicylate clarification method was used in order to avoid the long process of paraffin embedding protocol, and the male and female developmental stages in anthers and pistils were confirmed through Differential Interference Contrast microscopy. Five different growth parameters from pistils and anthers from different genotypes were registered during the mega and micro-sporogenesis and gametogenesis over more than 500 flowers. Pistil length was found easy to measure and effective to identify different developmental stages. This parameter was more reliable than anther length to infer the correct female developmental stage, providing an effective method to collect pistils at the desired stage.

P64 Microsporogenesis and anther development in *Helianthus maximilianii* Schrad. (Asteraceae)

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The wild perennial sunflower *Helianthus maximilianii* Schard. belong to Divaricati sections of the genus Helianthus. The material was collected at Kuban experimental station of N.I. Vavilov Research Institute of Plant industry. Whole flower heads on the different developmental stages were picked and fixed in FAA. The material was treated according to the classical method of permanent preparations for light microscopy.

The main indicators of the microsporogenesis and anther development processes in *H. maximilianii* are similar to the species *H. tuberosus* and *H. ciliaris*. Significant deviations between these species were not detected. The differences that were found are associated with the time of formation or reorganization of reproductive structures.

Anthers are tetrasporangiate, locules are combined in pairs in two tecaes. The anther shape and arrangement of microsporangia are similar in all three species. Anthers have long outgrowths of sterile tissue in the upper part, participating in the opening of anthers and the spread of mature pollen.

The archesporium initiation starts first on the abaxial anther side, when pistil is already formed.

The anther wall is formed according to the Dicotyledonous-type. The formed wall of the microsporangium includes four layers - the epidermis, endothecium, middle layer and tapetum. The middle layer is ephemeral. Tapetum is cellular with reorganization, which take place in moment of first meiotic division in microsporocytes. The walls of tapetal cells disintegrate, and 2-4-nucleate protoplasts migrate gradually inside the locule.

Microsporogenesis is of simultaneous type. The process of cytokinesis is accompanied by callose walls formation between the microspores within the tetrad.

The wall of a mature anther consists of epidermis and endothecia with fibrous thickenings.

Mature pollen grains are 3-cell with the characteristic sculpture of exine which is typical for sunflowers. Two small round sperms form in a result of generative cell division. The pollen grains differing by size within the same locule.

P66 Understanding pollen abortion in female kiwifruit

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Kiwifruit (the genus Actinidia) is unusual amongst crop plants in that it is dioecious, meaning there are separate male and female plants. The dioecious nature of kiwifruit reduces breeding efficiency and impacts upon commercial production as growers must dedicate orchard space to non-fruiting males.

Sexuality in kiwifruit is controlled by two genes in an approximately 0.5 MB male-specific *sex-determining region* (*SDR*). One of these genes, *Friendly Boy* (FrBy), has recently been found to be crucial for male fertility, and its absence in female kiwifruit alters tapetal development resulting in sterile pollen. While FrBy has now been identified, its biological role and why its absence leads to pollen abortion is not yet understood. In addition, there are a number of other genes on the SDR, some of which are likely to be required for pollen fitness.

We are performing low-input RNAseq on single male and female anthers and their isolated meiocytes/microspores at key developmental stages. We will identify genes with different expression in males and females and focus on those found in the *SDR*, to identify candidate pathways influenced by FrBy and genes required for male fitness.

Results from this project will help guide the development of hermaphroditic kiwifruit capable of rapid breeding through selfing. Further, understanding the gene expression dynamics of young sex chromosomes such as the kiwifruit *SDR* may show how plants evolve two distinct sexes from a hermaphroditic ancestor.

P67 *PECTIN METHYLESTERASE* genes control cell wall composition in the germline niche of cereal ovules and affect downstream grain development

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In seed plants, the ovule is a multifunctional organ that supports development of the embryo sac and its constituent germ cells, in addition to regulating maternal resource flow into the developing seed. These functions are dependent upon development of three main tissues; the nucellus, integuments and funiculus. In ovules of *Arabidopsis*, the nucellus is small and contains few cells, while in cereal crops such as barley (*Hordeum vulgare*), the embryo sac is embedded in a large multicellular nucellus. The role of a large nucellus is not fully understood, but must somehow balance germline differentiation with nutrient accumulation and subsequent programed cell death to facilitate grain development. We have been studying a unique feature of the nucellus in barley, whereby cells adjacent to the germline exhibit a specific cell wall profile. Differences are first observed as the germline precursor expands, when adjacent nucellar cells start to accumulate unesterified pectin, and eventually form a defined niche surrounding the embryo sac. Correspondingly, division of nucellar cells within this niche is less active compared to nucellar cells with a low level of unesterified pectin, possibly due to limitations imposed by cell wall rigidity. To investigate the underlying molecular mechanisms, a tissue-specific transcriptome was generated from different barley ovule tissues. This revealed a number of candidates potentially involved in nucellus differentiation including several pectin methylesterases (PMEs) and transcription factors (TFs). Promoter analysis suggested that putative *cis*-elements in promoters of *PME* genes expressed in the nucellus may represent potential ovule TF-binding sites. Dual-Luciferase assays showed that several classes of TF proteins are possible regulators of *PME* genes in the nucellus. To functionally verify the roles of candidate genes, CRISPR-Cas9 genomic editing has been applied to create mutants. Our research will provide novel insights for ovule and grain development in cereals.

P68 Diploid to haploid transition in *Arabidopsis* is driven by P-body mediated inhibition of translation

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Meiosis marks the transition between diploid and haploid life cycle phases. It is accompanied by extensive reprograming of the cell division machinery from mitosis to meiosis and, upon formation of haploid spores, back to mitosis. We show that this transition in *Arabidopsis* is driven by inhibition of translation, achieved via a novel mechanism involving P-bodies. During the second meiotic division, the germline-specific protein TDM1 is incorporated into P-bodies through interaction with the nonsense mediated RNA factor SMG7. TDM1 attracts eIF4F, the main translation initiation complex, via a specific interaction between its scaffold protein eIFiso4G2 and TDM1, thereby temporarily sequestering eIF4F into P-bodies and inhibiting translation. Accordingly, the failure of *tdm1* mutants to terminate meiosis can be overcome by chemical inhibition of translation. We propose a model in which TDM1-containing P-bodies act as a catalyst to remodel the translatome at the end of meiosis to facilitate cell fate transition to postmeiotic germline differentiation.

P69 Channelling identity: modulation of female germline development in *Arabidopsis* by cell-type specific polysaccharide deposits

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Intercellular communication is a fundamental component of multicellularity that allows cells to interpret positional cues and adopt distinct fates. During plant reproduction, female germline formation and development is thought to be dependent on dynamic communication with surrounding sporophytic ovule cells. However, in *Arabidopsis*, once the germline precursor (MMC) differentiates, β-1,3-glucan is deposited in its wall, possibly establishing a communication block with adjoining cells. To test the possible molecular basis for β-1,3-glucan deposition and germline isolation, fluorescent-based cell sorting was used to isolate and transcriptionally profile germline and somatic cell-types from the *Arabidopsis* ovule. This revealed differential expression of genes involved in intercellular signalling through plasmodesmata. Dominant cell-type specific expression of a plasmodesmata-localised β-1,3-glucanase enabled selective re-establishment of intercellular movement between germline and somatic cells. This led to deregulated expression of key ovule regulatory genes, and termination of downstream post-meiotic MMC development, in a sporophyte-dependent manner. These findings demonstrate a functional role for β-1,3-glucan during early female germline development and highlight the critical importance of temporal germline isolation for downstream stages of female gametogenesis.

P70 From sexuality to apomixis: Insight into dynamics of double fertilization and embryogenesis in *Zanthoxylum armatum* DC.

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Apomixis, the ability to produce asexual seeds, constitutes, along with outcrossing and selfing, one of the three major breeding systems in angiosperms. It is however, by far the least common of these three, inspite of its immense theoretical advantages. Reported in more than 300 species of about 40 families of flowering plants, the phenomenon is prevalent in members of Poaceae, Rosaceae, Asteraceae and Rutaceae. *Zanthoxylum armatum*, a dioecious member belonging to family Rutaceae has been found by our group to exhibit facultative apomixis. Commonly known as Indian Prickly Ash, the plant is of immense importance in medicine, household, commerce and ethnobotany. Due to increased market demands, dubious methods of collection and insufficient skills of harvest, there is a sharp decline of the species in the wild, adversely affecting its regeneration. Our studies on two subtropical populations of Jammu & Kashmir, India addressed following questions i)type of pollination prevalent in the species ii)dominant mode of its fruit set and iii)type of apomixis. Different pollen vability tests revealed *Z. armatum* pollen to be adequately viable with 94.82% pollen viability. Fruit set on open pollination, manual pollination and bagging of female inflorescences in both the populations is found to be averaging 69.39%, 52.44% and 8.05% respectively. In wake of these pollination experiment results, autonomous apomixis appeared to be the adjunct reproductive mechanism. Notwithstanding the adequate fruit set on open and manual pollinations, interestingly only 4% of the total open pollinated female flowers scanned showed meagre pollen load(1-6) and pollen germination(1-2) on the stigmatic surface. Fluorescence microscopic analysis of manually pollinated pistils showed pollen tube growth in the style 1 day after pollination advancing further towards the ovary. Microtome sectioning of open, manually pollinated and bagged pistils revealed different developmental embryological pathways in the species. Presentation will elaborate on these differences.

P71 Chromosome choreography in different populations of *Eremurus persicus* (Jaub. & Spach) Boiss.- a synaptic mutant of NW Himalayas

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Majority of higher plants reproduce sexually through the union of male and female gametes ensuring restoration of parental chromosome number along with sufficient variability in the offspring for adaptation and evolution. The core of meiotic process is a specialized nuclear division (Meiosis-I) in which homologs pair with each other (synapsis), recombine and then segregate from each other. Mutation in genes controlling synapsis affect normal pairing of homologues during prophase-I and give rise to synaptic mutants. These synaptic mutants show complete (asynapsis) / partial (desynapsis) lack of chromosome paring during meiosis. Failure to properly accomplish this elegant chromosome dance results in aneuploidy along with the increase in frequency of abnormalities and errors. As a mutation, this failure is reported in several members of Poaceae followed by leguminosae and liliaceae. Here we report this as a genetic feature of all the populations of *Eremurus persicus* (Jaub. & Spach) Boiss. (Asphodelaceae) a little known species of genus Eremurus. In India it is endemic to Trikuta hills in Reasi district of UT Jammu & Kashmir. The species is economically important and is used in folk medicine with a wide array of uses. We examined the male meiosis and reproductive output in 07 populations of this species. All the populations exist with diploid count of 2n=14. Meiotic course in majority of the pmcs in all the populations is not normal due to desynapsis. Instead of 7IIs most of the pmcs examined reveal varying number of loosely paired bivalents and enhanced frequency of univalents at metaphase-I. Unequal anaphasic segregation along with the formation of chromosomal bridges and laggards were also recorded. Various pollination treatments revealed high rate of fruit and seed abortion in all the populations. The correlation of the desynaptic behavior to this adverse effect is being addressed.

P72 Structural maintenance of chromosomes SMC5/6 complex is necessary for meiotic chromosome reduction in *Arabidopsis*

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Meiosis is a critical stage of plant sexual reproduction. Its key events include controlled induction of DNA double-strand breaks, exchange of segments between homologous chromosomes, and production of haploid spores. We will show that *Arabidopsis thaliana* Structural maintenance of chromosomes 5/6 (SMC5/6) complex is essential for normal progression of meiosis and healthy offspring. Initially, we found that the *SMC5/6* complex mutants produce about 15-20% paternally-induced triploid plants. The analysis of whole male reproductive development revealed a frequent migration of all chromosomes to one nuclear pole during meiosis, resulting in about 30% unreduced microspores. Fertilization with diploid pollen resulted in seeds containing a triploid embryo and tetraploid endosperm, representing in excess of the paternal genome, problems with endosperm cellularization, and frequent seed abortion. However, some of the aberrant seeds survived and gave rise to the aforementioned triploid offspring. In conclusion, we show a novel role of the SMC5/6 complex in the maintenance of gametophytic ploidy in *Arabidopsis*.

P73 Single-nucleic acid difference controls the pollen production in Japanese cedar, the largest source for severe pollinosis in Japan

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Pollinosis (pollen allergy) is a global problem caused by pollen from various plant species. Japanese cedar (*Cryptomeria japonica*) is a gymnosperm and has a large genome size (~10.8 Gb). Japanese cedar is the most important timber in Japan but also the largest source of severe pollinosis. Homozygous mutants (*ms4/ms4*) of *MS4* (*MALE STERILITY 4*) show abnormal pollen development after the tetrad stage and produce no mature pollen. In this study, we narrowed down the *MS4* locus by fine-mapping in Japanese cedar and found the gene *TKPR1* (*TETRAKETIDE α-PYRONE REDUCTASE 1*). *TKPR1* is known as an essential gene for the construction of the pollen wall in Arabidopsis thaliana and rice. Pollen wall phenotype and timing of the defect in the *ms4* mutant are similar with *TKPR1* mutants of *A.thaliana* and rice, which suggests that the causal gene of *ms4* is *CjTKPR1*. Sequence comparison of *CjTKPR1* revealed four nucleic acid differences, and interestingly only one SNP was non-synonymous (T to C at 244-nt position induces C82R) between wild-type and *ms4* mutant. Then, we conducted transformation experiments using *TKPR1* mutant of *A.thaliana* (*Attkpr1-1*/ *Attkpr1-1*). Pollen production was rescued by insertion of the *CjTKPR1* wild-type sequence but not by the *ms4*-type, indicating that the *CjTKPR1* sequence determines pollen production. Furthermore, the *CjTKPR1* wild-type sequence with a point mutation at 244-nt (T to C) could not rescue pollen production, but the *CjTKPR1 ms4*-type sequence with a point mutation at 244-nt (C to T) could recover pollen production. Taken together, we concluded that a single nucleotide substitution of *CjTKPR1* determines pollen production. Broad conservation of *TKPR1* beyond plant division could lead to the creation of pollen-free plants not only for Japanese cedar but also for a wide range of plant species.

P74 Identification and analysis of candidate genes regulating pollen number in *A. thaliana*

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Pollen number is an important factor for reproductive success. Compared with extensive studies on the cellular differentiation in pollen development, little is known about the molecular basis of this quantitative trait. Recently, we reported the first gene controlling male gamete number, *REDUCED POLLEN NUMBER1* (*RDP1*), from the predominantly selfing species *Arabidopsis thaliana*, which encodes a homolog of yeast Mrt4, the assembly factor of the ribosomal large subunit. In order to understand the underlying gene network, we aimed to identify genes that function together with RDP1. Here we present candidate genes suggested by preliminary results. We performed a differential gene expression analysis of tissue-specific RNAseq data with isolated microspore mother cells of *rdp1-3* and the wildtype using laser-assisted microdissection. We selected nine candidate genes and created mutants of these genes using the CRISPR/Cas9 system. In the mutants of four candidate genes, significantly different pollen numbers compared to the wildtype were found (approx. 10% difference). Two of them (encoding a transcription factor, and transmembrane protein) are putative positive regulators of pollen number while the other two (encoding a small peptide, and putative intramembrane protease) are putative negative regulators. With RT-qPCR we further confirmed that these four genes lay downstream of RDP1 and found no feedback regulation among the genes including *RDP1*. Next, we will assess the pollen number of complementation lines and backcrosses of these mutants to rule out the possible off-target effects from the CRISPR/Cas9 system. Our results will not only provide a more complete picture of pollen developmental processes but also help explaining the variation of male gamete number in different species in combination with further analyses.

P75 Hormonome dynamics during microgametogenesis in bicellular and tricellular pollen

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Plant microgametogenesis involves stages leading to the progressive development of unicellular microspores into mature pollen. Despite active and continuing interest in studying the development of male reproductive organs, little is still known about the hormonome at each ontogenetic stage.

In this work, we present the role of phytohormones during the process of microgametogenesis in species with bicellular pollen (*Nicotiana tabacum*, *N. alata*, *N. langsdorfii*, and *N. mutabilis*) compared to tricellular species (*Zea mays* and *Arabidopsis thaliana*). Advanced HPLC-ESI-MS/MS and transcriptomic techniques were used to identify endogenous hormone derivatives and genes throughout pollen ontogenesis. Their spectra changed dynamically during pollen ontogeny, indicating their important role in pollen growth and development. We found different dynamics in the accumulation of endogenous phytohormones during pollen development in bicellular and tricellular lineages and between the tetraploid *Nicotiana tabacum* and the other three diploid species, reflecting their evolutionary origin within the genus *Nicotiana*. We show here for the first time that certain forms of phytohormones, such as cis-zeatin- and methylthiol-type cytokinins, some derivatives of abscisic acid, phenylacetic acid, and benzoic acid, are involved in pollen development. Our comparative transcriptome analyses revealed DEGs and metabolic pathways involved in hormone signaling. Interestingly, we found that hormones from the auxin and ethylene signaling pathways were most abundant in the first two early ontogenetic stages of bicellular pollen development. Other signaling pathways studied were more than eight times less abundant.

Our results suggest that unequal amounts of endogenous hormones and the presence of specific derivatives may be characteristic of pollen development in bicellular and tricellular pollen as well as in different evolutionary plant groups. These results represent the most comprehensive study of plant hormones during pollen development currently available.

P76 The importance of cytokinins in the development of male gamethophyte

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Phytohormones cytokinins (CKs) have many unique functions during plant life. In male gametophyte reproductive development, CKs are involved in various processes such as anther cell wall formation, pollen production, stamen development, and anther dehiscence. However, the fundamental questions regarding CK production and the mechanisms that maintain CK homeostasis during pollen ontogeny have not been fully elucidated.

In our study, we examined in detail the endogenous profiles and dynamics of endogenous CKs during microgametogenesis in three model plant species, tobacco, including representatives of two different phylogenetic sections, *Nicotiana* (*N. tabacum*) and *Alatae* (N. langsdorfii, *N. mutabilis* and *N. alata*), *Arabidopsis* and maize. A total of eight to fifteen forms of classical isoprenoid CKs were detected during the pollen development in individual tobacco species, whereas a relatively lower spectrum of CK derivatives was revealed in *Arabidopsis* and maize. Regardless of pollen types, characterized by bicellular pollen in *Arabidopsis* and tobacco and tricellular pollen in maize, we found a dominance of CK phosphates along with CK bioactive and transport forms during pollen maturation in all species. Interestingly, we found CK methylthio derivatives in all analyzed samples, which are exclusively generated by the tRNA degradation pathway. To elucidate the maintenance of CK homeostasis at the molecular level, the RNAseq dataset of *N. tabacum* are searching for key genes of CK metabolism at all stages of pollen development. In addition, the effect of storage temperature on CK dynamics in mature pollen grains will be presented at the conference.

P77 The role of BELL1 and AINTEGUMENTA in megasporogenesis.

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The female germline development starts when within the nucella, the Megaspore Mother Cell (MMC) is specified and meiotically divides giving rise to four spores. Three of them degenerate and the only one surviving differentiates in the Functional Megaspore (FM). Meanwhile, the chalaza starts to develop the inner and outer integuments. Once FM is specified, it undergoes mitotic division distinctive of megagametogenesis process, at the end of which the seven-celled mature female gametophyte is formed.

The transcription factors BELL1 (*BEL1*) and AINTEGUMENTA (*ANT*) have been shown to be involved in the control of integuments development. Indeed, *ant-4* ovules cannot develop neither the outer nor the inner integument, whereas *bel1-1* ovules develop integument-like structures with nucellar identity.

We have investigated in the role of *BEL1* and *ANT* during the megasporogenesis since in both mutants this process is impaired. Indeed, the FM specification is altered and the megagametogenesis never occurred. Our results suggest that alteration in Auxin distribution, might cause defects in sporophytic tissues and leading to the altered Functional Megaspore specification.

P78 Characterization of ALBA family proteins in *Arabidopsis thaliana*

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Acetylation-Lowers Binding Affinity (ALBA) proteins belong to an ancient superfamily of DNA/RNA-binding proteins. In evolution, *ALBA* genes diversified and formed two Rpp20-like and Rpp25-like subfamilies in eukaryotes. Although, *ALBA* homologues were identified across a phylogeny tree, their function remains unclear in most model organisms. In plants, studies of the enriched number of *ALBA* family members revealed similar features to the animal homologues; association with RNA metabolism, mRNA translatability and stress response. We studied *ALBA* dynamics during reproductive development in *Arabidopsis* under standard conditions and following heat stress. Our data show *ALBA* genes involvement in heat stress response at the levels of gene expression and protein localization in generative organs. Exclusively, all ALBA proteins are presented in pollen development with a final localization in mature pollen-specific pattern that vary upon heat stress. Moreover, the stress-induced localization patterns of two Rpp25-like subfamily mostly diversified members ALBA4 and ALBA6 differ in correlation with poly(A)-binding protein 3 (PABP3) in mature pollen. Collectively, the dynamics and variations of the ALBA family reflects not only their redundancy but also their possible functional diversification in plants.

P79 Morphogenetic determinants of MMC formation in *Arabidopsis*

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In angiosperms, female meiosis takes place in a single cell per ovule, the MMC (Megaspore Mother Cell, or female Spore Mother Cell). The MMC is formed in apical sub-epidermal position in the ovule, as an enlarged anisotropic cell. Recent data generated by our groups suggest that MMC identity in *Arabidopsis* is determined by the geometrical and mechanical parameters of the ovule. Moreover, developmental plasticity exists within ovule primordia: several candidate MMCs initiate differentiation, then a gradual fate restriction occurs to form a single MMC where meiosis will occur. The penetrance and timing of this process depends on the genetic background. However, neither the precise developmental trajectory of candidate MMCs nor their coordination with ovule tissue growth are resolved in 3D. To address this, we exploit 4D imaging data (time lapse) focusing on early primordia, and 3D static images reporting on candidate markers of MMC identity. We developed a semi-automatic workflow combining image segmentation, cell annotation and cell tracking during development. We undertake a quantitative analysis of cellular parameters, over time, such as cell division, cell growth, cell shape and cell contacts, to identify features best characterizing and discriminating candidate MMCs from their neighbors, and their gradual fate restriction during primordia growth. Overall, our goal is to improve our understanding of the geometry and organ topology patterns predictive of the plasticity of MMC formation at ovule emergence.

P80 The blossoming relationship between bHLH proteins and light impacts male fertility in *Arabidopsis*

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Development of viable pollen is essential for plant reproduction, which relies upon the secretion of a cocktail of enzymes and molecules into the anther locule from the surrounding tapetum tissue. Tapetum development is regulated by a number of genes including the pivotal basic Helix Loop Helix (*bHLH*) transcription factors DYSFUNCTIONAL TAPETUM (*DYT1*) and ABORTED MICROSPORES (*AMS*), which interact competitively with *bHLH010*, *bHLH089* and *bHLH091* to regulate downstream targets involved in synthesis and secretion of pollen wall components. Disruption of these genes leads to a failure of pollen development and male sterility. Previous research has shown that *ams*, *dyt1* and *bhlh089 bhlh010 amiR-bHLH091* knockout mutants are completely male sterile, whereas the single and double *bhlh010*, *-089* and *-091* mutants are predominantly fertile due to a high level of functional redundancy. However, here we reveal that *bhlh089 bhlh091* and *bhlh089 bhlh010* double mutants exhibit environmentally sensitive sterility. We carried out RNASeq analysis, identifying a number of downstream genes that are differentially regulated in response to low light, and outline the potential mechanisms behind this environmental interaction. Since male sterile lines have long been used to produce hybrid crops that have enhanced vigor or heterosis, and with homologous genes in many crop species, we present *bHLH010*, *bHLH089* and *bHLH091* as novel gene targets for the environment-sensitive genic male sterility (EGMS) hybrid breeding system.

P81 Pattern formation during ovule development required tuned activity of *WUSCHEL-RELATED HOMEOBOX 9/STIMPY* in *Arabidopsis thaliana*

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WUSCHEL-RELATED HOMEOBOX (*WOX*) genes encode for a family of transcription factors, sharing important roles in a wide range of processes during plant development. In *Arabidopsis thaliana WOX9/STIP* gene is necessary for the correct patterning of the embryo and for shoot apical meristem maintenance. We have investigated the role of *WOX9/STIP* in ovule development by the analysis of loss-of-function and gain-of-function mutant alleles. Our results showed that *WOX9/STIP* is required for the correct formation of the outer integument and the anatropy of the ovule. *wox9/stip-2* knockout mutant is characterized by severe defects in outer integument development, hence determining a radialized ovule phenotype. In addition, alteration of *WOX9/STIP* expression in the ovule affects the correct differentiation and progression of the female germline. Finally, we show that the outer integument identity gene *INNER NO OUTER (INO)* is a *WOX9/STIP* target in ovules. Our results unravel an important role of *WOX9/STIP* during ovule development, contributing to dissect the regulatory networks determining ovule development.

P82 Investigating a Role for Autophagy in the *Arabidopsis* Self-incompatibility Pathway

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Many *Brassicaceae* species carry a self-incompatibility (SI) system, and species-specific pollen grains are initially accepted or rejected by the stigma by selectively providing resources to compatible pollen grains. Stigmatic resources are delivered to the site of compatible pollen contact via vesicle trafficking, ultimately leading to pollen hydration, germination and pollen tube growth into the stigma. When SI pollen is recognized, it is rejected before pollen hydration and germination can occur. The key SI regulators are well-known, the polymorphic pollen SP11/SCR ligand and stigma S Receptor Kinase, and some downstream signaling events have been identified. These include a rapid increase in cytosolic calcium levels, absence of a focused actin cytoskeleton, and our previous work on the ARC1 E3 ubiquitin ligase as a downstream signaling protein. In *Arabidopsis* species, we also discovered that autophagy is rapidly activated, potentially targeting the secretory response, but the specific role of autophagy in this pathway is unclear. The focus of this work is to investigate the requirement of autophagy in the SI pathway in two ecotypes of *Arabidopsis thaliana* (*Col-0* and **C24**). Through the introduction of genes for the SI regulators into these two ecotypes, a robust SI response can be reconstituted in these normally self-compatible species. To knockout autophagy in these SI lines, *atg7* mutants were also crossed in, and the impact of the loss of autophagy in the stigma was assessed by pollen hydration and germination assays with SI pollen. The onset of autophagy with SI pollinations was also directly observed in the stigmatic papillae using the GFP:ATG8a marker to fluorescent tag autophagosomes. Overall, we observed some changes to the stigmatic SI response with the loss of autophagy indicating that autophagy is required for the full rejection of SI pollen in *Arabidopsis*.

P84 Technical approaches towards characterisation of plasma membrane pollen-stigma compatibility factors – the utility of lipid nanodiscs for protein-protein interaction studies

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Successful sexual reproduction in plants relies on a complex network of interactions between cells of male and female reproductive tissues during pollination. Arguably, molecular discrimination of pollen and establishment of its compatibility status are two of the most important early molecular interactions that occurs at the pollen-stigma interface. Work in our lab has focused on characterisation of pollen-borne factors that regulate pollen-stigma compatibility. For instance, a group of small cysteine-rich pollen coat proteins in *Arabidopsis thaliana* and *Brassica spp.* termed the PCP-Bs (for Pollen Coat Protein – class B) have been shown to play a role in pollen-stigma compatibility by mediating pollen hydration. Despite much interest in uncovering the molecular basis for pollen-stigma communication over the last few decades, progress has been hampered by the difficulties in studying protein-protein interactions that occur between extracellular ligands and potential plasma membrane-localised targets. For instance, traditional yeast two hybrid approaches do not easily applicable to proteins that interact extracellularly and extraction of membrane proteins for direct characterisation/ interaction studies typically results in loss of protein structure and function. To address this, we have developed protocols that utilise styrene maleic acid (SMA) copolymers to embed total stigmatic plasma membrane proteins in lipid nanodiscs. SMA-lipid particles (SMALPs) contain the membrane proteins in their native lipid environment without incorporating detergents or exogenous proteins which may otherwise interfere with downstream protein-protein interaction studies. By using a plasma membrane marker and total protein analysis, we demonstrate successful incorporation of membrane proteins from both leaves and stigmas of *A. thaliana* into nanodiscs. The present work has utility in research towards the characterisation of plasma membrane pollen-stgima compatibility factors, but also extends to membrane protein interactions in other plant tissues.

P85 Two-photon imaging of species recognition in pollen tube attraction of *Arabidopsis thaliana*

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Sexual reproduction within the same species is essential for species maintenance. In *Arabidopsis* species, pollen tube attraction has been divided into multiple steps. Pollen tubes emerge on the septum from the transmitting tract, grow on the septum surface and funiculus, and enter into the ovule from micropylar opening. Under semi-in vivo pollen tube attraction assay, pollen tubes recognize ovular secreted signaling and change the direction to ovule in a species-preferential manner. Recently LURE cysteine-rich peptides with species-preferential activity and its homologs XIUQIU with more general activity have been reported as attractants derived from ovules. However, it is not so simple and remains unknown how ovular signaling works as the species recognition mechanism in the pistil. Here we established the new method using two-photon microscopy and enabled to quantify the paths of each pollen tube in the pistil. We have been working on the interspecific cross between *A. thaliana* (*At*) and the closely related species *A. lyrata* (*Al*) as a model. We quantified growth paths of *At* and *Al* pollen tubes in *At* pistils and showed that *Al* pollen tubes elongated normally, but the pollen tube emergence on the septum delayed compared with *At* pollen tubes. Besides, we investigated the paths of pollen tubes in the pistil of *myb98* mutant, confirmed the severe defect of micropylar guidance *semi-in vivo*. In *myb98* mutant pistils, pollen tubes also showed defective phenotype in the micropylar guidance and delayed in the emergence on the septum not as severe as *Al* pollen tubes in *At* pistils. Our detailed two-photon imaging suggests that secreted peptides from synergid cells, as well as ovular sporophytic cells, contribute to species recognition system from early steps of pollen tube attraction to ovules.

P86 The roles of Arabinogalactan proteins in pollen-pistil interactions: sweet dreams are made of this

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The reproductive process leading to seed formation in flowering plants is a complex mechanism, involving a series of signaling pathways with several well described players. Arabinogalactan-proteins (AGPs) are an important group of signaling molecules involved in plant reproduction. AGPs are hydroxyproline-rich proteins containing a high sugar content, widely distributed in the plant kingdom. In this work, we inspect the functional importance of the carbohydrate moieties of AGPs, using as a test case three AGPs: AGP25, AGP26, and AGP27, which are essential for *Arabidopsis'* reproduction (unpublished data) and hydroxyproline galactosyltransferases (Hyp- GALTs) enzymes which add first galactose to Hyp residues in the protein backbone. In this work, a set of GALTs designated as GALT2, GALT5 and HPGT1-3 (GALT 7-9) were used, and a quintuple mutant of these enzymes (*galt2galt5galt7galt8galt9*) was analysed. The quintuple mutant, with AGPs under-glycosylated, was studied concerning reproductive processes. The *galt25789* presented a lower seed-set and defects in the female gametophyte with abnormal callose accumulation inside the embryo sac at the micropylar region. Mutant ovules also presented defects at the integuments being highly noticeable at the micropylar region of the ovules. In addition, using immunolabelling analyse indicated a reduction in the amount of glucuronic acid in the mutant's ovary. The present work confirmed the importance of AGPs via its carbohydrate moiety, in ovule development and pollen-pistil specific interactions

P87 Molecular basis of pollen tube reception as a pre-zygotic barrier in inter-lineage crosses in *Brassicaceae*

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Pollen tube reception is a pre-zygotic barrier that prevents successful fertilization in crosses between plants of different lineages or species. For instance, interlineage crosses between *Arabidopsis thaliana* and *Sisymbrium irio* showed that nearly 70% of *A. thaliana* ovules experienced pollen tube reception defects when interacting with *S. irio* pollen tubes. LORELEI (LRE), a putative glycosylphosphatidylinositol-anchored protein, is a critical component of pollen tube reception in *Arabidopsis thaliana* reproduction. In lre, pollen tube reception defects are reminiscent of that seen in interspecific crosses involving *A. thaliana* and *S. irio*. To test if LRE is involved in pollen tube reception barrier in interlineage crosses, we cloned LRE homologs from *A. thaliana* and *A. lyrata*, representative members of Lineage 1 and *S. irio*, a member of the Lineage 2 of *Brassicaceae*. We validated by expressing each of these homologs from the *A. thaliana* LRE promoter and complementing pollen tube reception defects in *A. thaliana lre-7* mutant ovules. To directly test if LRE mediates the pollen tube reception barrier, we examined if pollen tube reception defects were reduced in interlineage crosses between *A. thaliana lre-7* mutant ovules carrying *S. irio* LRE interacted with *S. irio* pollen tubes. We found that there was a noticeable increase (up to 53%) in normal pollen tube reception in *A. thaliana lre-7* mutant ovules carrying *S. irio* LRE interacted with *S. irio* compared to *A. thaliana lre-7* mutant ovules carrying *A. thaliana* or *A. lyrata* LRE interacting with *S. irio* pollen tubes. Future work will focus on testing the hypothesis that the pollen tube reception barrier is overcome in ovules in an interlineage cross provided they contain a cognate LRE ortholog of the incoming pollen tube and identifying the domains in LRE that are critical for incompatible pollen tube reception in interlineage crosses.

P88 Signaling in Pollen Tube tip growth: how to trace and manipulate intracellular dynamics

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Pollen tubes (PT) are among the most rapidly growing cells on our planet. They elongate at an astonishing rate by polarized tip growth.

Their growth direction is finely regulated by the perception of and response to both biochemical and physical stimuli from the surrounding environment. While some of the biochemical factors that contribute to PT growth have been identified, it is yet uncertain how mechanical properties of the cell wall are connected and integrated with the intracellular events that control the extension at the tip.

This project tries to shed light onto the mechanical and biochemical principles underlying PT growth and navigation.

To measure the mechanical properties of the cell wall, we are making use of a system including micro fluidics (Lab-on-a-Chip) devices and a Cellular Force Microscope (CFM), based on micro-electro-mechanical systems (MEMS) sensor.

In addition, we are studying intercellular events and biochemical properties through the engineering of transgenic *A. thaliana* lines expressing light-inducible switches and biosensor proteins specifically in the PTs. More specifically, our purpose is to construct a Photo-Activatable system that will allow the control of Ca^{2+} concentration at the PT tips.

The integration of these two tools, together with the use of live imaging allows to follow responses of PTs to different stimuli. A better comprehension of pollen tube dynamics not only aims at understanding the functioning of a mechanism at the basis of plant productivity, growth and development, but also involves the construction of novel tools: the collaboration with mechanical engineering, micro- and nano robotics will be source of inspiration for the creation of autonomously growing and navigating soft robots with the potential to develop new medicine biotechnologies in the field of drug delivery system, laparoscopic surgery or diagnostics.

P89 Unraveling the role of methyl glucuronic acid of arabinogalactan proteins during Arabidopsis sexual reproduction

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Arabinogalactan proteins (AGPs) are highly glycosylated proteins involved in plant reproduction. Up to 90% of their molecules are constituted by arabinogalactan polysaccharides, composed of arabinose, galactose and minor sugars such as glucuronic acid (GlcA), fucose and rhamnose. Several efforts have been made to characterize the glycosyltransferases involved in AGP glycosylation, but many of them remain to be identified. Recently, it was proposed that AGPs bind and store Ca^{2+} through GlcA residues at the plasma membrane.

As the sugar moiety of AGPs seems crucial for their function, we explored the role of the glucuronosyltransferases (GlcATs). GlcATs appear to have an important mission on ensuring the presence of GlcA on AGPs for posterior binding to Ca^{2+}. Previously, five GlcATs were shown to transfer GlcA specifically onto AGPs and mutant plants showed a reduction in methyl GlcA substitution in AGP-enriched preparations that held less calcium than wild-type plants *in vitro*. Therefore, the aim of this work focused in exploring the importance of several GlcATs in the reproductive success of *Arabidopsis thaliana*.

GlcATs mutants were grown in Ca^{2+}-deficient and non-deficient media and mutants grown in Ca^{2+}-deficient medium had reduced size, smaller siliques, aborted ovules, non-viable pollen and non-fertilized ovules, leading to the hypothesis that GlcATs are involved in two processes signaled by Ca^{2+}: pollen development and pollen tube attraction to the ovule.

Finally, this work highlights the importance of the sugar portion for AGPs' function, providing a deeper understanding of AGPs during reproduction.

P90 JAGGER as a tool to search for possible AGPs interactors

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JAGGER (AGP4) belongs to the Arabinogalactan Protein (AGP) family, a group of heavily glycosylated proteins ubiquitously distributed in the plant kingdom. Several AGPs have been associated with important functions during plant reproduction. We described JAGGER as necessary to avoid the attraction of multiple pollen tubes into the embryo sac of *Arabidopsis thaliana* ovules. In *jagger*, an *AGP4* knock-out mutant, the pistils show impaired polytubey blockage as a consequence of survival of the persistent synergid.

To further investigate JAGGER mode of action, an RNA-seq was performed using *jagger* and wild-type flowers. AGPs structure makes them suitable candidates to act as signalling molecules in reproduction, especially their richness in sugars and their anchorage to the plasma membrane by GPI (glycosylphosphatidylinositol) anchors. In an attempt to identify possible AGPs interactors, we scrutinized the *jagger* RNA-seq data. Transcriptomic analysis revealed that members of cysteine-rich receptor-like kinase (CRK) genes were highly enriched among downregulated genes in *jagger*. Several receptor-like kinases were selected for further analyses. An increasing number of RLKs have been shown to regulate signaling in cell-to-cell communications during the reproductive processes in flowering plants. So, we hypothesize that RLKs may be involved in the AGPs signaling pathways. One of the RLKs selected was CRK4 (Cysteine-Rich Receptor-Like Protein Kinase 4).

CRK4 was shown to function in defence responses and programmed cell death in previous studies, but there were no studies regarding its involvement in reproduction. Our studies on knock-out *crk4* plants revealed a strong phenotype related to flower development: production of flowers without petals, abnormal flowers, small siliques and consequently a reduced seed set. In general, these plants show a phenotype resembling the ABC model mutants, specifically Class A genes. Further analyses are under way.

P91 Uncovering *Arabidopsis* TCTP1 role in pollen tube growth

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Translationally controlled tumor protein (TCTP) is a small multifunctional eukaryotic protein involved in cell growth, cell cycle and cell death regulation. *Arabidopsis thaliana* genome carries two TCTP paralogs. *AtTCTP1* expression is highly upregulated in pollen. Prior characterization of *tctp1* loss-of-function mutant produced conflicting results for its involvement in reproductive development. We pursued *tctp1* characterization with focus on events prior to fertilization.

Structural studies revealed that human TCTP dimerizes via a disulfide bridge between terminal cysteine residues (tC-s=s-C). The dimerization is essential for TCTP-mediated immune response. Multiple protein sequence alignment of TCTP from various eukaryotic organisms uncovered that tC-s=s-C disulfide bonding is conserved in animal and green plant lineages but not in yeast. We employed *AtTCTP1* expression in heterologous *E. coli* system to verify its dimerization. Our results point to TCTP1 role in pollen tube growth and targeting. We further confirmed *At*TCTP1 dimerization mechanism and plan to test its role in plant development and physiology.

P92 A Hot Topic: Transcriptomics of tomato thermotolerant reproduction

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With rising global temperatures comes the challenge of decreased food availability from unsuccessful plant reproduction. This is an exceptional challenge, as human staple foods like rice, corn, apples, or legumes are products of plant reproduction, and their yields are negatively impacted by heat stress. Elevated temperatures coincide with the reproductive phases of crops, and when exposed to acute heat stress, even just for a few hours, successful plant reproduction is dramatically affected.

Mitigating this problem requires understanding the molecular basis of plant reproductive failure due to heat stress. Tomato (*Solanum lycopersicum*) is used to study this phenomenon, as most cultivated varieties are thermosensitive (TS), but naturally occurring thermotolerant (TT) varieties exist and are well defined. After immediately exposing pollinated pistils of TS and TT varieties to acute heat stress (37C for 12 hours), the plants were evaluated for indicators of successful pollination. Pollinated pistils exposed to acute heat stress for 12 hours result in dramatically reduced fruit production in cultivated TS varieties but not in TT varieties. TT tomato varieties show enhanced pollen tube (PT) growth during heat stress because they mount an altered genetic response that confers a reproductive advantage over TS tomato varieties.

The basis of thermotolerance is investigated from the female pistil perspective, a widely understudied and uncharacterized tissue in tomato thermotolerance. This study identifies differentially expressed genes (DEGs) of unpollinated pistils exposed to heat stress. This study will determine if unpollinated TT pistils have TT properties, prior to the introduction of pollen. Using unpollinated pistils as a control, comparative transcriptomics of unpollinated pistils and self-pollinated pistils can be used to identify DEGs of in vivo PTs and/or pistils in response to heat stress. The identification of the pistil- and pollen-expressed genes may lead to understanding gene functions that confer reproductive thermotolerance of *S. lycopersicum*.

P93 Position analysis of the *cis*-regulatory elements in genes expressed during male gametogenesis

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Male gametophyte (pollen) development in angiosperms is a complex process that requires coordinated transcriptional activity of many different genes. With regard to the precisely orchestrated transcription, a necessity of various specific *cis*-regulatory elements in genes involved in pollen development is obviously ineluctable. In promoters of pollen-expressed genes, several regulatory motifs and boxes were identified: e.g., LAT56 motif, regulatory boxes 52/56 and GTGA motif. We utilised comprehensive transcriptomic studies of *A. thaliana* pollen development covering several developmental stages ranging from uninucleate microspores to 24-hr *in vitro* cultivated pollen tubes (UNM, BCP, TCP, MPG, SPC and SIV) and compared them with sporophyte transcriptome. Our bioinformatics pipeline allows us to precisely localize these pollen specific motifs in vicinity of transcriptions start site (TSS) in genes with high transcription in individual pollen developmental stages. Interestingly, we observed that some of pollen-specific cis-regulatory elements are localised not only upstream the TSS, but also in 5'-UTR with non-random distribution. The abundance of certain motifs significantly differs in between early pollen stages (UNM, BCP) and late pollen stages (TCP, MPG). Moreover, we found out that the presence of telo-box regulatory sequence in promoters of pollen-expressed proteins positively correlates with their strong early pollen expression. We closely characterised the position of telo-box motif to TSS, ATG translation start codon and other regulatory motifs in different pollen developmental stages. It is obvious that not only distribution, but also specific permutation of these motifs is needed for gene regulation in process of sexual reproduction.

P94 Transcriptome analysis of MADS mutants reveals regulatory mechanism for pollen germination in rice

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The MADS (MCM1-AGAMOUS-DEFFICIENS-SRF)-box protein is a transcriptional factor with a preserved domain called MADS-box that regulates downstream gene expression as a transcriptional factor. It regulates the expression of downstream genes by directly binding to the CArG motif of those promoters. The three *MADS* genes in rice, *OsMADS62*, *OsMADS63*, and *OsMADS68*, exhibit preferential expression in mature rice pollen grains. To better understand the transcriptional regulation of pollen germination and tube growth in rice, we generated the loss-of-function homozygous mutant of these three *MADS* genes using the CRISPR-Cas9 system in wild-type backgrounds. The triple knockout (KO) mutant showed a complete sterile phenotype without pollen germination. The triple mutant pollen grains were not stained with the potassium iodide solution, ruthenium red, and Calcofluor white. But their size and shape seem normal. Next, to identify downstream genes that are regulated by the three *MADS* genes during pollen development, we proceeded with RNA-seq analysis by sampling the mature anther of the wild-type and triple mutants. DEGs were selected based on their expression in the anthers and pollen grains, 274 upregulated and 658 downregulated genes were identified. In downregulated genes, there are 25 signaling factors, 23 Transcriptional factors, and 12 calcium signaling-related genes. Also, there are 45 genes that are highly related to cell wall modification and organizations. Furthermore, in downregulated genes, 156 genes have CArG motifs in the 2kb upstream promoters.

P95 Hybridization barriers: functional analysis of novel CRPs in *Zea mays* fertilization

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Small polymorphic proteins, being secreted from pollen/pollen tube (PT), embryo sac or sporophytic maternal tissues, often facilitate species-specific interactions to overcome pre-zygotic hybridization barriers in plants. The most well-known group of small polymorphic proteins involved in the fertilization process are small cysteine-rich peptides (CRPs). We are investigating novel CRPs that are highly expressed in the gametophytic tissue of *Zea mays* to elucidate their roles in various fertilization processes. ZmTOI1-3, which contain four cysteine residues and are enriched in pollinated silk, belong to a family of 17 ZmTOI members. *ZmLOI1-2*, belonging to a family of 21 *ZmLOI* members, are strongly expressed in silk/hair cells and contain eight conserved cysteine residues. Furthermore, *ZmPOI1* and *ZmPOI2* are highly expressed in PTs and belong to a family of 26 ZmPOI members with six conserved cysteine residues throughout the whole family. Phylogenetic analysis showed that the ZmPOI protein family can be classified into three clades, while ZmPOI1 and ZmPOI2 belong to the same clade. A further candidate CRP (ZmCRP) with four cysteine residues is a single copy gene in maize and does not occur in genomes of other plant species. To investigate the function of these candidates in *Zea mays*, *in-vitro* PT germination experiments were performed using pollen from RNAi- as well as CRISPR/Cas9-edited plants. PTs of mutants were less stable compared with those from wild-type plants. Subcellular localization analysis showed that ZmPOI1 and ZmPOI2 were localized in the cytosol and cell wall, while ZmCRP can be found in different populations of endosomes or vesicles. In conclusion, ZmPOI1-2 and ZmCRP are localized in different compartments along the secretion pathway. We will further investigate the function of these candidates and identify possible interaction partners to gain knowledge about the underlying molecular processes regulated by these candidates.

P96 Transcriptome analysis reveals Rac6-mediated signaling mechanism involved in the pollen germination of rice

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For successful reproduction in angiosperms, pollen germination and pollen tube growth should proceed normally. This process is regulated by the transcriptional regulation and cellular activities such as exocytosis, endocytosis, and actin dynamics, where various signaling proteins are key of the signaling pathways. The Rho family of GTPases functions as well-known signaling protein for multiple signal transduction pathways in eukaryotic systems. However, the molecular mechanisms of Rho family of GTPases in pollen germination remain highly unknown. Here, we identified *Rac6* gene that is the only gene expressed during the pollen development stage among the Rho family of GTPases in Rice. Homozygous mutant, *rac6*, created by CRISPR-Cas9 exhibited male sterility with the defects in pollen germination. As a result of the transcriptional analysis of *rac6* vs wild type anthers, 652 genes with the pollen preferential expression were upregulated compared to WT, and 608 were downregulated. These genes were functionally classified through gene ontology enrichment and MapMan analysis and visualized through protein–protein interaction (PPI) network. Subsequently, it was found that the activity of Rac6 can be modulated by post-translational modification (PTM) as well as GTP-GDP cycling to regulate calcium mediated-signaling, cell cycle or metabolism in pollen germination. Collectively, our result provides new insight on the signaling mechanism underlining the pollen germination.

P97 Flavonols protect tomato pollen from heat impaired germination, viability, and rupture by reducing levels of reactive oxygen species

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Rising temperatures impair the development of reproductive and vegetative tissues resulting in lower crop yields through an increase in reactive oxygen species (ROS). Plants can protect themselves from ROS through the localized synthesis of antioxidants, including flavonol metabolites. The tomato mutant, anthocyanin reduced (are), which has a defect in the gene encoding flavonol 3-hydroxylase (*F3H*) and reduced synthesis of flavonols, has impaired pollen function under optimal conditions, and is hypersensitive to heat stress. We identified impaired pollen germination and accentuated rupture in the are mutant that are accentuated at elevated temperatures. The levels of reactive oxygen species are elevated in the are mutant and increase more dramatically with temperature stress in the mutant than in wild-type. Chemical complementation with flavonols and genetic complementation with an *F3H* transgene reversed the effect of elevated temperature on pollen germination and pollen tube rupture in are, while overexpression of *F3H* conveyed thermotolerance. Consistent with plants modulating flavonol synthesis in response to stress as a protective mechanism, heat stress increased flavonols in pollen. We have performed RNA Seq in are and its parental line at optimal and elevated temperatures revealing substantial transcriptional changes during pollen germination and pollen tube elongation in are, with many fewer transcriptional changes in the parental line. These results demonstrated the protective capabilities of flavonols from heat-induced ROS and provide mechanisms for future development of approaches to safeguard plants against climate change.

P98 Visualisation of translational machinery in the male gametophyte of *Arabidopsis thaliana*

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In plants, translation regulation has an important role in pollen development and subsequent progamic phase, fertilisation and also in seed development. During early microgametogenesis in *Nicotiana tabacum*, transcripts are stored in large ribonuclear particles (RNPs) containing proteins of mRNA processing, protein synthesis and transport machinery. After pollination, the stored mRNAs are quickly released and translated in growing pollen tubes. In order to study translation regulation in *Arabidopsis thaliana*, we analysed the translational state of dry and activated mature pollen grains and pollen tubes by polysome profiling method. Moreover, we used superresolution confocal microscopy for localization of the translation machinery in pollen grains and growing pollen tubes of transgenic plants expressing tagged subunits of the *eukaryotic initiation factor 3* (*eIF3*) complex or ribosomal proteins.

P99 Knock out of a novel non-*S*-specific self-incompatibility factor of Petunia

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In Solanaceous self-incompatibility (SI), self-recognition of pistil and pollen is regulated by *S* locus-encoded proteins S-RNase and SLFs. Non-S-specific factors which unlinked to the *S*-locus are also known to be needed to maintain the SI system. It is important to isolate and analyze non-*S*-specific factors to understand the SI system, but not much is known about them. Recently, 2H12 was found in Petunia as a new non-*S*-specific factor. RNAi-mediated suppression of 2H12 resulted in breakdown of SI, which suggested 2H12 is required for SI in petunia (Sassa, this conference). To analyze the specificity/redundancy of 2H12, we performed genome editing of *2H12* with CRISPR-Cas9. We obtained two different mutants of *2H12*, and both mutants were induced frameshift mutations. Different from the RNAi results, both mutants did not result in the breakdown of SI, but pollen tube elongation at self-pollination was approximately 2-fold longer than the wild type. This result suggested that SI was weakened by the knockout of *2H12*. Different phenotypes of genome editing and RNAi may suggest redundancy in 2H12.

P100 The role of *Capsella* RBOHD and RBOHF in mediating pollen/stigma interactions

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Angiosperm reproduction is governed by a highly selective series of reproductive barriers. One such barrier includes the ability to reject incompatible or foreign pollen at the stigma by increasing the production of reactive oxygen species (ROS) via upregulation of respiratory burst oxidase homolog (RBOHs). *Capsella rubella* and *Capsella grandiflora* are relatively recently diverged sister species that differ in their modes of reproduction. *Capsella rubella* is self-compatibile (SC) while *Capsella grandiflora* is self-incompatibile (SI). Recent findings indicate that pollen hydration in both modes of reproduction is mediated by bursts of ROS when incompatible pollen interacts at the stigmatic surface. To determine the role of these bursts in the rejection of pollen in both *Capsella* species, we first used motif-based nucleotide searches to identify homologs of RBOHs from genomes of species in the family Brassicaceae, to which *Capsella* belongs. Moreover, we identified orthologs of RBOHD and RBOHF in *Capsella*, since both of these genes are known to generate ROS in stigmatic papillae of other Brassicaceae members. To manipulate ROS levels, we designed CRISPR constructs to induce knockout (KO) mutations or transcriptional knockdown (KD) of expression for these genes in both *Capsella* species. Once plant lines expressing these constructs are established, pollen hydration, germination, and pollen tube growth assays will be performed to determine the effects of the KO or KD on the ability to recognize self vs. non-self pollen in both species. If the function of these RBOHs is conserved in *Capsella*, it could suggest that a conserved mechanism establishes this critical reproductive barrier in all members of the Brassicaceae.

P101 Elucidating the structure and function of the *Papaver rhoeas* pollen *S*-determinant (PrpS)

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Self-incompatibility (SI) is a genetically controlled system used by approximately 50% of flowering plants, preventing self-fertilization and inbreeding. The well-studied *Papaver rhoeas* SI system comprises of two genetically linked *S*-determinants, the pistil expressed secretory protein (PrsS) and pollen expressed transmembrane protein (PrpS). Cognate PrpS-PrsS interaction within a self-incompatible scenario triggers downstream signalling pathways, leading to rapid pollen tube growth arrest and programmed cell death (PCD). Besides specifying SI, the PrpS-PrsS module also triggers growth arrest and cell death in vegetative cells. However, mechanisms of how the PrpS-PrsS module functions is still unknown and there are no PrpS homologues outside the Papaveracea databases. As a cognate interaction triggers a rapid influx of Ca^{2+} and K^{+} (and potentially H^{+}), we hypothesise that PrpS functions as a ligand-gated ion channel. *In silico* modelling work suggests that PrpS contains 7-transmembrane domains. Sequence alignment of multiple PrpS alleles and topological predictions, provide further clues of protein regions that may be vital for structural stability and PrsS binding. Conserved acidic amino acid residues hint at sites that may be involved in cation transport. Targeted mutagenesis studies, combined with previously identified non-functional PrpS mutants, will provide information on specific amino acids important for overall protein structure and function. PrpS is small (~20kDa), whereas ion channel receptors are usually multimeric plasma membrane proteins, this suggests that PrpS may be a subunit that multimerizes. Using blue native PAGE analysis, we will determine whether PrpS forms multimers and if this depends on interaction with its cognate ligand. Electrophysiological studies will enable us to establish the true functional nature of PrpS as a potential ion channel. Information on the structure and function of PrpS is essential to improve understanding of the initial signalling events triggered in the *Papaver* SI system.

P104 Self Compatibility is the norm in the salt-tolerant, maritime *Chrysanthemum arcticum* complex

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Classically, *Chrysanthemum* spp. express a tight sporophytic self incompatibility (SI) system which has been examined in great depth in ornamental (*C. xgrandiflorum*, *C. xhybridum*; 3 *S* loci) and pyrethrum (*C. cinerariifolium*, *C. coccineum*; 2 *S* loci) species. Reports of pseudo-self compatibility (PSC), the breakdown of SI, also occur in advanced breeding lines of both species. The sole chrysanthemum species native to the Americas, *C. arcticum* L. (=Arctanthemum) and its two subspecies (*C. arcticum* L. subsp. arcticum Kitamura; *C. arcticum* subsp. polaré Hultén) have the centers of origin and diversity in the State of Alaska (USA) only along the coastlines, although their range extends into northern Canada and two remnant populations in Kamchatka, Russia and Hokkaido, Japan. SNP data generated for these taxa show distinct differences in genetic structure within and among the species in the *C. arcticum* complex. Since most populations of this species are geographically isolated due to mountains, glaciers, and tundra SI would be detrimental in its small population sizes. Our objective was to assess pollen grain germination, pollen tube growth and seed set following self pollinations to determine the range in expression of SI in this species complex, along with assessment of its sporophytic status in SI genotypes. Pollen germination inhibition in the stigma or growth of pollen tubes within the stigmatic tissue of SI genotypes followed the classic sporophytic expression as found in other chrysanthemums. However, in a high percentage of the populations across species, pollen tube growth rates and self seed set showed the existence of self compatibility (SC). Self seed set even surpassed that of outcross pollinations. The implications of SC in this N. American chrysanthemum provide intriguing insights into the evolution of SI throughout the chrysanthemum complex.

P105 Role of callose in pollen tube invasive growth

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Pollen tubes serve as an ideal model system to study cellular morphogenesis, anisotropic growth mechanisms and cellular signaling in plant cells. The pollen tube cell wall plays an important role in regulating the growth process and protecting the sperm cells. Callose, a β-1,3-glucan, lines the pollen tube cell wall except for the apical growing region, and it constitutes the main polysaccharide in pollen tube plugs. These regularly deposited plugs separate the active portion of the pollen tube cytoplasm from the degenerating segments. They have been hypothesized to reduce the total amount of cell volume requiring turgor regulation, thus aiding the invasive growth mechanism. To test this, we characterized the growth pattern of *Arabidopsis* callose synthase mutants with altered callose deposition patterns. Mutant pollen tubes without callose wall lining or plugs displayed a wider diameter and slower growth compared to their respective wild type. To examine the pollen tube's ability to perform durotropism in the absence and presence of callose, we performed mechanical assays such as growth in stiffened media and quantified turgor through incipient plasmolysis. We found that mutants lacking callose plugs have lower invading capacity and higher turgor pressure when faced with a mechanical obstacle. This elevation in turgor pressure in the callose synthase mutants could potentially be enabled by increased levels of de-esterified pectin and/or cellulose in the tube cell wall. To support the hypothesis of a compensatory mechanism, we performed cell wall immunolabeling to detect alterations in cell wall polysaccharide content. Combined, the results reveal how callose contributes to the pollen tube's invasive capacity and thus plays an important role in fertilization

P106 Genetic and evolutionary dynamics of polysiphony in Solanaceae species

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The reproductive success of angiosperms played a major role in the evolution of life on Earth. Understanding its biology is a required step to decipher the role of plants in the evolution and establishment of ecosystems. One of the two sides that compose the complexity of plant reproductive system is pollen. Many aspects of pollen biology remain still poorly characterized. One of them is the dynamics that govern pollen germination. Our understanding of this process is limited and, unsurprisingly, its genetic characterization is confined to a few phenotypic classes. This is the case of polysiphony, the presence of multiple pollen tubes generated from the same grain. Its description is mainly confined in the pre-molecular biology era literature on non-model organisms. Our work aims to define the genetic basis and evolutionary dynamics across the Solanaceae species.

In *Physalis grisea* it appeared that the phenotype emerged as consequence of tetraploidization.

Polysiphony and its link to ploidy is not limited to this species. Indeed, a screening of tetraploid wild and domesticated tomatoes confirmed the presence of polysiphony in the *Solanum* family as well. Differences in the expressivity of the phenotype among different tomato ecotypes suggests a genetic basis that can be further explored. Interestingly, across the Solanaceae species this phenotype was found independent of the ploidy level. Indeed, in *Solanum eathiopicum*, a relative of eggplant, polysiphony is present in the F1 populations of different accessions, while absent in their progenitors. Polyploidization and hybridization both have been documented to have a global perturbation effect on the biology of the cell. The genomic resources on *Solanum* species recently generated by our lab can be a powerful tool to drive a comparative study across multiple Solanaceae species and decompose a common signature causing polysiphony.

P107 Self-incompatibility requires GPI anchor remodeling by the poppy PGAP1 orthologue HLD1

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Glycosylphosphatidylinositol anchored proteins (GPI-APs) are tethered to the outer leaflet of the plasma membrane where they function as key regulators of a plethora of biological processes in eukaryotes. Self-incompatibility (SI) plays a pivotal role in regulating fertilization in higher plants through recognition and rejection of 'self' pollen. Here we used *Arabidopsis thaliana* lines engineered to be self-incompatible by expression of *Papaver rhoeas* SI determinants for an SI suppressor screen. We identify HLD1/AtPGAP1, an ortholog of the human GPI-inositol deacylase PGAP1, as a critical component required for the SI response. Besides a delay in flowering time, no developmental defects were observed in *hld1/atpgap1* knockout plants, but SI was completely abolished. We demonstrate that HLD1/AtPGAP1 functions as a GPI-inositol deacylase and that this GPI-remodeling activity is essential for SI. Using GFP–SKU5 as a representative GPI-AP, we show that the *HLD1/AtPGAP1* mutation does not affect GPI-AP production and targeting but affects their cleavage and release from membranes in vivo. Our data not only implicate GPI-APs in SI, providing new directions to investigate SI mechanisms, but also identify a key functional role for GPI-AP remodeling by inositol deacylation in planta.

P108 Pollen tube growth at the stigma surface

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In the flowering plant *Arabidopsis thaliana*, the pollen tube, a tip growing cell, navigates thought the female organ (pistil) to deliver the immobile sperm cells to the ovules for fertilization. The pollen tube first partially penetrates the cell wall of the female epidermal cells also called stigmatic papillae, to make its path within this layer towards the cell bases. We recently showed that advancement of the pollen tube is closely linked to the mechanical properties of the invaded stigmatic cell and started to develop a framework for modeling pollen tube growth on the stigmatic surface. We aim at providing accurate experimental data using in vivo high-resolution imaging and innovative in vitro biomimetic experiments to orient our modeling effort and to get a mechanistic understanding of pollen tube growth guidance in a complex environment. Our hypothesis is that both the elongated papilla cell geometry and the cell wall elasticity would combine to provide a robust guidance mechanism. Our project associates the expertise of experimentalists and theoreticians with a pluridisciplinary background (biology, mathematics, physics) to dissect the initial step of a fundamental process, the sexual plant reproduction.

P109 ROPGEF in pollen germination in *Arabidopsis thaliana*

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Double fertilization is a fundamental mechanism in flowering plants. Because the sperm cells lost their motility in flowering plants, they are transported to the female gametes by pollen tubes, which germinate on the papilla cells of the stigma, grow through the transmitting tract until they reach the female gametophyte. Multiple cell-cell communication steps and molecular pathways are required to control pollen germination and growth. Many key players have been characterized in these processes, such as GUANINE EXCHANGE FACTORS (ROPGEFs) phosphorylated by RECEPTOR-LIKE KINASES (RLKs). ROPGEFs activate small RHOGTPase OF PLANTS (ROPs), leading to the initiation of pathways such as actin polymerization, calcium signaling, reactive oxygen species production, and vesicle trafficking. Even though polar growth is a well-understood process, pollen germination is not yet comprehended, and germination-specific players have not yet been characterized. In *Arabidopsis thaliana*, *ROPGEF8*, *9*, *11*, and *12* are specifically expressed in pollen. I could show that the pollen specific ROPGEFs display differential protein localization and have distinct functions during pollen germination and growth. ROPGEF8 and ROPGEF9 accumulate at the plasma membrane at the initiation site of pollen grains in a temporally biphasic manner. In contrast, ROPGEF11 and ROPGEF12 show accumulation in the growing domain only after germination and therefore I regard them as non-critical for the initiation of pollen germination. In line with this, the loss-of-function mutants of *ropgef8* and *ropgef9* exhibited severe decreases in pollen germination efficiency, aggravated in the double mutant *ropgef8ropgef9*. In contrast, mutants of *ropgef11* and *ropgef12* did not show any germination deficiency. These results suggest that ROPGEFs have a distinct function in pollen germination and that ROPGEF8 and ROPGEF9 are crucial during pollen tube germination.

P110 The regulation of ROPGEFs by AGC1 kinases in pollen germination of *Arabidopsis thaliana*

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An important step in the life cycle of angiosperms is sexual reproduction, where male and female gametes fuse to form a zygote in a process called double fertilization. To deliver the two nonmotile male gametes to the female gametophyte, plants relay on rapid tip growth of pollen tubes, which growth over a long distance through multiple different tissues of the flower. The growth regulation depends on multiple signalling pathways and is controlled by RhoGTPase OF PLANTS (ROP) signalling. ROPs are molecular switches that can be activated by the plant specific ROP GUANINE NUCLEOTIDE EXCHANGE FACTORs (ROPGEFs). In turn ROPGEFs are controlled by phosphorylation by RECEPTOR-LIKE KINASES (RLKs) such as POLLEN RECEPTOR KINASES (PRKs) and by cytosolic AGC1 kinases, which phosphorylate ROPGEFs in their catalytic core, the PRONE domain. *AGC1.5* and *AGC1.7* are highly expressed in pollen tubes and are crucial for normal pollen tube growth, but their specific role in pollen germination and their distinct effect on ROPGEFs remain unclear.

Here I show that mAbb0.5 AGC1.5 retracts from the pollen germination sites and overexpression constructs negatively affect pollen germination in a dose-dependent manner. In addition, *agc1.5/agc1.7* t-DNA-insertion double mutant are capable of germinating in a humid environment without any medium supply, indicating a growth inhibiting effect of AGC kinases. Phosphoproteome data of the *agc1.5/1.7* double mutant revealed one prominent phosphorylation site in the PRONE domain of multiple ROPGEFs, which suggest an undescribed regulatory mechanism of ROPGEFs. My data suggest that AGC1 kinases have an inhibitory effect on ROPGEFs by phosphorylation and are important for pollen dormancy. We hypothesize that AGC1 kinases are essential to keep pollen grains in a dormant state, which is revoked only in the presence of external germination signals that inhibit AGC kinases, triggering pollen germination specifically in favourable conditions.

P111 Molecular control of dominance/recessivity interactions between self-incompatibility alleles in *Arabidopsis*

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Self-incompatibility is a common genetic system preventing selfing and enforcing outcrossing in hermaphroditic plants. In the Brassicaceae, the *S*-locus receptor kinase (SRK) and the *S*-locus cysteine-rich proteins (SCR) determine the female and male specificities, respectively. While most plants are heterozygotes at the *S*-locus genes, in the vast majority of cases a single *S*-allele is expressed at the phenotypic level. In *Arabidopsis halleri*, the dominance hierarchy between *S*-alleles is controlled by a dedicated set of trans-acting small non-coding RNAs produced by dominant *S*-alleles that target specific sequences on the recessive *SCR* alleles, causing their transcriptional silencing. These molecular interactions are the first-ever documented case of "dominance modifiers", a class of genetic elements whose existence had remained elusive so far. To study the gene regulatory pathways by which these dominance modifiers achieve their function, we established a series of transgenic *A. thaliana* lines recapitulating the self-incompatibility phenotypic response. Some of the small RNAs are 24nt-long molecules, are partly loaded into the AGO4 protein and target intronic sequences, so we tested the role of the RNA-directed DNA methylation pathway DNA methylation. However, we found that the dominance modifiers were still able to transcriptionally silence their targets in genetically modified backgrounds in which we had inactivated core elements of this pathway. We also found no consistent evidence for differentially methylated regions along the *SCR* gene between dominant and recessive genotypic contexts, further arguing against the involvement of the canonical RdDM pathway. Overall, our results firmly establish that independent sRNA modifiers tightly control the transcript levels of *SCR* alleles, eventually bringing them down to a level where the self-incompatibility response is suppressed for the targeted allele. The exact mechanism by which this transcriptional control is realized, however, remains elusive.

P112 Impact of sexual selection on shaping sexual gene expression in *Arabidopsis lyrata* pollen

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Intrasexual selection is a component of sexual selection that describes the rivalry among conspecific members of one sex to fertilize the gametes of the opposite sex. In angiosperms, intrasexual selection could eventuate during the fertilization when pollen grains of conspecific individuals are deposited on the stigma and then grow a tube to reach the ovules. At this stage, traits like pollen germination and pollen tube growth might bring mating advantages to individuals. The transition from outcrossing to selfing in plants is frequent and is believed to lead to weaker sire-sire competition and thus relaxed sexual selection when compared to outcrossers. However, studies addressing the impact of sexual selection on pollen performance traits and sexual gene expression are relatively less studied and still under vivid debate. Here, we used *Arabidopsis lyrata*, a good system for varying degrees of mating strategies, and analyzed the variation in pollen performance traits to test whether different mating systems can affect intrinsic pollen competition ability. Further, we performed transcriptomics to elucidate the impact of sexual selection on shaping the sexual gene expression in *A. lyrata* pollen. Our results showed that pollen germination as a pollen performance trait is higher in outcrosser compared to selfer *A. lyrata*, but not pollen tube growth. Moreover, our transcriptomic results revealed that differentially expressed genes between outcrossers and selfers pollens are particularly involved in cellular functions such as vesicle-mediated transport, suggesting a potential impact of sexual selection on these functions known to be involved in pollen development.

P113 A novel non-*S*-specific self-incompatibility factor of Petunia

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Many solanaceous plants exhibit S-RNase-based self-incompatibility (SI) in which self/non-self discrimination in pistils and pollen is controlled by *S* locus-encoded proteins S-RNase and SLFs, respectively. While the *S* locus proteins have been extensively characterized, little is known about the nature and function of other SI factors which are not encoded by the *S* locus, non-*S*-specific factors. In order to identify new non-*S*-specific factors, we analyzed differentially expressed genes (DEGs) between SI and self-compatible (SC) Petunia plants having same *S* genotype. One of the DEGs, clone *2H12*, was selected and subjected to functional analysis. RNAi-mediated suppression of *2H12* of SI petunia resulted in breakdown of SI, suggesting that 2H12 is required for SI. *2H12* is expressed in pistil, and its suppression by RNAi did not affect the accumulation of the S-RNase. Further characterization of 2H12 would help our understanding of the mechanism of the S-RNase-based SI.

P114 Decoding the gametophytic translational role of eIF3E in *Arabidopsis*

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In flowering plants reproduction, a vegetative cell forms a polarized single cell extension, termed the pollen tube, responsible for delivering two non-motile sperm cells into the female gametophyte. Fast pollen tube growth is tightly regulated by utilization of transcripts formed during pollen maturation. A recent study has shown that translation initiation factors, particularly, eukaryotic translation initiation factor 3 subunits (eIF3), are present in mRNA storage compartment of mature pollen. Among all eIFs, eIF3 is considered to be the largest known complex involved in both sporophytic and gametophytic development. Here, we report that eIF3E subunit in *Arabidopsis thaliana* is required for male and female gametogenesis. Our current results suggest that loss-of-function *eif3e* not only affects pollen development post pollen mitosis I (PMI) and pollen germination, but also impacts on embryo-sac cell fate specification resulting in defect in fertilization. *eIF3E* is ubiquitously expressed and localized on the vegetative cell membrane and the cytoplasm. Concomitantly, regulators of mRNA translation, PABP5 co-localize with eIF3E in pollen and pollen tubes, suggesting a possible association of eIF3 with RNA in pollen. Moreover, Co-IP immunoprecipitation assay preliminary identified eIF3G1, eIF3A1, eIF3C1, eIF3I1, eIF3B1/2 as eIF3E associated subunits in pollen. Our findings unveil that although eIF3e is not a core subunit of eIF3 complex, it plays a vital nonredundant accessory role that is essential during pre and post fertilization events. Further structural studies will unveil the mechanistic properties of eIF3E on the control of mRNA transability.

P115 Characterisation of GPI-anchored proteins GDPDL 6/7 in pollen development and ovule attraction.

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Glycosylphosphatidylinositol (GPI)-anchored proteins (GAPs) are widespread in all eukaryotic organisms and make up about 1% of *Arabidopsis thaliana* genes. GPI-anchoring is a post-translational, C-terminal modification that targets a protein to the outer leaflet of the plasma membrane. GPI-anchor synthesis mutants are embryo lethal and some have shown to be essential for effective pollen-tube targeting and reception. These factors make pollen-expressed GAPs prime suspects to elucidate their role in pollen tube development and pollen-ovule intercommunication. We pre-selected pollen-expressed GAPs and identified Glycerophosphodiester phosphodiesterase-like (GDPDL) genes as potential regulators of fertilization in *Arabidopsis*. Both, *gdpdl6* and *gdpdl7*, showed reduced seed set at silique maturity. Further characterization revealed severe loss of seed set in *gdpdl6* as a result of loss of pollen tube attraction by mutant *gdpdl6* ovules as well as pollen abortion at anthesis. Reduced allele transmission was however only observed through the female gamete. Surprisingly, this suggests GDPDL6 plays a vital role in both male and female gametophyte development. Sporophytic GDPDLs have been implicated in cell wall deposition required for cell elongation in root hairs and hypocotyl. *GDPDL6* complementation resulted in the rescuing of phenotypes and the ability to recover homozygous T-DNA mutants. Thus, GDPDL6 is a highly pollen-expressed GAP but is required for both pollen development and by ovule for normal pollen tube attraction.

P116 An actin nucleator ARP2/3 complex role in *Arabidopsis thaliana* reproduction

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Pollen tube growth is an actin-dependent, finely controlled, and polarized process, during which vesicles deliver cell wall material into the apical part of the pollen tube. The clear zone is the most apical part of growing pollen tube, from which big organelles are excluded and where exocytosis of cell wall components takes place. Actin cytoskeleton plays a key role in establishment of the clear zone. Our results showed that mutants lacking functional ARP2/3 complex or its nucleation promoting factors (NPFs) have shorter and thicker pollen tubes. Mutants also have reduced clear zone in comparison with wild-type plants, which correlates with their slower growth. When we evaluated actin cytoskeleton structure in the subapical zone, we found that both actin density and bundling was higher in mutant plants. Finally, pollen tubes of *ARP2/3* mutants have modified composition of the cell wall. Our results suggest a role of ARP2/3 complex in polarized growth of pollen tubes through the control of actin and localized cell wall deposition.

P117 The functional characterization of a myosin XI adaptor that is indispensable in rice pollen tube growth.

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In angiosperms, pollen tube (PT) growth is an important process for successful double fertilization and it directly affects crop productivity by maintaining the seed setting rate. Due to the importance, many attempts were made to identify genetic resources related to PT growth in rice (*Oryza sativa* L.), but the research is very limited due to its difficulty to obtain homozygous mutants by traditional methods unlike the *Arabidopsis* (*Arabidopsis thaliana* L.).

In a screening of T-DNA insertional rice mutants, we identified a mutant that disrupted the Tethering protein of actomyosin transport in pollen tube elongation (TAPE) gene showed a severely distorted segregation ratio in genotyping assay. Its CRISPR/Cas9 knockout mutants showed short pollen tube with sterile phenotype. Through intensive functional characterization of the TAPE, we confirmed its expression, interactors, transcriptional regulation related to PT growth and concluded that TAPE is an essential myosinXI adaptor mediating actomyosin-driven transport during the rice PT growth. To the best of our knowledge, it is the first functional characterization of myosinXI adaptor in PT elongation among angiosperms. The findings presented here provide a clue to understanding the actomyosin-driven transport mechanism inside the PT elongation along with inside the other plant cell.

P118 Deciphering the gametophytic role of eukaryotic translation initiation factor, subunit M (eIF3M)

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In flowering plants, pollen tube growth is achieved through a massive activation and translation of mRNA stored in the mature pollen grain. Recent studies identified members of the eIF3 in the mRNA storage particles of mature pollen. eIF3 complex is a multitasking factor in the translation network. The major function of eIF3 is to scaffold the formation of the translation initiation complex. eIF3M, part of the eIF3 complex, plays a vital role in mRNA recruitment and polysome formation in humans and yeast. Moreover, eIF3M also plays a critical role in maintaining the structural integrity of the eIF3 complex. Here, we are using genetic and biochemical approaches to decipher the male gametophytic role of *eIF3m1* and *eIF3m2*, the two *Arabidopsis* paralog genes for the *eIF3m*. Loss of function of *eIF3m1* causes mature pollen defects and impacts on pollen tube germination rate. Furthermore, *eIF3M1/M2* overexpression lines driven by pollen-specific and native promoters resulted in alterations in the pollen tube growth phenotype (aborted and branching pollen tubes). T-DNA insertion lines transformed with translational fusion constructs showed partial complementation of the mutation. Pollen tube growth was defective under heat shock (28 and 37°C) in wild type, whereas, pollen germination ratio was increased in *eIF3M1-1* and *eIF3M1-2* T-DNA insertion mutants under the heat stress. eIF3M1 and M2 were localized in both the vegetative cell nucleus and the sperm cell membrane. Polysome analysis revealed translation defects in *eIF3m1/m2* double homozygous T-DNA lines and seedlings expressing the eIf3M2 fused with YFP driven by the native promoter.

P119 Synergid and filiform apparatus regulation of pollen tube reception

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Successful fertilization requires signaling between the pollen tube and the synergid cells of the female gametophyte. Synergids are highly specialized haploid cells that have a highly invaginated and membrane dense structure known as the filiform apparatus at their base. During pollen tube reception, synergids secrete pollen tube attractants and communicate with arriving pollen tubes to trigger pollen tube bursting and release of the sperm cells. Concurrently, the filiform apparatus serves as a site of accumulation for many essential reception regulating molecules such as reactive oxygen species and calcium; and proteins such as FERONIA, NORTIA, LORELEI, ANJEA, and HERK. The filiform apparatus is critical for successful reception; with abnormalities in its development often leading to disruption of pollen tube reception, such as in *myb98* knockout mutant ovules that are unable to attract pollen tubes. Despite previous research efforts, many questions remain about the detailed structure of the filiform apparatus and its composition, as well as how the pollen tube interacts with the synergids during reception. To address these unknowns, our research employs the use of forward genetic screening to identify novel regulatory molecules, as well as new advanced forms of microscopy to analyze synergid and filiform apparatus ultrastructure before and during pollen tube reception. Progress toward imaging the filiform apparatus in 3D will be presented.

P120 ECS1 and ECS2 identified as the first molecular factors regulating polyspermy

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Reproduction is the key to evolutionary success ensuring survival of species on earth. In sexually reproducing animals, gamete fusion is critical, defining successful fertilization. In many animals, a single egg cell is challenged by millions of sperm cells. Given that polyspermy in animals is typically lethal, organisms have evolved egg cell blocks ensuring successful fertilization by a single sperm only.

In plants, there are mechanisms already implicated at the level of sperm transport, which is mediated by pollen tubes. However, plant ovules infrequently attract supernumerary pollen tubes. In flowering plants, polyspermy remained uncharted territory as we simply lacked the tools to detect this event. Nakel *et al*., 2017, established a HIPOD system (high-throughput polypaternal breeding design), which identified for the very first time, polyploid plants induced through polyspermy.

Why is this important? Polyspermy empowers plants to sneak extra copies of DNA only to the egg cell 'behind the back' of DNA-sensitive endosperm essentially bypassing triploid block as demonstrated by Mao *et al*., 2020. This polyspermy induced polyploidization might be of selective advantage under distinct environmental conditions as additional copies of genes could take up auxiliary or new functions.

It is therefore tempting to speculate whether polyspermy is an adaptive trait. In a quest to investigate polyspermy induced triploid plants, we identified *ecs1 ecs2* double mutants, which have previously been implicated in cleaving pollen tube attractant LURE1 (Yu *et al*., 2021). Interestingly, we observed that these double mutants exhibit a fertilization defect and concomitant formation of haploid offspring. Here we discuss a third phenotype, unravelling ECS1 and ECS2 as the first molecular factors to regulate polyspermy.

P121 Analysis of phosphoinositides during synergid and filiform apparatus regulation of pollen tube reception

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Successful fertilization requires signaling between the pollen tube and the synergid cells of the female gametophyte. Synergids are highly specialized haploid cells that have a highly invaginated and membrane dense structure known as the filiform apparatus at their base. During pollen tube reception, synergids secrete pollen tube attractants and communicate with arriving pollen tubes to trigger pollen tube bursting and release of the sperm cells. Concurrently, the filiform apparatus serves as a site of accumulation for many essential reception regulating molecules such as reactive oxygen species and calcium; and proteins such as FERONIA, NORTIA, LORELEI, SCYLLA, ANJEA, and HERK. The filiform apparatus is critical for successful reception; with abnormalities in its development often leading to disruption of pollen tube reception, such as in *myb98* knockout mutant ovules that are unable to attract pollen tubes. Despite previous research efforts, many questions remain about the filiform apparatus, its composition, membrane properties, and how it is able perform its functions during pollen tube reception. To further elucidate these properties, our research employs the use of an array of phosphoinositide biosensors; targeting of phosphoinositide biosynthesis and metabolism; and targeting of phosphoinositide signaling. Our research seeks to investigate the role of phosphoinositides during stages of synergid and filiform apparatus development, regulation of pollen tube guidance, and regulation of pollen tube reception.

P122 Evolution and function of secreted small cysteine-rich EC1 proteins

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Sexual reproduction in flowering plants is based on a unique process known as double fertilization. Upon landing on the stigma, the pollen grain germinates. The emerging pollen tube grows towards the ovules and bursts at its tip upon arrival, releasing two sperm cells. The embryo is formed when one sperm cell unites with the egg cell, while the other sperm cell fuses with the central cell, which develops into the endosperm and nurtures the embryo.

Meanwhile, a few players essential for male and female gamete attachment and fusion have been discovered in *Arabidopsis thaliana*. In the sperm these are the fusogen HAP2 (HAPLESS 2), the adhesion factor GEX2 (GAMETE EXPRESSED 2) and fusion facilitators DMP8 and 9 (DUF679 Membrane Protein 8 and 9). In the egg cell, secreted small cysteine-rich proteins termed EC1 (EGG CELL 1) are essential for double fertilization. When the sperm cells arrive at the fusion site, EC1 proteins are secreted from vesicle-like structures, triggering HAP2 to shift from the endomembrane system to the sperm cell's plasma membrane, allowing the gametes to fuse.

Here we will present our studies on the evolution and function of EC1 proteins. After generating CRISPR/Cas9 knockout lines for the five *Arabidopsis EC1* genes we analyzed their phenotypes regarding sperm cell adhesion and fusion, resulting in drastically decreased seed set. Evolutionary conservation of EC1 proteins and related secreted small cysteine-rich proteins is investigated by testing their capacity to restore the reproductive phenotype in the *ec1* quintuple mutant. Our functional studies also include the heterologous expression and purification of EC1 proteins in order to identify important domains, EC1-interacting molecules on the sperm cell surface as well as downstream effectors."

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P123 Double fertilization in *Asparagaceae* family: F-actin highway for long distance movement of sperm cells

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As a special feature, the central cell nucleus of the female gametophyte in the *Asparagaceae* family is polarized at its chalazal end, below the antipodal cells. Because of that, when double fertilization occurs, the second sperm must travel about 200µm across the central cell to get its target. Although it is known that immotile sperms are transported by female actin coronas and star-shaped structures, most of the current knowledge on double fertilization was obtained from model species, where the distance between the egg cell and the central cell nucleus does not exceed 10 µm. To date, it is not known whether *Asparagaceae* embryo sacs display conventional F-actin structures or new ones that support such a long trip. In order to visualize F-actin structures involved in the transport of sperms for kariogamy in *Asparagaceae*, ovules from *Agave salmiana* were stained with rhodamine-phalloidin, clarified with methyl salicylate, and analyzed by confocal microscopy. The central cell nucleus of mature embryo sacs appeared surrounded by a dense coat of actin filaments from which several parallel actin cables extended until they reached the ovular apparatus at the opposite end of the embryo sac. During double fertilization, a bundle of several actin cables was formed; this bundle conspicuously wrapped the sperm cell in transit to the central cell nucleus. Once karyogamy events were completed, these structures disassembled. Taken together, these observations suggest that these structures work as a highway for the movement of the non-motile sperm across the central cell cytoplasm to fuse its nuclei. The large size of the central cell, the long distance that sperm must travel for karyogamy and, the thick actin structures that *Asparagaceae* species possess make them suitable for more detailed studies of the fertilization process.

P124 Delayed pollen tube growth in Calotropis procera is controlled by environmental changes

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In *C. procera*, as in many other *Apocynaceae*, the nectar from these flowers is secreted from the nectaries located inside the stigmatic chamber, with the excess flowing via the capillary system into special reservoirs (cucculi). The nectar has two functions: it is used as a reward to attract pollinating insects; and it serves as the germination medium for pollen grains. Under natural conditions the nectar concentration is subjected to a large variability, ranging from 22-68% sucrose.

In the present study we followed the process of pollen germination under various experimental sucrose concentrations simulating the nectar. We found that the optimal concentration of a sucrose medium for pollen germination is 20%. However, if the already-germinated pollen grains are subjected to high sucrose concentration for different periods of time (between one and three hours), elongation of the pollen tubes is inhibited. In all the experimental groups, the pollen tubes renewed their elongation following a reduction of the sucrose concentration to 20%.

This phenomenon of increased sucrose levels causes delayed fertilization, as already well known in animals (Blandau and Young 1939); and in the plant it enables it to postpone fertilization until conditions improve and thus to thrive under the extremely high temperatures and fluctuations in relative humidity that characterize its habitat.

P125 Heteranthery - a plan for staggered pollen presentation in two species of *Crotalaria* inhabiting N-W Himalayas, India- its relative implication

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Flowering plants display unsurpassed diversity in the morphology of their reproductive structures, including the stamens. Stamens within a flower are usually akin in appearance, however some species possess two, or occasionally three, structurally distinct types that often differ in reproductive function. This peculiar morphological differentiation of stamens and anthers within a flower is referred to as heteranthery. It occurs in at least 20 families distributed among 12 orders including *Commelinaceae*, *Fabaceae, Lythraceae, Melastomaceae* etc. Functional significance of this feature has also been elaborated in several species. Genus *Crotalaria* of family *Fabaceae* is a classical example displaying this mechanism. Present work carried out on two species of genus *Crotalaria* i.e., *C. medicaginea* and *C. mysorensis*, forming natural population at an altitude of 426-1015 masl in subtropical regimes of Northwestern Himalayas is aimed to address relative functional significance of this dimorphism. The dimorphism of anthers that includes shape, size, dehiscence time as well as size of pollen is associated with staggered presentation of pollen in both the species. As expected, this aids in delayed selfing and assurance of seed set. However, while heteranthery plays extremely significant role in the reproductive success of *C. mysorensis*, extent of contribution is relatively much lower in *C. medicaginea*. Presentation will elaborate on this comparative implication.

P126 How to set a perfect parthenocarpic fruit?

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Fruit set, the switch from quiescent ovary to developing fruit, is normally established during and soon after fertilization of the ovules inside the quiescent ovary. Despite the progress made, the molecular mechanism underlying fruit set is still only partly understood. We showed that the tomato AGAMOUS-like-6 (SlAGL6) loss-of-function mutant (*slagl6CR-sg1*) is capable of fertilization-independent setting of normal, yet seedless (parthenocarpic) fruit. *SlAGL6* is preferentially expressed in the quiescent ovary ovules and sharply decline upon pollination. We found that *slagl6CR-sg1* ovules were enlarged due to integument over-proliferation and failed to differentiate an endothelium, the integument's innermost layer, upon maturation. A causal relation between this abnormal phenotype and slagl6 loss-of-function is inferred from the observation that *SlAGL6* is predominantly expressed in the immature ovule integument and upon ovule maturation its expression shifts to its innermost layer the endothelium. RNA-Seq of unfertilized *slagl6CR-sg1* ovules indicated that their transcriptome underwent reprogramming that resembles the transcriptional changes occurring in wild-type ovules following fertilization. The *SlAGL6* gene encodes a MADS-box transcription factor and thus is hypothesized to function by complexing with other MADS-box proteins. Screening yeast two-hybrid library of quiescent ovaries with the SlAGL6 full-length protein resulted in the identification of eight MADS-box transcription factors as candidate partners that complex with SlAGL6. CRISPR knockout of corresponding genes revealed that one mutant exhibited a parthenocarpy syndrome that closely resembled that of *slagl6CR-sg1*. This includes the absence of a typical endothelium in mutant ovules and the precocious post-fertilization reprogramming of their integument. Taken together, our results suggest that a protein complex containing SlAGL6 acts from within the ovule integument to inhibit ovary growth beyond anthesis. That by suppressing components of the fertilization-induced ovule reprogramming underlying fruit set.

P127 Maternal regulation of double fertilization in *Arabidopsis thaliana*

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In ovules, fertilization of both egg and central cell is required to assure a proper seed development. The sporophytically expressed genes *SEEDSTICK (STK)* and *ARABIDOPSIS Bsister (ABS)* encode for MADS-domain transcription factors, that act in concert for ovule integument differentiation. In the *abs stk* double mutant the innermost layer of ovule integuments named endothelium does not properly differentiate leading to a partial sterility and defective seed development. *abs stk* fertilization defect is sporophytically controlled as in plants heterozygous for both genes fertilization occurs as in wild type plants.

Furthermore, the *abs stk* double mutant plants are characterized by starch accumulation in the central cell and more in general in the female gametophyte. In this work, we were able to restore fertilization efficiency and seed formation by modulating starch accumulation and degradation pathways. Our results suggest that a fine regulation of starch metabolism in the ovule is required to allow fertilization. Here, we identified STK and ABS as maternal regulators of this process, acting in a non-cell autonomous manner.

P128 *bHLH* genes in the MBW complex, more than a flavonoid biosynthesis genes

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The seed is formed by the seed coat (diploid tissue), the embryo (diploid) and the endosperm, the nutritive tissue (triploid). A crosstalk between the endosperm and the seed coat is required for proper seed development. In this crosstalk, the endothelium, the innermost layer of the seed coat, which is in direct contact with the endosperm, plays a crucial role. But on the other hand, seed development is sensitive to parental dosage due to a postzygotic barrier known as triploid block. The endothelium is known to play a key role in triploid block because the mutation of endothelium-specific genes partially or completely rescue the triploid block in paternal excess unbalanced crosses. The MYC-bHLH-WD40 (MBW) complex is involved in flavonoid biosynthesis in the endothelium but also in several other plant developmental processes. Here we investigate the role of *bHLH* genes (i.e., *TT8, EGL3, GL3*) members of the MBW complex in balanced and unbalanced crosses. We propose here: 1) their specific roles in triploid and 2) expression patterns in several species of angiosperms allow us to hypothesize about the conservation of their function. Our results show that the diverse functions of the *bHLH* genes evolved from ancestral roles in flavonoid biosynthesis into multiple aspects of plant development. Finally, the use of wt and *tt8* mutant plants, which allows the morphological characterization and RNAseq of seeds obtained from balanced and unbalanced crosses, constitutes an important step towards a better understanding of the maternal role in the triploid block.

P131 Changes ingene expression and metabolites composition during the development of peaseed coat and embryo

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In angiosperms, the mature seed consists of an embryo, a seed coat (SC), and, in many cases, and endosperm. Seed development involves the coordinated activities of three genetically distinct entities: the embryo, the endosperm, and the maternal plant. In contrast to knowledge about embryo and endosperm we have relatively little knowledge of SC especially at thegenomics level. We have analyzed the gene expressionand metabolite profileduring the seed development using the panel of cultivated and wild pea genotypes. Collected material (SC from seeds of 10-15-20 and 25 days after pollination) was used for chemistry, transcriptomics, protein-enzymatic activitiesand anatomical analysis.Isolated RNA from four developmental stages was subjected to transcriptome sequencing. Reads were annotated, mapped to pea genome and RNA atlasand analysed forpairwise differential gene expression.This revealed developmentally regulated clusters of genes as well as differences between wild, dormant and domesticated, non-dormant genotypes.Selected genes were studied in details by RT-qPCR.

Chemical composition analysis done by laser desorption-ionization mass spectrometry (LDI-MS) followed by multi variate statistical analysis, specifically principal component analysis for dimension reduction which showed significant differences among developmental stages. Synoptic view on the metabolomics data was provided by heatmaps of log ratio transformed data. A marked segregation of developmental stages 1-3 and 4-5 was observed in heatmaps obtained from LDI-MS measurements in negative ionization mode. Significant changes in the hydroxylated long chain fatty acids profile in seed coats of dormant genotypes occur during seed development. LDI-MS in imaging mode (MSI) was used to visualize the differences in chemical composition on the seed coat surface.

This work presents the first comprehensive gene expression and metabolites profiling of pea seed coat development. Moreover,the comparison of wild and cultivated genotypes allows analysis of the gene

expression in relation toseed development, dormancy and also domestication.

P132 Analysis of pea pod dehiscence in relation to domestication

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The transformation of wild plants into domesticated crops is the central process of early agriculture and one of key points in human history. Domestication can be viewed as an accelerated evolution, the result of human and natural selection. It triggered changes representing adaptations to cultivation and harvesting named domestication syndrome. These include loss of germination inhibition and increase of seed size, linked to successful early growth of planted seeds as well as loss of pod dehiscence. Despite of crucial position of legumes, as protein crops, in human diet, comparably little is known on their domestication.

The main objective of the study is to identify genetic, structural a chemical basis of one key trait of legumes domestication, the pod dehiscence, by comparative analysis of wild and domesticated pea genotypes. Several pairs of wild versus domesticated forms were selected, together with primitive forms (landraces) representing transitory steps. These pairs as well as mapping populations of crosses are morphometrically, histologically and physiologically characterized and assessed for pod dehiscence phenotype. Genetic mapping revealed single *Dpo1* locus and placed it into chromosome 5/LGIII. Comparative anatomical and histochemical staining of lignin compounds have revealed differences in lignin deposition, being higher in dehiscent genotypes. Similarly, transcriptomic analysis using MACE approach on dissected pod suture identified 132 down- and 16 were up-regulated genes in indehiscent libraries. Selected candidates have been subsequently tested by RT-qPCR. Currently new RNAseq analysis is underway.

P133 miRNA regulation of egg and zygote polarity

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Cell polarity is critical for differentiation and morphogenesis in both plants and animals. In *Arabidopsis*, the egg cell is polarized with an apical nucleus and basal vacuole. After fertilization this polarity is lost, and then restored when the nucleus moves to the apex, the zygote elongates, and the vacuole reforms at the base. Subsequently, the zygote divides asymmetrically to form an apical cell that will produce the embryo proper, and a basal cell that will form the suspensor. The mechanism by which the polarity of the zygote is restored after fertilization is not well understood.

Our laboratory has recently shown that the miRNA processing enzymes DICER LIKE 1 (DCL) and SERRATE (SE) are required for the apical-basal polarity of the zygote, and for proper asymmetric division. Loss of DCL1 or SE results in zygotes that lack correct polarity of the nucleus and vacuole, and divide symmetrically. This symmetric division of the zygote results in embryos with a duplication of cell fates, and which arrest several days after fertilization. The objectives of this project are to determine whether the altered polarity of the zygote in *dcl1* and *se* mutants originates in the egg cell, and to characterize the effect of loss of miRNA function in the egg cell and zygote using molecular markers for cellular compartments and cell identity. The results of these experiments will provide important information on the role of miRNAs in establishing the first two cell lineages in plant embryogenesis.

P134 Dynamics of mitochondrial distribution during development and asymmetric division of rice zygotes

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Mitochondria are highly dynamic organelles that actively move and change their localization along with actin filaments during the cell cycle. Studies of mitochondrial dynamics and distribution in plant cells have mainly been conducted on somatic cells, and our understanding about these aspects during the formation and development of zygotes remains limited. In this study, mitochondrial nucleoids of rice egg cells and zygotes were successfully stained by using N-aryl pyrido cyanine 3 (PC3), and their intracellular localization and distribution were demonstrated. Mitochondria in rice egg cells were small and coccoid in shape and were primarily distributed around the nucleus. Upon gamete fusion, the resulting zygotes showed mitochondrial dispersion and accumulation equivalent to those in rice egg cells until 8 h after fusion (HAF). Around 12 HAF, the mitochondria started to disperse throughout the cytoplasm of the zygotes, and this dispersive distribution pattern continued until the zygotes entered the mitotic phase. At early prophase, the mitochondria redistributed from dispersive to densely accumulated around the nucleus, and during the metaphase and anaphase, the mitochondria were depleted from possible mitotic spindle region. Thereafter, during cell plate formation between daughter nuclei, the mitochondria distributed along the phragmoplast, where the new cell wall was formed. Finally, relatively equivalent amounts of mitochondria were detected in the apical and basal cells which were produced through asymmetric division of the zygotes. Further observation by treating the egg cell with latrunculin B revealed that the accumulation of mitochondria around the nuclear periphery in egg cells and early zygotes depended on the actin meshwork converging toward the egg or zygote nucleus.

P135 *STK* target genes crosstalk in *Arabidopsis thaliana* ovules and seed development

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The lifecycle of flowering plants relies on essential events of fertilization and seed formation. This vital element – the seed – will maintain and multiply the plant's progeny.

Over the years the studies on ovule and seed development underlined the undeniable function of the integuments during several stages. The developing gametophyte, the seed coat and its fertilization products interact continuously, although it remains uncertain how the crosstalk between the new generation and the mother plant is settled.

During ovule and seed developmental stages, the MADS-box transcription factor SEEDSTICK (STK) plays a vital role since the ovule starts to develop until the mature seed is established. It is expressed in the placental tissues and along the ovule primordia. When the integuments begin to evolve, it appears in the nucellus and funiculus and, during seed mature stage, is detected in the funiculus, outer integument and in the inner integument outer layer.

STK has recently revealed its direct target genes, by RNA and CHIP sequencing analysis, being one of them the arabinogalactan proteins (AGPs), known for long time to be involved in several plant development processes. AGPs are the perfect allies along the study of the reproductive structures, as they are basal units of plant cell walls, performing common functions in cell differentiation and organogenesis.

In this work, it was possible to observe that *stk* plants produce smaller and rounder seeds, less mature green seeds per silique and shorter siliques than WT plants. Moreover, their funiculus abscission zone does not constrict. The expression levels and patterns of *AGP9, AGP21, AGP24 and AGP31*, putative STK target genes, were quantified and observed in WT and *stk* plants. The results point towards a down-regulation of the *AGP31* gene in the *stk* plants, supported by the absence of its expression in *stk* mutant flowers, similarly to the *AGP24* gene.

P136 The Role of HAP2 in fertilization and initiation of fruit development in tomato

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As the human population grows and climate change progresses, it is increasingly important to understand the molecular and cellular mechanisms responsible for food production. HAP2 is a broadly conserved, sperm-expressed, transmembrane protein that drives gamete fusion in *Arabidopsis* and is thus critical for seed and fruit production in that model species. However, HAP2 and gamete fusion have not been studied in crop plants so we do not yet understand the relationship between gamete fusion and seed and fruit production in species that produce our food. We used CRISPR-CAS9 to generate *hap2* loss-of-function mutants in tomato, Solanum lycopersicum. CRISPR-CAS9 transgenic plants actively producing *hap2* mutations are completely sterile: flowers abort by abscission at a specialized structure in the pedicel and fruits fail to initiate. These results suggest that a threshold number of gamete fusion events within the ovary is required to initiate signaling to the pedicle that blocks floral abscission. We have established two loss-of-function alleles that we maintain as heterozygous plants; one is an early frameshift, the other an in-frame deletion of 77 N-terminal amino acids. Analysis of genetic transmission in crosses between heterozygous mutants and wild-type plants suggest that mutant alleles completely block sperm function. We are currently focused on using tomato *hap2* mutants to determine the molecular pathways initiated by gamete fusion that promote fruit initiation and production.

P137 Key regulatory genes involved in the sporophyte and gametophyte development in *Ginkgo biloba* ovules revealed by *in situ* expression analyses

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In *Arabidopsis thaliana*, the role of the most important regulatory genes involved in ovule development is widely known. In non-model species, and especially in gymnosperms, the ovule developmental processes are still quite obscure. In this study, we characterized the expression domains of several key developmental genes in *Ginkgo biloba* ovules. In particular, we have studied *AGAMOUS* (*AG*), *AGAMOUS-like 6* (*AGL6*), *AINTEGUMENTA* (*ANT*), *BELL1* (*BEL1*), *Class III HD-Zip*, and *YABBY* Ginkgo genes. We analysed their expression domains through in situ hybridizations on two stages of ovule development: the very early stage that corresponds to ovule primordium, still within wintering buds, and the late stage at pollination time. We have provided an overview of the changes in the expression domains of these key regulators that occur during Ginkgo ovule development. The major findings are 1) the studied expression patterns of these orthologous genes reveal that they may generally play similar functions during ovule development also in distantly related species such as Ginkgo and Arabidopsis, despite the differences among their reproductive structures. 2) The possible control of the sporophyte on the gametophyte development could also be acting in Ginkgo, since the expression of most of these regulators is circumscribed in the female gametophyte region or in the contact area between the sporophyte and the developing gametophyte at pollination time. This observation has lead us to hypothesize that there is a possible communication between the sporophyte and the gametophyte, as occurs for Arabidopsis. This kind of studies may offer a contribution to the discussion on the evolution of the gene network that regulates ovule development across seed plants, considering also its impact on the morphological evolution of land plant reproductive structures.

P138 Regulation of tomato fruit growth and development by a plant-specific splicing regulator

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Following fertilization, fleshy fruit development proceeds initially by cell division and then by cell expansion in ovaries to yield commodities like tomato. The molecular mechanisms underlying fruit development are tightly regulated and involve various pathways which are controlled at multiple levels of regulation of gene expression. Different developmental stages are characterized by alterations in transcriptome and proteome landscapes. As in many developmental processes, a large number of genes is alternatively spliced during fruit development. What are the central regulators of alternative splicing in tomato fruits and what is their function, particularly in the early stages following fertilization? We used tomato (*Solanum lycopersicum* cv. Moneymaker), a model organism for fleshy fruit development, and generated mutants for two plant specific Serine/Arginine-rich proteins, namely RS2Z35 and RS2Z36 by CRISPR/Cas9. Members of the SR-protein family have been shown to act as the major splicing regulators and these two genes show increased transcript levels in early stages of fruit development. Despite their high similarity in sequence, only mutants of RS2Z36 were affected in their fruit shape compared to the wild type or *CR-rs2z35* mutant. *CR-rs2z36* mutants exhibited a more elongated fruit with a significantly higher fruit shape-index (max. height to width). Furthermore, this increase could be traced down to the earliest stages of development (2-4 days after anthesis). Histological analysis also revealed an increased shape-index of cells at the stage of anthesis in the pericarp, explaining the overall elongation in the mutant fruits. Transcriptome analysis revealed that RS2Z36 affects the transcript levels but also the alternative splicing of many genes. Particularly interesting, many of RS2Z36 targets code for cell wall related proteins. Thereby our results highlight the importance of a splicing regulator for the early stages of fruit development.

P139 Unveiling new STK interactors in Arabidopsis funiculus during seed development

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Seeds are intricate units, vital not only to establish the next plant's sporophytic generation, but also important to Humans, as their major source of calories. It is of pressing demand clarify the processes inherent to plant's sexual reproduction in order to increase high quality seed production.

The funiculus is a stalk structure that connects the maternal plant to the developing seed, allowing the direct transport of nutrients, sugars and signals. Even though this sporophytic tissue has an important biological function, the current knowledge on the genetics and molecular processes involving the funiculus is still limited. Mutants' morphological analysis revealed that alterations in the funiculus engender deformations on the overall developmental pattern of the seed. *seedstick* (*stk*) mutants showed thicker and longer funiculi compared to the wild type (wt), along with smaller seeds and a defective seed abscission zone, which results in decreased seed dehiscence. STK encloses for a MADS-box transcription factor and continues to be one of the few genes known to affect funiculus development.

Using an RNA-sequencing approach, we identified several genes deregulated on the *stk* funiculi and enriched for cell wall biogenesis. The transcriptome was validated by quantitative PCR and candidate genes were selected for further analysis. The results obtained so far from the phenotypic analysis as well as from the promoter reporter lines point to a role of these genes in fruit/seed development and/or seed abscission. This study will contribute to decipher the molecular regulation of STK in the funiculus and how is it affecting the development of the seed.

P140 Diversity and functions of monounsaturated fatty acids in seeds of the Brassicaceae family

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Collectively, oleaginous seeds display a wide range of fatty acid structures. The acyl-acyl carrier protein desaturases (AADs) are plant-specific desaturases that contribute to this diversity by introducing carbon-carbon double bonds (also referred to as unsaturations) at various positions within the aliphatic chains of saturated fatty acids, yielding monounsaturated fatty acids (MUFAs). The omega (ω) nomenclature indicates the number of carbon atoms separating the double-bound from the methyl end of the acyl chain. Different classes of MUFAs can thus be defined: the ω-9 fatty acids are abundant in seed oils of most oleaginous species, the unusual ω-7 fatty acids are specifically stored in the endosperm of *Arabidopsis thaliana* and *Brassica napus*, two members of the Brassicaceae family. While the uses of these different MUFAs exhibiting contrasted physicochemical properties have increased over the last decade, their in planta functions remain poorly understood. We are currently exploring the genetic diversity of the AADs and the biochemical diversity of the MUFAs they produce by coupling genome analysis with transient expression of AAD coding sequences in leaves of Nicotiana benthamiana before fatty acid profiling by GC-MS. In parallel, we characterize the diversity of fatty acids stored in seeds of different species of the Brassicaceae family. Considering that previous studies have revealed contrasting fatty acid profiles for embryo and endosperm oils, we analyze the endosperm fraction separately from the embryo. These analyses have shown that ω-7 fatty acids are abundant in the endosperm of many Brassicaceae seeds. Our research perspectives will consist in investigating the biological functions of these unusual MUFAs during seed development, storage, and germination.

P141 Analysis of seed coat-imposed dormancy in pea

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In angiosperms, the mature seed consists of the embryo, seed coat (SC) and in many cases, the endosperm. All these compartments collaborate during the seed development. The embryo represents the next generation, the endosperm is nourishing tissue, and the maternal plant contributes the protective and dispersal functions of the seed coat and pericarp. In contrast to knowledge about embryo and endosperm we have relatively little knowledge on SC especially in legumes. The maternally derived SC is responsible, in part, for the evolutionary success of the seed. The fact, that the SC is the only protective barrier separating the embryo from external environment, makes it essential for seed survival. In wild legume species, the SC regulates dormancy by restriction of water and gas income. Little is still known about the molecular basis of the dormancy evolution and SC function especially in case of physical dormancy as found in legume. Moreover, dormancy related to SC permeability was removed during the legume domestication .

We isolated and sequenced RNA from SC samples of 5 developmental stages (13-17-23 and 28 days after pollination and dry stage) of 4 contrasting pea genotypes (cultivated cv Cameor, JI92 and wild JI64, JI1794). We identified differentially expressed genes in SC between wild and cultivated genotypes during the seed development. In addition, we identified differences in SC anatomy. Water loss experiment was performed to compare SC permeability between genotypes. In pea, dormancy causative factors connected with SC permeability are yet to be identified.

The knowledge on seed coat mediated imbibition would help to secure uniform germination, which is important for subsequent crop establishment and yield. The work provides important knowledge and can be extended to other economically important legumes such as chickpea, lentil and soybean.

P142 BBM regulates IAOx metabolic pathway genes

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The BABY BOOM (BBM) AINTEGUMENTA-LIKE AP2/ERF domain protein is expressed during embryo and root development, where it promotes (meristematic) cell proliferation and tissue patterning. Ectopic expression of *BBM* induces dose-dependent cell proliferation in Arabidopsis seedlings. A relatively low level of *BBM* represses cell proliferation, a medium level induces organogenesis and a high level, somatic embryogenesis. We used RNA-seq to and dexamethasone + cycloheximide-treated *35S::BBM-GR* seeds to identify early BBM transcriptional target genes. Three genes in the indole-3-acetaldoxime metabolic pathway (*IAOx*), *PAD3*, *FOX1*, *CYP82C2*, were down-regulated in imbibed seeds after 8 hours of BBM-GR activation. The IAOx pathway is found exclusively in the Brassicaceae. IAOx can be converted into IAA and defense metabolites like camalexin and glucosinolates. Loss-of-function mutants for *FOX1* and *PAD3* reduced somatic embryo formation and increased ectopic shoot formation in a *35S::BBM-GR* background. These results suggest that *PAD3* and *FOX1* expression levels define BBM-induced developmental outcomes and that modulation of IAOx metabolism can alter plant developmental processes.

P143 Unravelling the process of thermoregulation during the seed development in *Brassica napus*

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Global warming and its effect on crop yield are among the most significant problems faced in the 21st century. Over the past decades, the gradual rise in global temperatures has reached above 1°C, affecting the yield of important crops like wheat, rice, and maize. *Brassica napus* (rapeseed or canola) comes from the agronomically important Brassicaceae plant family and is the second most widely produced oilseed worldwide. Studies on the development of plants with rising temperature conditions provide knowledge about the temperature influence on crop yield. This project studies the development of three *B. napus* cultivars, Westar, Topas, and DH12075, in three temperature regimes, 21°C, 28°C, and 34°C, in long-day conditions. Characterizing the thermomorphogenesis of *B. napus* grown in long-term heat stress conditions identified accelerated plant growth, reduced fertilization rate and increased seed abortion rate. The accelerated embryo development, defective embryo and seedling development, and pre-harvest sprouting phenotypes, probably resulting from reduced seed dormancy, reduce the viable seed set. From an RNA-Seq analysis and hormonal profiling data followed by RT-qPCR studies, we identified that the ABA and auxin hormonal pathways are misregulated in plants grown at high temperatures. Interestingly, some of the genes involved in the pre-mRNA splicing are upregulated in heat-stressed plants. Also, seed oil content measurements show the reduced quality of seeds developed at higher temperature conditions. Further studies in this research area will pave the way toward producing thermotolerant varieties of *B. napus* with better crop yield, thus contributing to improved global food production.

P144 Impact of temperature during vernalisation and seed development on *Brassica napus* seed yield

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In *Brassica napus*, seed properties including seed size, the number of seeds produced, and seed oil content are all factors contributing to the yield. The effects of temperature on seed properties have been seen in *Arabidopsis thaliana*, but analogous work has yet to be carried out in Brassica crops. It is known that yield stability in Brassica napus is impacted by temperature during seed development and vernalisation. For oilseed rape production to withstand the year-on-year variation in temperature caused by climate change, it is vital to produce crops with reliable yield across a wider temperature range. In this work, phenotyping and transcriptome approaches have been used to work towards a solution to this issue.

In a large-scale phenotyping experiment, a *Brassica napus* diversity set was grown under different combinations of vernalisation and seed maturation treatments, allowing the effects of each aspect to be studied independently as well as for associative transcriptomics to be used to identify genes related to differences in response.

From this experiment, it was found that seed size in *Brassica napus* is significantly impacted by temperature during vernalisation and seed maturation, although the effect of vernalisation length was not significant when considering the diversity panel as a whole. These effects were also found for the number of seeds produced per pod. The overall trend revealed in this experiment suggested that warmer temperatures (above 10°C during vernalisation and 24°C during seed maturation) had a detrimental effect on seed yield parameters. However, differences in temperature response were also found to exist between different crop types of Brassica napus.

Through genetic analysis, genetic regions linked to changes in seed weight and number have been identified. Work is ongoing to identify the genetic pathway involved in the effect of temperature on thousand grain weight and seed number.

P145 Effect of paternal genome excess on the developmental and gene expression profiles of polyspermic zygotes in rice

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Fertilization is a characteristic event of eukaryotic unicellular and multicellular organisms that combines male and female genetic materials for the next generation. In the diploid zygote generated by the fusion between haploid male and female gametes, parental genomes function synergistically to ensure the faithful progression of zygotic development and embryogenesis. In previous study, the effects of parental genome imbalance on zygotic development were clarified by producing polyploid zygotes with an imbalanced parental genome ratio via the in vitro fertilization of isolated rice gametes. The developmental profiles of the polyploid zygotes showed that polyploid zygotes with a paternal gamete/genome excess exhibit arrested development, whereas polyploid zygotes with a maternal excess develop normally. These observations indicate that paternal and maternal genomes synergistically influence zygote development via distinct functions. In this study, to clarify how paternal genome excess affects zygotic development, the developmental and gene expression profiles of polyspermic rice zygotes were analyzed. The results indicated that polyspermic zygotes were mostly arrested at the one-cell stage after karyogamy had completed. Through comparison of transcriptomes between diploid and polyspermic zygotes, 36 and 43 genes with up-regulated and down-regulated expression levels, respectively, were identified in the polyspermic zygotes relative to the corresponding expression in the diploid zygotes. Notably, *OsASGR-BBML1*, which encodes an AP2 transcription factor possibly involved in initiating rice zygote development, was expressed at a much lower level in the polyspermic zygotes than in the diploid zygotes. Thus, the developmental dysfunction of polyspermic zygotes was predicted to be due to the misexpression of genes important for initiating zygotic development.

P146 Identification and functional characterization of the RNA-binding proteome during early embryogenesis in *Arabidopsis*

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The determination of cell fate depends to a large extent on post-transcriptional gene regulation in eukaryotic cells and involves RNA processing, transportation, localization, translation, and degradation. These processes are controlled by various RNA-binding proteins (RBPs), which interact with RNAs and form ribonucleoprotein complexes (RNPs). We have detected RNPs containing *WUSCHEL RELATED HOMEOBOX2* (*WUS2*) and *WUS8* mRNAs, which localize to granule structures in zygotes and early embryos. Moreover, these granules showed a polar distribution in apical and basal cells of the globular embryo. However, the composition of these granules and the regulatory mechanism of the RNA interactome involved in RNA localization and asymmetric division of the Arabidopsis zygote and early embryo is still unclear due to technical limitations. In this study, we used the construct pH_*35Sp:RKD2-GFP* to induce the formation of calli, which display an egg cell/zygote/stem cell-like transcriptome (“RKD2-callus”; Sprunck et al., 2019; bioRxiv). Here, we report the RNA binding protein repertoire of this RKD2-callus. In total, 37 RBPs were detected in non-crosslinked tissue, while 359 RBPs could be identified after UV-crosslinking. Furthermore, we found that ~82% of these are predicted to be involved in RNA processes and 126 RBPs are linked to RNP formation. We further show that 15 RBPs are significantly downregulated while 27 RBPs are upregulated 14 h after fertilization. We anticipate, that this RNA interactome capture method in the RKD2-callus will provide more clues to identify WOX-related RNPs, which were also detected in the RKD2-callus. The latest results from this study will be presented.

P147 Optimizing methods for phenotyping and expression pattern analysis in developing seeds of *Arabidopsis* and *B. napus*

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The efficiency of plant reproduction, including seed development, is crucial for crop production. Early stages of embryo development may be affected or stopped by stress factors such as fungal infections, lack of water in the soil, or high temperatures during reproduction, leading to a drop in yield. Therefore, scientists need tools to study and visualize the changes in development caused by these factors.

Imaging embryonic development in *Arabidopsis thaliana* or *Brassica napus* is not as simple. Phenotypic analyses are routinely performed in Arabidopsis after clearing the seeds a few hours with a chloral hydrate solution, using a DIC-equipped light microscope. However, in *B. napus*, the clearing of young seeds may take a few weeks to observe a heart-staged embryo. We attempted to develop a protocol to visualize the *B. napus* young embryo within its seed by X-ray computed microtomography (µCT). The samples were stained with iodine after alcohol fixation allowed for the visualization of the seed structures within the seed coat without any cellular resolution in the embryo.

The challenge is even more significant when studying the expression pattern of fluorescently tagged proteins in a whole seed. The seed coat possesses various apoplastic barriers and produces secondary metabolites that render the seed difficult to clear while maintaining the integrity of the fluorescence. We modified the ClearSee Alpha protocol for whole seeds up to the embryonic torpedo stage in *Arabidopsis* and the globular stage in B. *napus* to visualize fluorescent protein expression. ClearSee Alpha clearing also appears to be better than chloral hydrate for clearing *B. napus* seeds for morphology analysis.

This work presents a few methods for imaging embryos in whole seeds in both species.

P148 RNA-seq analysis for identification of novel regulators of asymmetric division of *Arabidopsis* zygote

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The body axis formation is essential process for plant development. *Arabidopsis* zygote is highly polarized after fertilization and divide asymmetrically to become 1-cell embryo. The apical-basal axis is established by this division. However, the molecular mechanism of asymmetric division is almost unknown because few mutants have been identified due to genetic redundancy and embryo lethality.

We recently found that transcriptional activation of post-fertilization is crucial for zygote polarization. WRKY2 transcription factor activated by paternal MAP kinase signaling, cooperating with maternal transcription factors HDG11/12, promotes the transcription of regulators asymmetric division. *wrky2 hdg11/12* zygote show severe defect of asymmetric division. Therefore, we aim to identify genes failed to be transcribed in *wrky2 hdg11/12* zygote as key factors of asymmetric division by RNA-seq analysis. To achieve that, we established the method to isolate 1-cell embryos from the ovule. We screened several mutants of downregulated genes in *wrky2 hdg11/12*, and successfully identified several candidates of key regulator.

P149 Towards the function of the embryonic imprinted gene *mee1* in maize

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Genomic imprinting is an epigenetic phenomenon that leads to the biased expression of alleles depending on their parental origin. In flowering plants, imprinted gene expression occurs mainly the endosperm and to a lesser extend in the embryo. While imprinted expression of some genes is essential for reproductive success changes in expression bias of the majority has no obvious phenotypes. However, the biological roles of the majority of imprinted plant genes are unknown. Elucidation of their function is important to get a better understanding on the evolution and biological significance of plant imprinted genes. In contrast to most plant imprinted genes, *maternally expressed in embryo 1* (*mee1*) shows a highly restricted expression pattern. *Mee1* is exclusively expressed in central cells and from the maternal allele in young filial organs. The maize genome encodes a single copy of mee1 on chromosome 5. Homology searches with the hypothetical MEE1 protein of 99 amino acids in length indicated it to be angiosperm-specific.

In situ immunolocalization with a polyclonal antibody, raised against a N-terminal peptide of the putative *MEE1* sequence, revealed highly specific signals in filial tissue and central cells, indicating that mee1 actually encodes a protein. Reduced expression of *MEE1* by RNAi in transgenic plants led to defective, aborting seeds, indicating that MEE1 is essential for seed development. Transient expression of *MEE1:GFP* fusion proteins in aleuron cells revealed a localization in multi vesicular bodies (MVB) and a colocalization with catalase and peroxisomes. Immunolocalization combined with TEM supported the localization in MVB and indicated lipid bodies to be involved. Together these initial results might indicate an involvement of MEE1 in specialized fatty acid metabolism or storage initiation during seed development. MVB association and highly restricted expression would also be compatible with functioning in signaling networks in maternal-filial organ development.

P150 Unravelling ovule developmental patterns in gymnosperms: involvement of *KAN*, *BEL* and *C3HDZ* genes in the development of the ovule in *Taxus baccata*

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Ovule and seeds development are crucial processes in seed plant reproduction. Most of the studies at molecular level have been focused to describe developmental mechanisms in ovule and seed formation in angiosperms. However, for a complete comprehension of the evolutive trajectories that have led to seed plants, gymnosperms have to be included. In this context, the study of *Taxus baccata* suggests once more that gymnosperms could have ovule and seed peculiar developmental patterns, involving many families of regulator genes. Some of these are known to be involved in the regulation of the development in different plant groups along the lineage of vascular plants. During a collaboration between the Botanical Garden of Padova and the NY Botanical Garden in the frame of the MSCA-RISE project EVOfruland, we focus our attention to the conserved families of *KANADI*, *BEL* and *Class III HD ZIP*. Genes belonging to these families have been recognized in the ovule transcriptome obtained from *Taxus baccata* reproductive and vegetative material both from Padova (IT) and from NY (US). In situ hybridization experiments have demonstrated that some of these genes are expressed during the early-stage development of the ovule with partially overlapping domains. These gene are particularly expressed in correspondence of actively proliferating tissues, and differently to what observed in angiosperms, the expression of these genes is not clearly associated with an in-out tissue differentiation, but instead with an apical-basal tissue differentiation. These observations, together with those obtained from other gymnosperm species, can contribute to understand the developmental patterns subtending ovule formation in gymnosperms.

P151 Studying the role of nascent polypeptide-associated complex subunit β on seed germination under stress conditions

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Nascent polypeptide-associated complex (NAC) is a heterodimeric complex composed of an α- and a β-subunit. It binds to the newly synthesized polypeptide chains emerging from the ribosome in the eukaryotic cells. In our experiments, *Arabidopsis thaliana* was chosen for studying the function of the NAC complex due to its well-annotated genome, which contains five homologous genes encoding the α-subunit and two homologues for the β-subunit.

The NACβ function was studied in more detail. The double homozygous mutants in both *NACβ* genes (that carried no functional allele of any *NACβ* gene) were acquired by conventional crosses of two publicly available T-DNA insertion lines. These homozygous mutants showed several phenotypic differences from wild type plants: they had shorter siliques containing less seeds, their flowers contained an abnormal number of flower organs, and their leaves were light green because they contained less chlorophyll than the wild type ones. Moreover, the lines with overexpressed *NACβ1* or *NACβ2* under 35S promoter were obtained that did not show any notable phenotype.

To further investigate the growth and development of the *nacβ1nacβ2* double homozygous mutants and the lines overexpressing *NACβ1* or *NACβ2*, we tested seed germination of these plants under various abiotic stresses. The seeds were sown in vitro on half Murashige-Skoog (MS) medium with several concentrations of mannitol or sodium chloride. As a control, a parallel germination of plants was observed on half MS medium without added stressing substances. As such, these experiments proved the influence of the absence or overexpression of NACβ subunit on seed germination rate under various abiotic stresses.

P152 The supernumerary B chromosomes of *Aegilops speltoides* undergo precise elimination in roots early in embryo development

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Not necessarily all cells of an organism contain the same genome. Some eukaryotes exhibit dramatic differences between cells of different organs, resulting from programmed elimination of chromosomes or their fragments. Here, we present the first analysis of programmed chromosome elimination in plants. Using the goatgrass *Aegilops speltoides* as a model, we demonstrate that the elimination of B chromosomes is a strictly controlled and highly efficient root-specific process. At the onset of embryo differentiation B chromosomes undergo elimination in proto-root cells. Independent of centromere activity, B chromosomes demonstrate nondisjunction of chromatids and anaphase lagging, leading to micronucleation. Chromatin structure and DNA replication differ between micronuclei and primary nuclei and degradation of micronucleated DNA is the final step of B chromosome elimination. This process might allow root tissues to survive the detrimental expression, or overexpression of 23 B chromosome-located root-specific genes with paralogs located on standard A chromosomes.

P154 The effect of micropylar endosperm on early embryo development

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Proper seed development requires coordinated growth among the three genetically distinct components, the embryo, the endosperm, and the seed coat. This growth coordination is partly achieved through the interaction between the embryo and the surrounding endosperm. Like many angiosperms, Arabidopsis has a nuclear type endosperm. During the initial syncytial stage, the endosperm differentiates into three functional domains: micropylar, peripheral, and chalazal domains. The subsequent endosperm cellularization requires the Fertilization-Independent Seed (FIS)-Polycomb Repressive Complex 2 (PRC2). After endosperm cellularization, the endosperm ceases to grow and is eventually absorbed by the embryo. We discovered two putative invertase inhibitors (InvINH1 and InvINH2) that are involved in the acceleration of embryo growth after endosperm cellularization. *InvINH1* and *InvINH2* are preferentially expressed in the micropylar endosperm that surrounds the embryo. After endosperm cellularization, *InvINH1* and *InvINH2* are down regulated in a FIS-PRC2-dependent manner. We hypothesized that FIS-PRC2 complex represses *InvINH1* and *InvINH2* to increase invertase activity around the embryo, making more hexose available to support the accelerated embryo growth after endosperm cellularization. In support of our hypothesis, embryo growth was delayed in transgenic lines that ectopically expressed InvINH1 in the cellularized endosperm. We also discovered a group of transcription factors as the regulatory connection between the FIS-PRC2 complex and *InvINH1*. Our data suggested a novel mechanism for the FIS2-PRC2 complex to control embryo growth rate via the regulation of invertase activity in the micropylar endosperm.

P155 Impacts of heat stress on *Medicago* seed maturation

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Legumes are important crop species as they can produce highly nutritious seeds for human food and animal feed. Production of high-quality seeds (e.g. high germination rate, germination vigor, long storability) represents a key challenge to improve crop production. In a climate change context, it becomes even more crucial, as the acquisition of these seed quality traits is highly sensitive to environmental conditions, which impact seed and seedling performances.

In *Medicago*, we showed that seed quality traits are acquired during seed maturation at specific developmental stages. Moreover, sub-optimal conditions during seed development impact the timings of acquisition of different seed maturation processes, leading to dramatic changes of germinative quality (i.e. rate, vigor and homogeneity) and seed storability. The goal of the project is to decipher the impacts of heat stress on Medicago seed maturation leading to final seed quality.

In our study, we first identified physiological impacts of heat stress in mature seeds in relation to temporal (i.e. different developmental stages) and spatial (i.e. different seed tissues) changes of transcriptomes and epigenomes in order to unravel seed stress responses. Then, in parallel, we performed a genome-wide association study with the *Medicago* HAPMAP collection to identify genes associated with heat seed stress tolerance or sensitivity. Finally, by combining both approaches, we intend to identify and functionally characterize key genes related to heat stress response or heat stress adaptability in seeds.

P156 Time to sleep or to germinate? A case of legumes seed dormancy.

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Timing of seed germination is one of the key steps in plant life. It determines when plants enter natural or agricultural ecosystems. Plants have evolved various mechanisms to control the entry of the quiescent seed protecting embryo into vulnerable environment. Understanding of the genetic basis of local adaptation has relevance to climate change, crop production as well as understanding of the speciation. Along with other traits, seed dormancy has been removed during domestication. We have used a comparative anatomy, metabolomics and transcriptome profiling of pea seed coats in order to identify changes and genes associated with loss of seed dormancy in relation to domestication. In parallel, we tested adaptation to environmental conditions influencing dormancy release and the timing of legume seed germination, using wild pea (*Pisum sp.*) with relevance to crop and Medicago truncatula models. Level of Medicago seed dormancy correlated with increased aridity, suggesting that plastic responses to external stimuli provide seeds with strong bet-hedging capacity and the potential to cope with high levels of environmental heterogeneity. Similarly, in pea, dormant accessions were found in the environment with higher annual temperature, smaller temperature variation, seasonality and milder winter. Genome-wide association analysis of sequenced Medicago lines identified candidate genes associated with dormancy release related to secondary metabolites synthesis, hormone regulation and modification of the cell wall. Analysis of chemical composition of pea seed coat using mass spectrometry identified differences in the profile of proanthocyanidins, glycosylated flavonoids and fatty acids, related to impermeability for water. RNA sequencing identified several dozen differentially expressed genes between dormant and non-dormant pea seeds, and genome wide approach applied to RIL mapping population yielded candidate loci

regions. This analysis has been recently extended to chickpea and lentil crops, and has therefore applicability to other economically important legume species.

P158 Dynamics of endoreplication and programmed cell death in developing barley seeds

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Seeds are complex biological systems comprising three genetically distinct tissues nested one inside another, i.e. embryo, endosperm, and maternal tissues. Cereal grains represent a special type of seeds, with the largest part being formed by endosperm, a specialized triploid tissue for embryo protection and nourishment. We used the diploid cereal crop barley to study the dynamics of nuclear ploidy and programmed cell death (PCD) of the three major seed tissues from pollination until fully desiccated state. We found that the cell cycle was under strict developmental control during seed formation. After an initial wave of active cell division, some endoreplication occurred in all seed compartments. However, most endoreplication was found in the endosperm, where, specific and selective loss of endoreplicated nuclei occurred during terminal stages of seed development. This was accompanied by a reduced endosperm nuclear genome stability and progressive loss of cell viability. Our study shows, that the nuclear ploidy changes and cell death in endosperm and non-endosperm tissues are linked phenomena that frame the grain development in cereals.

P159 Maternal genome dominance in early *Arabidopsis* embryogenesis

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Previous studies have found conflicting results regarding the relative contributions of the maternal and paternal genomes to early *Arabidopsis* embryogenesis. Using *embryo defective* (*emb*) mutants to test for functional contributions of maternal and paternal alleles, we show that for 30 of 35 different *EMB* genes, the maternal allele plays a more important role in early embryogenesis than the paternal allele. Since parental contributions to early embryogenesis have previously been shown to vary between hybrids, we used these 35 *emb* mutants to test for the effect of hybridization of different ecotypes on the penetrance of maternal effects. Our tests of Col x Ler, Col x Cvi, Col x Bur and Col x Tsu show that the Col x Tsu hybrid is functionally almost indistinguishable from isogenic Col, and that Col x Ler and Col x Cvi hybrids are significantly different from isogenic Col. From these results, we conclude that the Col x Tsu hybrid is a good proxy for producing allele-specific transcriptomes with parent-of-origin contributions similar to isogenic Col.

We generated and analyzed embryo transcriptomes using the Col x Tsu hybrid. Zygote-1cell and octant stage embryos showed thousands of genes with reciprocal maternal transcript bias, regardless of the direction of the cross. This maternal bias decreased strongly at the globular stage, and was absent at later stages. Interestingly, the maternal transcript bias in zygote-1cell and octant embryos showed no correlation with the ratio of egg and embryo transcript abundance, suggesting that there is preferential transcription of maternal alleles after fertilization. In agreement with this, we found that reads mapping to sequence variants in introns (a proxy for de novo transcription) showed a strong maternal bias in pre-globular embryos. Together, these results support a predominant role for the maternal genome in early *Arabidopsis* embryogenesis.

P160 Response of high-moisture cowpea (*Vigna unguiculata*) seeds to drying temperature

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Cowpea is characterized by low field emergence percentage and this has been associated with field deterioration that occur subsequent to attainment of maximum seed quality but before the seeds are considered safe for harvesting and post-harvest handling. Physiological maturity of cowpea seeds occur at high seed moisture content, about 46%, which potentially could predispose the seed to drying shocks and poor physical characteristics. The response of such high-moisture seeds to varying drying temperature were evaluated with respect to biochemical composition and physiological quality. Seeds were harvested at three maturity stages at 18, 21 and 24 DAA corresponding to 63, 46 and 38% seed moisture content, respectively. Both fresh and dried seeds were subjected to laboratory tests to determine moisture content, viability, vigour and biochemical composition. Fresh seeds were subjected to 3 drying temperature regimes of 40, 45 and 50oC for 48 h. Seeds harvested at 21 DAA had the highest ($P<0.05$) standard and accelerated ageing germination percentages. For any maturity stage, seeds dried at 40°C had higher ($P<0.05$) crude protein and lower ($P<0.05$) carbohydrate contents and vice versa for seeds dried at 50°C. For seeds harvested at 21 DAA, viability was not affected when seeds were dried at 40°C. However, a 5°C increase in drying temperature from 40 to 45°C resulted in a 6% loss in vigour as measured by accelerated aging germination. High moisture cowpea seeds harvested at moisture contents below 50% could be safely dried at 40°C without any impairment of physiological quality.

P161 Distinct parental mechanisms of receptor pathway activation shape the *Arabidopsis* embryo

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In flowering plants, many asymmetric cell divisions are controlled by a MAP kinase signaling pathway including the MAP3K YODA. In the early *Arabidopsis* embryo, this YODA-dependent pathway is activated by two distinct mechanisms: by canonical activation of an evolutionarily conserved receptor complex and by the Brassicaceae-specific membrane-associated protein SHORT SUSPENSOR that activates *YODA* independent of receptor activation.

We will present genetic data that clearly identifies the receptor kinase that functions upstream of *YODA* in the early embryo. Furthermore, we will give mechanistic insight in the evolution of this receptor kinase/MAP kinase signaling pathway as well as the regulation of YODA signaling on a molecular level. In addition, we will discuss the distinct parental contributions to embryonic patterning and their possible selective benefits.

P162 The role of pollination in controlling *Ginkgo biloba* ovule development

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The main molecular mechanisms and genes responsible for the switch from ovule integuments into seed coat have been studied in *Arabidopsis thaliana* in which the process is activated upon the fertilization of the central cell within the female gametophyte. By contrast, in gymnosperms, the processes that trigger the switch from ovule into seed integument are still unknown. Besides, in gymnosperms pollination and fertilization events are temporally separated, in particular in *Ginkgo biloba* four months passes between the two. Interestingly, the single ovule integument of Ginkgo acquires the typical features of the seed coat long before fertilization takes place. That suggest that the pollen arrival could be the crucial event that leads to the further progression of the ovule development and to the subsequent transformation of the ovule integument into the seed coat. A morphological atlas describing the stages of ovule development is presented, and its production was essential for molecular studies. We investigated whether pollination triggers the transformation of the ovule integument into the seed coat by performing transcriptomics and metabolomics analyses on ovules just prior and after pollination. Omics analyses allowed to describe the changes occurring in Ginkgo ovules during this specific time frame, and highlighted the crucial role of the pollen arrival on the progression of ovule development. In particular, soon after the pollination has happened the metabolic pathways involved in the lignin biosynthesis and in the production of fatty acids are activated, suggesting that the middle and the outermost layers of the Ginkgo ovule integument (respectively sclerotesta and sarcotesta) start to differentiate into the seed coat layers long before fertilization. The next step forward is to understand which pathways are activated or down-regulated upon pollen arrival, since we observed that after the pollination time frame has passed, Ginkgo ovules that have not received the pollen abort.

P163 Development and regeneration of wheat–rice hybrid zygotes produced by *in vitro* fertilization system

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Hybridization plays a decisive role in the evolution and diversification of angiosperms. However, the mechanisms of wide hybridization remain open because pre- and post-fertilization barriers limit the production and development of inter-subfamily/intergeneric zygotes, respectively. To bypass these barriers, hybridization between wheat and rice using in vitro fertilization (IVF) system was examined in the study. Several gamete combinations of allopolyploid wheat–rice hybrid zygotes were successfully produced, and the developmental profiles of hybrid zygotes were analyzed. Hybrid zygotes derived from one rice egg and one wheat sperm cells ceased at the multicellular embryo-like structure stage. This developmental barrier was overcome by adding one wheat egg cell to the wheat–rice hybrid zygote. In the reciprocal combination, one wheat egg and one rice sperm cells, the resulting hybrid zygotes failed to divide and degenerated. However, doubling the dosage of rice sperm cell allowed the hybrid zygotes to continuously develop into plantlets. Although the regenerated plants derived from wheat–rice hybrid zygotes were successfully obtained, rice chromosomes appeared to be progressively eliminated during early developmental stage of hybrid embryos. The elimination of rice chromosomes gave rise to the hybrid plants displaying wheat plant morphology and approximately 20% of regenerated plants showed abnormal morphology. These results suggest that hybrid breakdown can be overcome through optimization of gamete combinations, and the present hybrid will provide a new horizon for utilization of inter-subfamily genetic resources.

P164 The importance of *Arabidopsis* PHOSPHOLIPID N-METHYLTRANSFERASE (PLMT) in glycerolipid metabolism and plant growth

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Phosphatidylcholines (PCs) are a major class of phospholipids essential for plant postembryonic growth in plants. In Arabidopsis, three copies of the phospho-base Nmethyltransferases, PMT1, PMT2, and PMT3 are known to account for PC biosynthesis because the triple-knockout mutant is devoid of PC biosynthesis and shows lethality in postembryonic but not embryonic development. Arabidopsis also has another distinct phospholipid N-methyltransferase (PLMT) homologous with yeast and animal PLMT that methylates phospholipids to produce PC. However, the role of this enzyme remains unclear as the knockout mutant of *plmt* does not show morphological defects nor decreased PC content. We demonstrated that PLMT is ubiquitously expressed in different organs and localized at the endoplasmic reticulum (ER), where PC is produced. Overexpression of *PLMT* in planta increased the content of phospholipids including PC and affected the vegetative but not reproductive growth. Although silique lengths were shorter, pollen remained viable and larger fresh seeds were produced. Intriguingly, triacylglycerol content was increased in the mature seeds with altered fatty acid composition, suggesting that PLMT might be a functional enzyme in PC biosynthesis and plays an organ-specific role in developing seeds to supply phospholipids conversion into triacylglycerol where rapid accumulation of storage lipid occurred.

P165 The loss of polyphenol oxidase function is associated with hilum pigmentation and has been selected during pea domestication

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Seeds as means of species survival and dispersal are often deposited in unfavourable environments. While in soil, they need to protect vulnerable embryo from various biotic and abiotic stresses. During evolution, seeds have developed protection by physical, chemical and biochemical means. Besides, domestication has altered the property of the seed coat impacting seed dormancy.

We used a range of genetic, transcriptomic, proteomic and metabolomic approaches to determine the function of pea seed *polyphenol oxidase* (*PPO*) gene during the development of the seed coat of wild, dormant in comparison to cultivated, non-dormant pea genotypes. We show that while in wild, dormant pea seeds, the *PPO* gene is functional and highly expressed, in cultivated, non-dormant pea genotypes the PPO is truncated. The gene and protein expression as well as PPO enzymatic activity is down regulated in domesticated pea. Majority of domesticated peas have allele associated with the loss of hilum pigmentation indicating selection during domestication. The functionality of the *PPO* gene relates to the oxidation and polymerization of gallocatechin in the seed coat. Additionally, imaging mass spectrometry supports the hypothesis that hilum pigmentation is conditioned by the presence of both phenolic precursors and sufficient PPO activity.

This is the first report of PPO functionality in relation to the domestication in legumes and support the findings in rice, barley and foxtail millet. Selection for nutritional and visual seed quality traits acted during domestication and subsequent crop improvement affecting digestion, palatability. It can be expected that similar selection acted in other legume crops such as faba bean or soybean.

P166 Endosperm cellularization is initiated by a family of auxin related factors

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The endosperm is a reproductive tissue derived from the fusion of a haploid sperm cell with a predominantly diploid central cell, which sustains and supports embryo development. Failure in endosperm development results in the arrest of embryo growth and seed abortion.

In *Arabidopsis thaliana*, like in most angiosperms, endosperm development occurs in two phases. In the initial phase, endosperm nuclei proliferate quickly without forming cell walls, resulting in a coenocytic tissue. Then, at a tightly controlled time, a wave of cellularization starts from the micropylar region to reach the chalazal endosperm. At the end of the process, most of the endosperm is cellularized and nuclear divisions cease.

The timing of the second phase is critical for seed development, for reasons that remain unknown. Thus, delayed cellularization like in response to interploidy and interspecies hybridizations results in embryo arrest and seed abortion.

Previous work from our group identified auxin as a critical factor determining the timing of endosperm cellularization. Auxin biosynthesis is initiated from the paternal genome and responsible for the first nuclear division of the endosperm. Interestingly, auxin levels cease at the time of cellularization, while conversely endosperm cellularization failure correlates with increased auxin levels[1,2].

Here, we report the identification of auxin related factors triggering endosperm cellularization. These factors are specifically expressed from the maternal genome and are expressed during a sharp developmental time window corresponding to cellularization initiation. In contrast to auxin, increased expression of these proteins can induce early cellularization, which can initiate outside the micropylar domain. We propose that these auxin related factors act antagonistically to the auxin pathway and are used by the maternal genome to arrest nuclear proliferation of the endosperm, initiating the second phase of endosperm development.

[1] Figueiredo et al, 2015.
[2] Batista et al, 2019.

P167 Regulation of Ovule Initiation in *Arabidopsis thaliana* by EPIDERMAL PATTERNING FACTOR-Like Proteins

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Organ initiation and patterning in plants requires coordinated cell-to-cell communication to control cell growth and differentiation. In *Arabidopsis thaliana*, multiple ovules initiate asynchronously along the elongating placentae from stage 9 to stage 11 of floral development. Previous research has established a role for the phytohormone auxin in governing the site of ovule initiation, and for CUP-SHAPED COTYLEDON (CUC) transcription factors in regulation of ovule spacing. ERECTA family receptor-like kinase signaling via the EPIDERMAL PATTERNING FACTOR-like 2 (EPFL2) secretory peptide ligand regulates ovule density and spacing during Arabidopsis fruit development, but the mechanisms underlying this regulation remain unclear. We further investigated the mechanism of ERECTA family signaling and EPFLs during ovule initiation in Arabidopsis. Our experiments established that EPFL1 and EPFL2 show unique, cell-type specific expression throughout the process of ovule initiation. These two ligands positively regulate ovule number in a partially redundant manner without significantly affecting placenta lengths. To characterize the mechanisms of this regulation, auxin signaling and expression of genes controlling ovule initiation were inspected in the *epfl1 epfl2* mutant. In addition, we used a genetic analysis to investigate molecular mechanism of EPFL function. Ultimately, this research further characterizes the mechanisms by which EPFL-mediated signaling positively regulates the initiation and spacing of ovules in Arabidopsis thaliana.

P168 Towards a better understanding of LEC2 role(s) by functional analysis of variants

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LEAFY COTYLEDON 2 (LEC2) is a transcription factor (TF), known to be a master regulator of seed development and embryogenesis. LEC2 controls various aspects of seed development, from embryo formation to storage compounds accumulation or acquisition of desiccation tolerance. Beside its role in sexual reproduction, LEC2 is also involved in the induction and maintenance of somatic embryogenesis capacity of plant tissues. The very well conserved B3 DNA binding domain has been well described and the B2 protein-protein interaction domain of LEC2 has been characterized in our team. The aim of our work is to obtain a better comprehension of the structure and function of LEC2 thanks to functional analyses in *Arabidopsis thaliana*, transient expression in *Physcomitrella patens* and *Nicotiana benthamiana*, and yeast assays. Prediction data based on the protein sequence, coupled with transient expression in tobacco leaves have allowed us to characterize the nuclear localization domain of LEC2. The transcriptional activation domains (AD) have also been identified through a predictive tool published, ADpred (https://adpred.fredhutch.org/), based on deep neural network and confirmed with yeast transcriptional activation assays. By using a transient expression system in moss protoplasts developed in our lab, allowing the quantification of the transcriptional activity, we have shown that LEC2 is unable to activate its targets without AD (ΔADLEC2). However, the expression of *ΔADLEC2* in *lec2* background displayed a WT phenotypic reversion, leading us to the new hypothesis of a double function for LEC2, with and without activation domains. This double function could explain the roles of LEC2 in somatic embryogenesis in one hand, and in seed maturation in the other. In our model, LEC2 could act as a partner of LEAFY COTYLEDON 1 (LEC1), a pioneer transcription factor able to modify the chromatin and allow accessibility to other TFs, by guiding it towards its targets.

P169 Impacts of heat stress on seed maturation and seed quality

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Production of high-quality seeds (e.g. high germination rate, germination vigor, long storability) represents a key challenge to improve crop production. In a climate change context, it becomes even more crucial, as the acquisition of these seed quality traits is highly sensitive to environmental conditions, which impact seed and seedling performances.

In previous studies, we showed that seed quality traits are acquired during seed maturation at specific developmental stages. Moreover, sub-optimal conditions during seed development impact the timings of acquisition of different seed maturation processes, leading to dramatic changes of germinative quality (i.e. rate, vigor and homogeneity) and seed storability. Here, we will describe physiological impacts of heat stress in mature seeds in relation to temporal (i.e. different developmental stages) and spatial (i.e. different seed tissues) changes of transcriptomes and epigenomes in order to unravel seed stress responses. Then, in parallel, we performed a genome-wide association study with the *Medicago* HAPMAP collection to identify genes associated with heat seed stress tolerance or sensitivity. Finally, by combining both approaches, we identified and functionally characterized a key gene related to heat stress adaptability of seed germination vigor and homogeneity, called *MIEL1*.

P170 Embryo sac development differences in oilseed rape cultivars may influence final seed yield

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The female gametophyte of a seed plant containing the egg cell and the polar nuclei which give rise respectively to the embryo and the endosperm on fertilization, is known as the embryo sac. Its correct development influences fertilization and subsequent seed development. The structure and development of embryo sac has been studied in detail in Arabidopsis thaliana, but less is known about the embryo sac in various crop species.

Brassica napus, also known as oilseed rape, is the second most important oilseed crop worldwide. Understanding its seed development is thereby crucial to maximise seed yield and its productivity. Different *B. napus* cultivars are grown worldwide depending on their environmental requirements, and are classed as Winter, Spring or Semiwinter oilseed rape groups. We studied a total of 9 cultivars with varying yield, 3 from each group, to determine if seed development is impaired at pre- or post-fertilization stages. Our results revealed differences in embryo sac development which may be influencing subsequent successful fertilization. Differences in their embryo development were also observed between the 3 cultivars for each studied group, which may be responsible for very different final seed yield. Moreover, pollen germination, seed number, area and seed distribution within green fully developed pods were analysed, uncovering differences in seed packing. We are currently exploring differences between the main and secondary inflorescences, as well as uncovering different strategies between the 3 oilseed rape groups depending on their vernalisation requirements.

Overall, we conclude that the cultivars from the 3 groups appear to have differences in the development of their embryo sac which might be influencing final seed yield, making this a trait worth to be further studied in more detail. This knowledge can be used to develop better yielding cultivars and hence, improve crop production.

P171 Deciphering the molecular switch of seed desiccation tolerance

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Drought has challenged food security worldwide, urging the development of drought-tolerant crop varieties. Crops do not withstand severe drought at the vegetative stage, but produce seeds that survive extreme dehydration. Seeds maturation is a crucial process during which seeds acquire the capacity to tolerate desiccation. The ability to tolerate extreme dehydration (desiccation tolerance, DT) is tightly regulated, being switched on during seed maturation and off shortly after germination (DT switch). In *Medicago*, the DT is acquired between 16 and 20 DAP and is lost immediately after protrusion of radicle. However, DT can be re-induced during the early stage of germination by applying a mild stress. We identified numerous genes potentially implicated in DT. In *Arabidopsis* leaf, majority of these genes are present in chromatin with a specific state, which is characterized by high level of H3K27me3 and low expression of genes. We propose combining cutting-edge molecular methods on developing seeds and our capacity to re-induce DT in germinating seeds of the model legume, *Medicago truncatula*, to understand the nature and timing of the DT switch. Then, using the same experimental models, we intend to characterize the minimal core set of genes associated with the DT mechanisms, including regulatory genes and pioneer genes to switch on/off desiccation tolerance in plants.

P172 Combinations of maternal-specific repressive epigenetic marks in the endosperm control seed dormancy

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The endosperm is a reproductive tissue specific for flowering plants that has critical roles for seed development by supporting embryo growth. The endosperm is a product of double fertilization, resulting from the fusion of a haploid sperm cell with the diploid central cell. Many endosperm-expressed genes are specifically active only from one of the parental alleles. This phenomenon is termed genomic imprinting and a consequence of parental-specific epigenetic regulation. We could show that paternally-biased gene expression is significantly correlated with the combination of three repressive epigenetic modifications on the maternal alleles; trimethylation of histone H3 on lysine 27 (H3K27me3), dimethylation of histone 3 on lysine 9 (H3K9me2) and CHG methylation (CHGm). Interestingly, H3K27me3 and H3K9me2 are generally not co-occurring in vegetative tissues, suggesting a specific function of the coexistence of both repressive marks in the endosperm. However, the biological meaning and molecular function of the triple repressive marks remain to be elucidated.

We found that genes with triple repressive marks are continuously silenced throughout endosperm development, while genes with single H3K27me3 tend to be induced during germination. Consistently, genes with single H3K27me3 are enriched for ethylene-responsive genes that are necessary for germination. Our data suggest that endosperm-specific triple repressive marks are established before fertilization and prevent the activation of genes, while genes with single H3K27me3 are activated during germination and therefore control the timing of this process. Impediments to germination result in seed dormancy; our data thus reveal a new function of imprinted genes to control seed dormancy, a highly important adaptive trait of great agronomic relevance.

P173 Functional role of PEG2 in the formation of hybridization barriers in *Arabidopsis thaliana*

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Flowering plants exhibit the unique feature of double fertilization, resulting in the formation of embryo and endosperm. The endosperm provides nourishment, supports embryo growth and germination. Proper endosperm development is crucial, as its failure leads to embryo arrest and seed lethality. Hybridization of plants with varying ploidy levels leads to the establishment of an interploidy hybridization barrier in the endosperm termed as the 'triploid block'. Deregulation of imprinted genes in the triploid block is linked to its establishment. *PEG2* is an imprinted paternally expressed gene (PEG) that has been shown to establish a postzygotic interploidy hybridization barrier in triploid seeds of Arabidopsis thaliana. The transcript of *PEG2* acts as a sponge for the small interfering RNA (siRNA) siRNA854 in the endosperm, likely preventing its interaction with other target genes, causing the triploid block. In the *Col-0* accession, siRNA854 causes translational repression of *PEG2* owing to incomplete sequence complementarity between the target sequence and siRNA854. The target site is highly conserved across the accessions of A. thaliana albeit with natural sequence variations. We found that, though the intraspecific sequence variation is non-causal for the accession-specific triploid block phenotype, the accession-specific expression levels of *PEG2* correlate with frequencies of collapsed seeds obtained in the paternal excess crosses. We also found that a Crispr/Cas9 generated *peg2-2* allele that lacks the siRNA854 target site, suppresses the triploid block, while *peg2-3*, containing the siRNA854 target site, is unable to suppress the triploid block. Therefore, the 21-bp long target sequence in *PEG2* is essential for the formation of the triploid block. This reveals a concerted function between a PEG and a siRNA in the formation of the triploid block.

P174 Histone deacetylases regulate heat stress induced haploid embryogenesis in *Brassica napus*

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Haploid embryogenesis refers to the ability of male or female reproductive cells to regenerate in vitro into a complete plant via embryogenesis. How these cells initiate embryogenic growth in response to inducing signals is poorly understood. We have shown that histone deacetylases (HDAC) repress heat-stressed-induced in vitro haploid embryo development from immature pollen in Brassica napus[1]. Through comparative RNA-seq and ChIP-seq analysis of embryogenic samples vs pollen samples we identified genes that are regulated by histone acetylation during haploid embryogenesis. Also, our work revealed that mutation of selected HDAC genes can enhance the rate of microspore embryogenesis and bypass the requirement of heat stress for this process.

[1] Hui Li et al, 2014. Plant Cell

P175 Functional requirement of RNA Polymerase IV in seed coat development in Capsella

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The seed coat provides a protective layer for the developing embryo against adverse external biotic and abiotic factors and influences seed dormancy and germination. Coordination and exchange of signals between seed coat, endosperm and embryo is critical for proper seed formation. The seed coat is derived from the ovule integuments, in which seed coat development is blocked by the sporophytically active Polycomb Repressive Complex2 (PRC2) before fertilization. The initiation of seed coat formation is triggered by unblocking PRC2 through signals generated after double fertilization. Auxin produced in the endosperm after fertilization was shown to be sufficient to remove PRC2 and to initiate seed coat development. Here, we show that mutating the largest subunit of RNA polymerase IV (Pol IV) in Capsella rubella, CrNRPD1, causes a seed coat defect. Pol IV produces precursors for small RNAs that direct DNA methylation, which requires the function of Pol V. Interestingly, mutants depleted for CrNRPE, encoding a subunit of Pol V, form normal seed coats. Small RNA sequencing revealed that NRPD1-dependent small RNA loci in the endosperm are genes encoding for auxin biosynthesis and transportation, which are required for seeds coat development. Our data suggest a functional role of NRPD1-dependent small RNAs in the regulation of auxin production to control seed coat development.

P176 Isolation and characterization of small RNA-containing vesicles from maize pollen

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Plant sperm cells are unique, because they are cells within a cell. It is known that vegetative cell-generated small RNAs (sRNAs) can regulate gene expression in sperm cells. However, how sRNAs move from vegetative cells to sperm cells is still unknown. In order to understand the mechanisms of sRNA trafficking in pollen, we isolated vesicles from maize pollen and characterized them by RNA and proteomic analyses. Intact membrane vesicles from mature pollen from Zea mays B73 were purified and fractionated by sucrose density gradient ultracentrifugation. The RNA analysis of the fractions revealed that the 1.10 g/ml density fraction contains mainly short sRNAs while the higher density fractions contain various lengths of RNAs. LC/MS analysis of those fractions showed that the 1.10 g/ml fraction was enriched in Argonaute proteins, DEAD-box ATP-dependent RNA helicases and Annexins, that were shown to be sRNA-binding proteins in the Arabidopsis leaves exosomes-like extracellular vesicles. Also, some components of COPII vesicles were enriched in this fraction. Negative staining TEM of that fraction showed that two types of vesicles were budding from the endoplasmic reticulum-like fragments, type 1; low electron density ≈50-65 nm vesicles and type 2; high electron density ≈70-120 nm vesicles. Exosomes are 40-150 nm diameter extracellular vesicles derived from multivesicular bodies and play an important role for cell-to-cell communication by transporting sRNAs to neighboring or distant cells. COPII vesicles are known to be ≈60 nm in diameter and to bud from endoplasmic reticulum exit sites (ERES). Thus, the vesicles containing short sRNAs might be exosome-like vesicles derived from ERES. Further experiments are needed to clarify whether short sRNA-containing vesicles originate from ERES and are destinated to sperm cells.

P177 Maize Argonautes are required for maintaining DNA methylation in the female reproductive organ

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Sexually reproducing organisms generate the germ lineages through meiosis. During plant germline development, various types of small RNAs are dynamically changed and global DNA methylation is also reprogrammed. Here, we demonstrate that maize Argonautes (AGOs) are required for female organ development via epigenetic regulation. Several AGOs are expressed in the female reproductive organs. Of those, MAGO2 accumulated in the ovule and epidermal layer of the immature ear. To demonstrate the function of MAGO2 in the female organs, transcriptomic analysis was performed in immature ears from WT and *MAGO* knock-down (*MAGOKD*) plants. Interestingly, approximately 10,000 genes were differentially expressed in the *MAGOKD* ears. Since AGOs are involved in RNA-directed DNA methylation (RdDM), we deduced that huge changes of transcripts might be occurred by DNA methylation. Through bisulphite sequencing, we found that overall chromosomes were hypermethylated in the *MAGOKD* ears. Especially, CHH methylation was significantly increased in the *MAGOKD* ears and that was enriched in promoter regions of genes. Our results demonstrate that an Argonaute-dependent RdDM is critical in plants to sustain female germlines by controlling the genes.

P178 MET2a and MET2b DNA methyltransferases are required for transgenerational methylome stability

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In plants, DNA methylation patterns are relatively stable through generation. However, several changes of DNA methylation occur during sexual reproduction. Such process is thought to be necessary to ensure genomic stability by maintaining the proper silencing of transposable elements (TEs). MET1 is the main DNA methyltransferase that ensure TEs silencing in the model plant Arabidopsis thaliana. Indeed, in a *met1* mutant, TEs reactivation causes a pleiotropy of phenotypes due to the presence of new insertions. In wild Arabidopsis assession, it was hypothesized that a homologue of MET1, MET2a might play a role in TEs silencing. In this paper, we study in detail the function of MET2a as well as its closely related homologue MET2b. We showed that MET2a and MET2b proteins are detectable in mature central cell, where they are addressed to the cell nuclei. MET2a and MET2b are likely functional DNA methyltransferases as they could partially complement the *met1* mutation. MET2a and MET2b are regulating the ovule transcriptome prior to fertilization. Interestingly, the plant methylome is not globally affected by mutations affecting *met2a* and/or *met2b*. Their effect on the methylome is revealed when introduced in a met1 background where we could identify several DMRs which were surprisingly mostly hypermethylated DMRs. Finally, we show that *met2a, met2b* lead to an increased transgenerational silencing of the *FWA* locus or transgene. Overall, our work suggests that MET2a and MET2b are involved in a non-cell autonomous regulation of the embryo DNA methylation.

P179 MicroRNA function and RNA regulation transitions during the maturation of pollen

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microRNAs play important roles controlling the development of eukaryotic organisms. Both animal and plant microRNAs are essential for the spatio-temporal regulation of development but together with this role, plant microRNAs also control transposable elements and stimulate the production of epigenetically-active small interfering RNAs. This last role is evident in the plant male gamete containing structure, the male gametophyte or pollen grain, but how the dual role of plant microRNAs is integrated during its development is unknown. Here, we provide a detailed analysis of microRNA dynamics during pollen development and their genic and transposable element targets using small RNA and mRNA cleavage (PARE) high-throughput sequencing. We uncover the microRNAs loaded into the two main Argonaute proteins in the mature pollen grain, AGO1 and AGO5. Furthermore, the use and comparison of different RNA sequencing strategies (PARE, polyA mRNA, total RNA and sRNAs) allowed us to uncover RNA regulatory layers that might be important for RNA regulation during pollen maturation. Our results indicate that the developmental progression from microspore to mature pollen grain is characterized by a transition from microRNAs focused on the control of development to microRNAs regulating transposable element activity and that both posttranscriptional modifications of RNAs and RNA binding proteins are important components of pollen maturation.

P180 Structure and dynamics of the network of dominance interactions among self-incompatibility alleles in *Arabidopsis halleri*

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Genetic dominance is a basic property of inheritence systems. According to Fisher (1928), dominance between alleles would result from the intervention of genetic elements, referred to as “dominance modifiers”. To date however, documented examples of dominance modifiers have remained scarce, with only a single well-understood example: the dominance hierarchy between alleles controlling pollen specificity in the sporophytic self-incompatibility system of the Brassicaceae. In *A. halleri*, this dominance hierarchy is controlled by small non-coding RNAs (sRNAs) that are linked to the self-incompatibility locus (S-locus). They regulate in heterozygous individuals the relative transcript levels of the two S-alleles in pollen and function through molecular interactions between the sRNAs produced by the dominant alleles and their target sequences on the recessive alleles. However, previous models for the evolution of dominance between S-alleles assumed a simplistic genetic basis for dominance modifiers viewed as single, independent genetic entities. Here, we combined phenotypic, genomic and theoretical approaches to study the evolution of these dominance modifiers and their targets. We used controlled pollination assays to characterize the phenotypic dominance network between 11 S-alleles of *A. halleri*. We inspected the genomic sequence of these 11 S-alleles to identify the mutations by which the molecular network evolved through changes in sRNA regulators and/or their target sites. Finally, we used stochastic simulation models to compare the strength of natural selection on mutations creating new regulatory interactions as a function of 1) their level of pleiotropy, 2) their molecular nature (on the sRNA or on the target), 3) the initial level of dominance of the allele on which they appear (dominant vs. recessive S-alleles). Overall, our results reveal the extent to which details of the genetic architecture of dominance modifiers as a two-components system have important consequences for their evolution.

P181 Epigenetic landscape in the repeat-based holocentric genome of *Rhynchospora pubera*

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Epigenetic regulation of the eu- and heterochromatin domains and thereafter the genome architecture can differ between monocentrics and holocentrics, due to the presence of multiple centromeres in the latter. Relatively very less information is available about the epigenetic landscape of holocentromeres and almost no such research has been conducted earlier in a holocentric plant. To shed light on this aspect, we conducted epigenetic studies using chromatin immunoprecipitation sequencing (ChIP-seq) and enzymatic-methyl sequencing in the holocentric plant model, *Rhynchospora pubera*. With the availability of a reference genome assembled by our group, we could demonstrate that the centromere-specific protein, RpCENH3 is associated with the satellite repeat sequence, Tyba. Tyba arrays of 17.8kb average length (Tyba monomer length is 172bp) constitute the holocentromeres of *R. pubera*. Such sequence-dependent holocentromeres are not yet reported in other holocentric models. High mCpG, low levels of H3K9me2 (heterochromatin-specific histone mark), and depletion of H3K4me3 (euchromatin-specific histone mark) are the epigenetic clues that mark the holocentromeres of *R. pubera*. The centromere-specific clues reported recently in the repeat-based monocentric plant *Arabidopsis thaliana*, like high H3K9me2 and high mCHG in the bordering regions than in the core of monocentromeres are strikingly similar in the holocentromeres of *R. pubera* as well. Through this study we describe, despite the different centromere types/distribution, epigenetic regulations observed in the repeat-based centromeres of both mono- and holocentric species may be evolutionarily conserved.

P182 tRNA fragment biogenesis during male gamete biogenesis is linked to transcriptome reprogramming

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RNA silencing is an evolutionary-conserved mechanism that protects genomes against invading RNAs and/or regulates gene expression at the transcriptional and post-transcriptional level. To exert these functions, small RNAs (sRNAs) act through a diversity of pathways. tRNAs are known for their indispensable function linking transcription and translation, but they can also produce different classes of functional sRNAs: tRNA-derived fragments (ranging from 18 to 28 nts and termed tRFs) and tRNA halves (ranging from 30 to 35 nts).

Here we have investigated the accumulation pattern of tRNA-derived sRNAs and their effects at the post-transcriptional level during *Arabidopsis thaliana* male gametogenesis. Using sRNA sequencing of different pollen developmental stages, we identified that tRNA halves accumulate to high levels in the microspore and mature pollen grain. These pollen-accumulating tRNAs halves derive from specific tRNAs precursors and show a correspondence between highly accumulated tRNA fragments and the abundance of RNA modifications. In parallel, RNA sequencing from mature pollen indicates that accumulation of tRNA halves correlates with a decrease in the activity of genes mediating RNA post-transcriptional modifications and an increase in the expression of members of the RNase T2 family, opening the possibility of RNA modification as a regulatory pathway for the production and processing of tRNA fragments. Additionally, we studied ribosome dynamics in the mature pollen grain using 5'P-sequencing. This analysis showed that the pollen grain displays a differential ribosome-protection pattern in gene coding transcripts which could be related to ribosome stalling and a higher presence of initiation complexes. This pause of translation is maximum at pollen maturity correlating with maximum tRNA halve production. In summary, our data indicates that the Arabidopsis pollen grain experiences a programmed degradation of the tRNAs that might influence the stability and translation of mRNAs during male gametogenesis and fertilization.

P183 H3K27me3 epigenome and transcriptome correlation analysis reveal key networks controlling ovule developmental stages during pollination of *Ginkgo biloba L.*

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Most results of the molecular network controlling ovule development were investigated based on the studies in angiosperms, while is still largely unexplored in non-model plants, mainly in Gymnosperms.

Polycomb repressing complex 2 (PRC2) proteins are rated among the master regulators in several aspects of plant development. They are responsible for the tri-methylation of lysine 27 of Histone 3 (H3K27me3), causing the transcriptional repression of target genes [1].

In this context, the focus of the present work was to identify the target genes involved in *G. biloba* ovule development with respect to the pollination stage, mediated by H3K27me3 epigenetic mechanisms.

To evaluate the effect of pollination signal on the ovule growth, both pools of pollinated and unpollinated ovules were collected, considering four stages: the pre-pollination stage, the pollination drop stage, and three post-pollination drop stages, respectively 1, 6 and 8 days after the emission of the pollination drop.

ChIP-seq was performed to precisely determine the location of H3K27me3- marked regions across the genome on the pollinated ovules. The obtained results were correlated with the RNA-seq data. 4307 Differential Methylated Genes (DMGs) and 4838 Differentially Expressed Genes (DEGs) were identified among the steps. Weighted gene co-expression network analysis (WGCNA) was used to identify RNA-Seq Modules (RSM), gene clusters, highly correlate with ovule development trait.

DMGs were then compared with RSM to discern those modules that may be biologically regulated as a group by trimethylation of H3lysine27. Several Flavonoids and Auxin DEGs, (the two most impacted pathways), were also target of PRC2 complex. Taken together, a model can be drawn with PRC2 complex playing pinpointing roles for the determination of flavonoid and auxin accumulation profiles at defined developmental phases.

[1] Hennig and Derkacheva, 2009.Trends in Genetics

P184 Epigenetic reprogramming of the seed coat: a role for brassinosteroids?

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Seed development in flowering plants starts with a double fertilization event,where two parental sperm cells fertilize a central cell and an egg cell,leading to the formation of a diploid embryo and a triploid endosperm. The fertilized products are surrounded by seed coat,which is derived entirely from maternal ovule integuments. Although fertilization of the maternal gametes is necessary to drive seed development, fertilization independent seed(fis) mutants can develop seeds without fertilization(autonomous seeds). *FIS* genes code for proteins forming Polycomb Repressive Complexes(PRC) and the PRC2 complex, which deposits repressive H3K27me3 marks in target loci, is responsible for repressing seed development genes in the maternal gametes and in the integuments. Interestingly, removing gametophytic and sporophytic components of PRC2 complex results in autonomous endosperm and seed coat development,respectively. A synergistic effect is seen when both components are removed. We have previously shown that auxin produced after fertilization removes sporophytic PRC2 from the integuments, allowing for the seed coat to develop. However, how seed coat-related target genes become active following PRC2 removal remains to be understood. This suggests that there are additional signals apart from auxin which are required for formation of a functional seed coat. Our data indicates that Brassinosteriods (BR) plays an important role in seed coat development along with timely removal of H3K27me3 marks from integuments by JUMONJI (JMJ) type histone demethylases.

We aim to understand the mechanisms that follow PRC2 removal from the integuments and are necessary for the timely development of the seed coat. For this we screened BR mutants for defects in seed coat development and tested for genetic interactions with members of sporophytic PRC2s. We found that lack or excess of BR resulted in defects in seed development which was partially rescued by global loss of H3K27me3,establishing a link between BR activity and epigenetic reprogramming of the seed coat.

P185 Switch on drying: Epigenetic aspects of desiccation tolerance acquired during seed development

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During seed maturation phase, seeds acquire desiccation tolerance (DT), which is the capacity to survive extreme dehydration, a drastic loss of water content to levels below 10%. This allows seeds to survive in dry conditions for a certain period of time before germination. Subsequently, the DT is lost with a developmental transition from germination to seedling establishment. The seed maturation program is significantly affected by environmental stresses, however the timing of DT acquisition in developing *Medicago truncatula* seeds is robust under several stress conditions, indicating that the tolerance is an important trait for plants and is genetically switched on at specific times in the maturation program. Some of the seed DT-related genes are also known to be commonly implicated in desiccation of the vegetative part in resurrection plants, suggesting that understanding of molecular switches that regulate DT intrinsicly in seeds may contribute to improve drought stress tolerance in plants. However, our understanding of molecular mechanisms that underlie DT in seeds remains fragmentary, especially regarding the epigenetic factors.

Here, we performed RNA-Seq of maturating seeds before and after the DT phase in Medicago and detected differentially expressed genes potentially involved in the DT process. These genes were then compared with previous DT-related transcriptome datasets and those commonly detected were redefined as DT-core genes that may play essential roles in cell survival during desiccation. A cluster enrichment of these DT-core genes was confirmed by ChIP-seq of specific histone marks as well as ATAC-seq, suggesting that the expression of some DT-core genes is epigenetically controlled during seed maturation. The regulatory mechanism of DT-core gene expression known to be important for seed DT, such as genes related to the metabolism and signaling of the plant hormone abscisic acid will be also discussed.

P186 It takes a family: Exploring the duplication history and expression of CrRLL1L family members involved in reproduction

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The *Catharanthus roseus* RECEPTOR-like KINASE 1L (CrRLK) family is broadly expressed in *Arabidopsis*, although several members of the family receive and transmit signals during pollen hydration, pollen tube growth, and pollen tube reception. To better understand the evolutionary relationships among reproductively active CrRLKs, we used motif-based searches to exhaustively identify CrRLK homologs from across land plant genomes, aligned the putative homologs, and inferred phylogeny. The resulting tree revealed two super clades of CrRLKs, each of which contained multiple angiosperm-specific sub-clades of reproductively active CrRLKs. We detected positive selection along some post-duplication branches of the tree, suggesting that neofunctionalization or escape from adaptive conflict may have influenced the diversity of CrRLKs in angiosperms. Finally, we used publicly available RNA-seq data to recover the expression evolution of reproductively associated CrRLKs and to infer their ancestral expression patterns. Our analyses revealed that CrRLKs known to act in a coordinated fashion during reproduction in *Arabidopsis* are ancient paralogs, and that some of the roles characterized in Arabidopsis likely evolved in proto-angiosperms.

P187 Constraints on interspecific hybridization intensity of *Cirsium oleraceum-rivulare-palustre-acaulon* complex (Asteraceae)

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The study was conducted to determine possible causes that allow or limit the interspecific hybridization intensity of *Cirsium oleraceum-rivulare-palustre-acaulon* complex based on data of different field sites in Central Europe and herbarium records. Among the six possible hybridizing pairs of four Cirsium species studied, frequency of natural hybrids expressed by 641 herbarium specimens are 31.20 % of *C. oleraceum* x *C. rivulare*, 25.43 % of *C. oleraceum* x *C. palustre*, 22 % of *C. oleraceum* x *C. acaulon* and 21.22 % of *C. palustre* x *C. rivulare*. *Cirsium acaulon* rarely produce hybrids with *C. rivulare* (0.15 %) and *C. palustre* (0 %). The hybridization potential between species-pairs is estimated based on geographic, ecological similarities and floral phenological overlap constituting the pre-mating reproductive barrier. Compared to the species pair *acaulon-rivulare*, the species pairs *oleraceum-acaulon* and *oleraceum-rivulare* are 40-times higher in their geographical distributions and 12-times higher than oleraceum-palustre. However, no hybrid *C. acaulon* x *C. palustre* was detected. The ecological similarity between the potentially hybridizing species pairs are oleraceum-rivulare > palustre-rivulare > oleraceum-palustre > oleraceum-acaulon > acaulon-rivulare > acaulon-palustre. The floral phenological overlap between species pairs is as follows, oleraceum-acaulon > oleraceum-palustre > acaulon-palustre > palustre-rivulare > acaulon-rivulare > oleraceum-rivulare. Despite limitations, frequent natural hybridization suggests interspecific pollen competition plays a key role in determining the intensity of hybridization in each hybridizing pair. Future analyses of pollen-pistil interactions, in vivo pollen tube growth rates, will provide the best possible explanation for a wide variation in Cirsium hybrid intensity.

P188 Effects of pollinator behavior on the mating system of a moth-pollinated threatened tree species

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Wrightia tomentosa is a small deciduous tree species, which is known to occupy the warmer parts of India. The species has become threatened in the wild due to indiscriminate cutting for its ivory-white valuable wood. The floral architecture and nocturnal anthetic period imposes strong selection for specialized pollination by moths. The flowers are visited by two different types of moths: settling moth and hawkmoth. We have investigated whether the foraging behavior of the two types of moths seems to have a significant influence on the mating pattern in the species. Breeding system was established through natural- and manual-pollination experiments. Various parameters of floral visitors such as foraging behaviour, flower-handling time, number of flowers visited per tree, number of trees visited in a bout were recorded. Molecular markers (AFLP and RAPD) were further employed to estimate relative proportion of selfed and outcrossed progeny in the open-pollinated fruits. All the flowering individuals at the site were self-compatible. However, spontaneous autogamy was not favoured primarily due to constraints imposed by floral contrivances such as herkogamy and agglutination of pollen near the line of dehiscence by sticky pollenkitt material. Amongst the two groups of moths, settling moths were primarily involved in geitonogamous pollination and hawkmoths in xenogamous. Application of genetic markers revealed that the open-pollinated progeny in *W. tomentosa* were indeed the product of mixed-mating and were predominantly outcrossed.

P189 Elimination of viroid parasitic RNAs from pollen

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Viroids are plant parasitic RNAs of unknown origin possibly representing living fossils from RNA world being single stranded, non-coding, predominantly circular RNAs fully adapted to plant bichemical machinery. Their fast sperading, resistant structures and difficult eradication makes viroids very dangerous plant pathogens inducing specific diseases including strong developmental distortions in some hosts. Some viroids are not transmissible through pollen to seeds, while some are transmissible. This suggests that evolution led either to their eradication from- or to viroid adaptation to male germline. In this study we analyzed the molecular background of elimination of two hop (*H. lupulus*) viroids from tobacco pollen, apple fruit crincle viroid (AFCVd) and citrus bark craking viroid (CBCVd). Our results show that in pollen the natural viroid replication pathway proceeds, but it is dramatically depressed in comparison to somatic tissues. Simultaneously, specific and unspecific viroid degradation with some preference for minus chains occurred in pollen, as detected by analysis of vd-sRNAs, by quantification of viroid levels using strand-speific viroid RT-qPCR and by detection of bigger degradation products forming “comets” on northern blots. The levels of both viroids subsequently dropped during pollen development. This decrease correlated with accumulation of mRNA of several factors involving in RNA degradation identified by wide RNA or protein profiling like AGO5, DICER-like factors and TUDOR S-like nuclease. It is proposed in this study based on quantification results that functional status of pollen, as tissue with high ribosomal content could play some role during suppression of AFCVd replication involving transcription factors IIIA and its processing regulator, ribosomal protein L5. Suppressed AFCVd propagation was maintaining viroid levels only as traces in pollen tubes and viroid was not transferable from AFCVd-infected pollen to seeds during the last steps of development and fertilization process.

P190 Sphingolipids and plant sexual reproduction: The role of ceramidase in the evolution of siphonogamy in angiosperms

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Sphingolipids are ubiquitously present in all eukaryotic cells and in a few bacteria. They are thought to be essential for membrane structure and are also involved in signal transduction. Ceramide, sphingosine, sphingosine-1-phosphate (S1P) and their derivatives are examples of a class of well-studied bioactive sphingolipids. Ceramidases hydrolyze ceramide to yield sphingosine and fatty acid. Thus, they play an essential role in sphingolipids homeostasis. In previous study, we identified a Golgi-localized alkaline ceramidase TOD1 (TurgOr regulation Defect 1), as a key turgor regulator in *Arabidopsis thaliana* pollen tubes (Chen et al., 2015, Nature communications). The mutation of the *TOD1* gene leads to the reduced pollen tube growth potential in pistil. Here, we provide evidence that *OsTOD1* is also preferentially expressed in rice pollen grains and pollen tubes, which is similar to *AtTOD1*. *OsTOD1* knockout results in reduced pollen tube growth potential in rice pistil. Both *OsTOD1* genomic sequences with its own promoter and CDS under *AtTOD1* promoter can partially rescue the *attod1* mutant phenotype. Furthermore, *TOD1s* are present in seed plants, albeit with diversified functions between gymnosperms and angiosperms: TOD1s from angiosperms can partially rescue *attod1* mutant phenotype, while TOD1s from gymnosperms are not able to complement attod1 mutant phenotype. Our data suggest that TOD1s in angiosperms function as a conserved turgor pressure regulator in pollen tube growth, which may co-opt the pollen tube to the new role as siphonogamy mediated by fast growing pollen tubes.

P191 RNA-seq-based bulked segregant analysis of the causal U-genome gene for hybrid incompatibility between tetraploid wheat and wild wheat relative *Aegilops umbellulata*

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Aegilops umbellulata Zhuk. is a diploid wild wheat relative with UU genome, and the U-genome donor of many wild wheat relatives. It provides the ability to produce allohexaploids with AABBUU genome through triploid F1 hybrids with ABU genome obtained by intraspecific crossing with tetraploid wheat. About 50 % of Ae. umbellulata accessions show one of three types of hybrid incompatibilities in triploid ABU F1 hybrids; seed production failure, severe growth abortion (SGA), or grass-clump dwarfism (GCD). The symptoms observed depend on the accession of *Ae. umbellulata*. The microarray analysis of the hybrids suggested that GCD is a novel hybrid incompatibility in wheat relatives.

To identify a causal gene for GCD, we developed molecular markers tightly linked to the U-genome causal gene using RNA-seq-based bulked segregant analysis. Two *Ae. umbellulata* accessions were crossed: one accession shows GCD in the ABU hybrids and the other shows normal growth phenotype (WT) in the hybrids. Then, the F1 plant was used as the pollen parent and crossed with Ldn to generate a segregating population of the ABU triploids. The RNA samples derived from hybrids showing GCD and WT were bulked, and calculated SNP-indexes based on their RNA-seq reads. The reference genome of Ae. tauschii was used as a hypothetical genome of *Ae. umbellulata*. A large number of positions with high SNP-index were densely assigned to chromosome 6UL, and the causal locus was mapped to 5.4 cM interval. RNA-seq analyses in crown tissues of the ABU hybrids revealed potential candidate genes showing differential expression between GCD and WT at this interval and also characterized the expression pattern of GCD. Identification of Gcd1 will contribute to elucidating molecular mechanisms of post-zygotic reproductive barriers, and to utilizing efficiently the *Ae. umbellulata* genes in further wheat breeding.

P192 Evaluation of the phenological performances of the almond and peach trees in response to climatic variations.

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Climatic factors, have high importance in determining the phenological stages of fruit trees, especially flowering and dormancy phases, due to their important role in some agricultural practices and crop management.

However, few studies have been conducted on the effect of climate on flowering and dormancy of almond and peach trees, especially in mild climate areas.

This study aims to simulate the dormancy phases, which are closely involved in determining the flowering time of almond and peach species in response to temperature variations.

Seven varieties of almond trees most grown in Morocco (Marcona, Fournat, Belona, Soleta, Laurrane, Ferragnès and Ferraduel) and ten varieties of peach trees (Halawa, PL2, peche plate, Early bomba, Earlu queen, Summer sweet, Septembere sun, Sugar time, Honey cascade, Zeffyr) planted in the region of Meknes, have been chosen for this study during two seasons 2020/2021 and 2021/2022.

The evolution of flower bud dormancy for each cultivar will be determined using the experimental protocol described by Tabuenca Abadia (1967). Regular flower bud samples from each ten days are divided into two groups, the first one is destined to weigh fresh and dry weights (dehydration at 75 °C for 48h). The second is placed separately in pots containing water, and introduced in a forcing chamber (temperature 25 °C, photoperiod of 16 hours and relative humidity 90%). After 10 days of forcing the flower buds are weighed in the same way as the first group to obtain the fresh and dry weight.

After the realization of the curves, the date of dormancy breaking will be determined statistically. An application of simulation approaches (using platforms dedicated to phenological modeling) with the aim of developing and validating a model or models explaining the thermal requirements involved in the processes of dormancy and flowering of fruit trees.

P193 Endosperm evolution by duplicated and neofunctionalized Type I MADS-box transcription factors

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MADS-box transcription factors (TFs) are present in nearly all major eukaryotic groups. They are divided into Type I and Type II that differ in domain structure, functional roles, and rates of evolution. In flowering plants, major evolutionary innovations like flowers, ovules, and fruits have been closely connected to Type II MADS-box TFs. The role of Type I MADS-box TFs in angiosperm evolution remains to be identified. Here, we show that the formation of angiosperm-specific Type I MADS-box clades of Mγ and Mγ-interacting Mα genes (Mα*) can be tracked back to the ancestor of all angiosperms. Angiosperm-specific Mγ and Mα* genes were preferentially expressed in the endosperm, consistent with their proposed function as heterodimers in the angiosperm-specific embryo nourishing endosperm tissue. We propose that duplication and diversification of Type I MADS genes underpin the evolution of the endosperm, a developmental innovation closely connected to the origin and success of angiosperms.

P194 Resilient genetic systems and extra range dispersal of *Artemisia* L.- A proliferate in NW Himalaya, India

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Artemisia L. is a cytologically flexible and medicinally important genus of tribe Anthemideae of family Asteraceae. It is widely distributed mainly across the Northern Hemisphere, with a major center of diversification in Asia. Worldwide, more than 500 species of this genus have been reported, out of which a total of 45 exist in India; 20 among these being reported in J&K state, India, where the present work has been carried out.

Compilation is based on more than 5 years of detailed probing of the meiotic and breeding system of 8 species and across 41 populations of Artemisia sprawling diverse altitudinal regimes of NW Himalaya, India. These include *Artemisia maritima* L, *Artemisia scoparia waldst&Kit*., *Artemisia nilagirica (Clarke)Pamp*., *Artemisia glauca Pall.ex Willd*, *Artemisia sieversiana Ehrh.ex Willd*, *Artemisia gmelinii Weber.ex Stechm*, *Artemisia vestita Wall* and *Artemisia tournefortiana Reichb*. Studies revealed a spectrum of chromosome races and breeding system variation. Only three species turned out to be stable diploids, rest showed extensive cytological variation at population level. *Artemisia maritima* is the only species capable of setting seeds by self pollination also, rest all are predominantly out crossed. We have tried to correlate this variability with the dispersal potential of these species highlighting the differences between endemic, restricted in distribution, and widely spread among these.

P195 Recombination landscape divergence between populations is marked by larger low-recombining regions in domesticated rye

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The genomic landscape of recombination plays an essential role in evolution. Patterns of recombination are highly variable along chromosomes, between sexes, individuals, populations, and species. In many eukaryotes, recombination rates are elevated in sub-telomeric regions and drastically reduced near centromeres, resulting in large low-recombining regions. The processes of recombination are influenced by genetic factors, such as different alleles of genes involved in meiosis and chromatin structure, as well as external environmental stimuli like temperature and overall stress. In this work, we focused on the genomic landscapes of recombination in a collection of 916 rye (*Secale cereale*) individuals. By analysing population structure among individuals of different domestication status and geographic origin, we detected high levels of admixture, reflecting the reproductive biology of a self-incompatible, wind-pollinating grass species. We then analysed patterns of recombination in overlapping subpopulations, which revealed substantial variation in the physical size of low-recombining regions, with a tendency for larger low-recombining regions in domesticated subpopulations. Genome-wide association scans for low-recombining region size revealed a major quantitative-trait-locus at which an ortholog of histone H4 acetyltransferase *ESA1* was located. Rye individuals belonging to domesticated subpopulations showed increased synaptonemal complex length, but no increase in crossover frequency, indicating that only the recombination landscape is different. Furthermore, the genomic region harbouring rye *ScESA1* showed weak patterns of selection in domesticated subpopulations, suggesting that larger low-recombining regions might have been indirectly selected for during domestication to achieve more homogeneous populations for agricultural use.

P196 Ploidy as an imperfect postzygotic reproductive barrier in speciation: drivers and consequences of inter-ploidy gene flow in natural populations

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Whole genome duplication (polyploidisation) is a dominant force in sympatric speciation, particularly in plants. It has traditionally been assumed that ploidy acts as an instant hybridization barrier between diploids and their polyploid derivatives. Generally, triploids arise as a result of crosses between diploid and tetraploid individuals of the same or related species. Such crosses often result in the failure of seed formation, a phenomenon called the 'triploid block'. Triploid block can result in a high degree of instant postzygotic reproductive isolation between tetraploids and their diploid progenitors, since backcrossing to either parent will produce mainly nonviable progeny. Triploid hybrids in natural populations often do not survive until maturity. However, recent flow cytometric and population genetic surveys of natural populations have challenged this view by reporting signs of widespread inter-ploidy introgression in multiple ploidy-variable species. Here, we review novel knowledge concerning the variations in the strength of triploid block across multiple angiosperm families and the mechanisms driving such variations. We revisit the developmental basis of the triploid block to account for the causes of angiosperm-wide variation in triploid block manifestation. We also determine the variation in realised interploidy gene flow across species from our surveys involving natural progeny arrays and flow cytometry screening for the occurrence of unreduced pollen. However, a scarcity of studies in a natural diploid-autotetraploid system and a virtual lack of integration with population genomic approaches leaves the underlying mechanisms and levels of realized interploidy gene flow in nature largely unknown. Finally, we discuss how knowledge of natural ploidy frequency and variation in the strength of reproductive barriers have increased our understanding of the interploidy gene flow and the evolutionary significance of polyploidy. Finally, we discuss potential consequences of such genome permeability on polyploid speciation.

P197 Reduction in fertility after moderate heat stress application during silking of maize ears

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The reproductive phase in flowering plants is highly sensitive to ambient temperature stresses, with even a single hot day sometimes being fatal to reproductive success. Heat stress caused by climate change is predicted to reduce the productivity of crop plants. Many studies of heat stress on crop plants have shown that pollen development and fertilization often belong to the most sensitive reproductive stages. Less attention has been paid to heat sensitivity of female reproductive organs. We applied moderate heat stress to study the sensitivity and contribution of female organs to reproduction under heat stress in maize. We focused on the elongated stigma tissue (silk), which is in direct contact with ambient environment at the silking stage. We showed that moderate heat stress on female organs caused increased cell death and decreased cell vitality of silk hair cells. However, moderate heat stress on silk didn't affect pollen germination and early pollen tube growth inside the silk, but caused late growth arrestment of pollen tubes and may also cause micropylar guidance defects. Moreover, moderate heat stress during silking before and after pollination caused severe seed set reduction. Our findings demonstrate that a short moderate heat stress affects silk cell vitality and the pollen tube growth inside the silk, which consequently leads to severe yield losses in maize. To study the mechanism of heat stress response in detail, we will perform RNA-seq of heat stressed silks, to find the heat tolerant or susceptible genes.

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P198 Strategies to enhance pollination in the Field Bean, *Vicia faba*

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With pollinator populations in decline, it is vital that we develop crops which are both attractive and beneficial to pollinators. The field bean, *Vicia faba*, is one economically important crop which benefits greatly from bee pollination. Bee pollination enhances the yield of *V. faba* and can decrease yield variability. To be able to advise breeders on which floral traits may help to attract pollinators, we need to determine how much floral variation exists between current *V. faba* lines, and which traits influence pollinator behaviour.

Previous research has identified variation in some floral traits of this crop, which can influence pollinator attraction. This project seeks to further explore strategies for optimising field bean flowers, to provide maximum energetic reward to pollinators for minimum foraging energy expenditure. Current work has focused on identifying variation in floral traits of novel commercial *V. faba* lines, and the influence of *V. faba* wing spots on the behaviour of *Bombus terrestris*, the buff-tailed bumblebee. Future work will investigate the responses of pollinators to extremes of variation in specific floral traits in both laboratory conditions and on a field scale.

P199 Proteasome-mediated haploidisation of *Arabidopsis thaliana* via nanobody- specific degradation of EYFP-tagged CENH3

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The ability to generate haploid plants significantly accelerates the crop breeding process, while significantly reducing the time and expenses. We demonstrate that the nanobody-driven directed degradation of the EYFP-CENH3 fusion protein can be used for the generation of haploid plants. We show that recombinant anti-EYFP nanobody-Fbox or anti-EYFP nanobody-BTB proteins can recognize EYFP-tagged CENH3 in planta and make it accessible for the ubiquitin-proteasome pathway. We demonstrate that in crosses of the ♀*cenh3.1* mutant complemented by the EYFP-CENH3 construct with ♂anti-EYFP nanobody expressing lines the maternal genome undergoes elimination due to the degradation of the EYFP-CENH3 via anti-EYFP nanobody-targeted E3-ubiquitin ligase. In reciprocal crosses, no haploid induction was observed. Analysis of pooled F1 seeds by flow cytometry revealed 7.6% and 4.8% haploids in crosses with plants expressing the anti-EYFP nanobody-F-box or anti-EYFP-nanobody-BTB, respectively. To confirm complete haploidization, a Single Nucleotide Polymorphism (SNP) analysis based on next-generation sequence reads was performed. No remains of the pollinator genome were detected. Haploidization occurs independently in embryo and endosperm cells and only certain embryo/endosperm ploidy ratios can survive. The application of this method for the generation of haploid crop plants will be discussed.

P200 Gamete-specific degradation of centromere-specific histone 3 (CENH3) for haploid induction in *Arabidopsis thaliana*

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The availability of a robust haploid induction methodology would be helpful to accelerate the breeding program for crop plants. Haploid production through uniparental genome elimination through different modifications of CENH3 has been successfully demonstrated in Arabidopsis. However, the method was only applicable to a few crops with low haploid induction frequency. Recently, it was shown that either egg-cell specific removal of modified CENH3 or dilution of endogenous CENH3 in the egg cell was the reason behind genome elimination. Therefore, we are interested in targeted degradation of CENH3, specifically in egg cells, to check whether it could lead to the induction of haploid plants. For the targeted degradation of the CENH3 protein we selected a nanobody-guided proteasomal degradation approach. Arabidopsis plants carrying a null mutation for endogenous CENH3 complemented with EYFP-CENH3 were used in this study to use an anti-GFP nanobody for targeted protein degradation. Two different heterologous E3-ligases namely Nslmb and SPOP, fused to anti-GFP nanobody (VHHGFP4) expressed under the control of EC1.1 promoter were being evaluated for egg-cell specific degradation of EYFP-CENH3. Transgenic plants carrying engineered E3-Ligases were obtained and genotyped for different components of the gene constructs. The transgenic plants will be crossed with wild-type plants and haploid induction rates for different E3-Ligases will be evaluated.

P201 Development and application of a phenotyping toolbox to investigate the temporal dynamics of the unpollinated wheat stigma

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In the absence of pollination, female reproductive organs, such as stigmas, senesce leading to an irrevocable loss in the reproductive potential of the flower. Thus, research to understand the dynamics of stigma survival in wheat could be key to achieving higher seed yields in hybrid seed production.

To advance our knowledge of stigma survival and senescence in wheat, we developed a high-throughput phenotyping approach using light microscopy, image analysis tools and machine learning techniques. We successfully implemented these approaches on field-grown male-sterile wheat cultivars to identify natural variation in the dynamics of stigma shape and size post-anthesis. We found (1) the presence of distinct stigma growth patterns among cultivars; (2) this trait to be robust across different environments and; (3) the existence of a common underlying developmental programme. This knowledge will equip researchers with a new framework to advance our mechanistic understanding of female fertility in wheat.

P202 Cytological analysis of cell invasion during gametophyte interactions in *Arabidopsis*

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Just before fertilization in flowering plant reproduction, pollen tubes (PTs) signal with and invade synergid cells, to allow for PT burst and sperm cell release. The integrity of the PT cell wall before synergid invasion is regulated by autocrine signaling through perception of Rapid Alkalinization Factors (RALFs) by the PT-expressed receptors of the *Catharanthus roseus receptor-like kinase1-like* (*CrRLK1L*) family such as *ANXUR1/2 (ANX1/2).* Then, during reception of the PT by the synergids, the synergid-expressed CrRLK1L receptors such as *FERONIA,* mediate burst of the PT likely through RALF paracrine signaling. While it was shown that a dominant acting mutation (R240C) in the cytoplasmic kinase, *MARIS (MRI)* can suppress *anx1/2* PT growth defects, it is unknown whether *MRI* acts downstream of *FER* in synergid cells.

Here we show that MRI[R240C] cannot suppress the PT bursting defects of *fer-1* nor the root hair or dwarfism defects. However, we found that MRI-GFP localizes to plasma membrane and offers a useful tool for spatial analysis of the PT-synergid cell invasion process – an event that has been unclear due to imaging limitations. Here we use this marker line among others in combination with live cell multiphoton imaging of PT reception and show that the pollen tube enters synergids around the filiform apparatus, forming an invagination of the synergid membrane. This cell invasion is reminiscent of a fungal invasion where the host plasma membrane surrounds the fungal haustorium. The synergid membrane invagination likely increases the intercellular communication between the PT and synergid through CrRLK1L signaling and allows for rapid degradation of the PT cell wall leading to it's burst.

P203 Transcriptome landscape of endosperm in developing barley seeds

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Cereal grains are the major source of food and feed. The largest part of cereal seeds is occupied by triploid endosperm, representing a specialized tissue for embryo protection and nourishment. In order to understand molecular and cellular mechanisms governing cereal seed development, we used barley (*Hordeum vulgare*), which is a diploid temperate zone cereal crop. To provide a spatiotemporal information about the seed developmental process, we performed transcriptomic study of endosperm, embryo and seed maternal tissues at early, middle, and late stages of barley grain development. Analysis of differential gene expression and co-expression networks pointed out to the major biological processes going on in different grain tissues at different times after fertilization, such as cellularization, differentiation and storage components synthesis. Furthermore, we defined a set of tissue-specific marker genes, which can be used to follow tissue origin and stage of seed development. This opens new avenues towards functional analysis of barley seed development.

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P205 Analysis of cell membrane adhesion molecules by live-cell imaging in animal cultured cell

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Fertilization is achieved by recognition, adhesion, and fusion of gametes. Fertilization-related factors are identified, for example, adhesion factor GAMETE EXPRESSED 2 (GEX2) and fusogen GENERATIVE CELL-SPECIFIC 1/ HAPLESS 2 (GCS1/HAP2) in plant, but it remains to be elucidated how cell membrane could adhere and fuse through molecular interactions. To dissect molecular dynamics and function of factors involved in gametic interactions, such as gamete adhesion and fusion, we developed a system to analyze function of fertilization factors by live-cell imaging in animal cultured cells. We focused on the Fusion assay system developed by Valansi *et al.* (2017). BHK animal cultured cells were transformed with a plant fusogen gene GCS1/HAP2 or a *Caenorhabditis elegans* somatic cell fusogen gene Epithelial Fusion Failure 1 (EFF-1), which resulted in promotion of their cell-to-cell fusion. Furthermore, by live-cell imaging, we found that fusion occurred predominantly between cells both expressing a fusion factor. Interestingly, a mammal fertilization factor IZUMO-expressing cells promoted multinucleation. This result implies that IZUMO can be involved in not only adhesion but also fusion. In addition, for analysis of the molecular interactions, we developed mixing assay which mix the different genes transfected cells. To investigate adhesion, it is necessary to observe before and after adhesion by live imaging. When BHK cells transfected E-cadherin known as an adhesion factor attached with each other E-cadherin accumulated at the interface. It was suggested that adhesion could be evaluated using accumulation as an index. When IZUMO-expressing cells attached with IZUMO receptor JUNO-expressing cells, IZUMO accumulated at the interface of JUNO-expressing cells. We will discuss future directions of our live-imaging studies by using and improving our system.

P206 Unraveling the role of membrane-protein interfaces in the regulation of pollen tube morphogenesis

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Minor signaling lipids at the plasma membrane have been implicated in the regulation of many cellular events, including cytoskeletal dynamics, membrane trafficking and stress responses. In plants, signaling lipids typically show rapid turnover but detailed information about their spatio-temporal distribution and downstream targets is still largely missing. Here we show that specific localization of anionic lipids defines functionally distinct domains of pollen tube plasma membrane. To identify the landscape of peripheral membrane proteins that bind to minor anionic membrane lipids, we employed the vesicle-cosedimentation assay coupled with mass spectrometry. By combining microscopic, biochemical, and computational approaches, we will present the evidence that multiple anionic lipids are involved in the membrane recruitment and regulation of pollen tube exocytic and endocytic machinery at the plasma membrane.

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P207 DIACYLGLYCEROL KINASE 5 regulates polar tip growth of tobacco pollen tubes

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Pollen tubes require a tightly regulated pectin secretion machinery to sustain the cell wall plasticity required for polar tip growth. Involved in this regulation at the apical plasma membrane are proteins and signaling molecules, including phosphoinositides and phosphatidic acid (PA). However, the contribution of diacylglycerol kinases (DGKs) is not clear. We transiently expressed tobacco DGKs in pollen tubes to identify a plasma membrane (PM)-localized isoform, and then to study its effect on pollen tube growth, pectin secretion and lipid signaling. In order to potentially downregulate DGK5 function, we overexpressed an inactive variant. Only one of eight DGKs displayed a confined localization at the apical PM. We could demonstrate its enzymatic activity and that a kinase-dead variant was inactive. Overexpression of either variant led to differential perturbations including misregulation of pectin secretion. One mode of regulation could be that DGK5-formed PA regulates phosphatidylinositol 4-phosphate 5-kinases, as overexpression of the inactive DGK5 variant not only led to a reduction of PA but also of phosphatidylinositol 4,5-bisphosphate levels and suppressed related growth phenotypes. We conclude that DGK5 is an additional player of polar tip growth that regulates pectin secretion probably in a common pathway with PI4P 5-kinases.

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P208 Fixing complex genotypes in *Brassica* crops

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The long-term goal of my research program is to introduce apomixis into Canola (*Brassica napus*), one of Canada's most important crops. In this light, my lab studies the genus *Boechera*, a wild relative of Canola.

In an unprecedented step, we have recently completed telomere to telomere polished chromosome assemblies of a sexual and apomictic Boechera genome, and in a comparative analysis involving 50 sexual and apomictic genotypes, we have identified 533 apomixis-specific DNA polymorphisms which are consistent with polygenic control of apomixis in *Boechera*. Interestingly, from a total of 533 apomixis-specific DNA polymorphisms, 514 (96%) are found adjacent to the centromere of a single chromosome. In a second study initiated in 2014, a two-pronged approach has been taken to (i) generate backcrosses using apomictic *Boechera* for the simultaneous discovery of all genetic factors for apomixis initiation, and (ii) test the transferability of apomixis into multiple *Brassica* crops. The key advantage of our study is the use of several well-characterized natural and synthetic apomictic hybrid lines of Boechera which fulfill all necessary requirements for the successful transfer of apomixis via backcrosses and intergeneric crosses without ploidy barriers into a *Brassica* crop. Specifically, we have generated 12 diploid unbalanced apomictic hybrid *Boechera* lines which produce viable haploid pollen, for backcrosses into numerous diploid sexual *Boechera* mother lines. Each backcross leads to the enrichment of the sexual recipient mother genome while selecting for the apomictic phenotype of the apomictic donors. We select for diploid unbalanced apomictic *Boechera* lines which produce haploid pollen (i.e. vector for apomixis-inducing genetic factors, 2C:5C seed ploidy) in each generated backcrossing generation, while discarding all polyploid, aneuploid, sexual or diploid balanced apomictic (i.e. diploid pollen producers) progeny. This process has led to the establishment of 12 near isogenic lines (NILs).

P209 The exocyst complex regulates pollen maturation and polar growth of pollen tubes

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Pollen development and especially pollen tube tip elongation relies on a precise regulation of highly polarized secretion at the plasma membrane that requires the vesicle tethering complex exocyst. To sustain this process several pollen-specific exocyst subunits paralogs evolved in plants.

EXO70A2 is the main exocyst EXO70 paralog in *Arabidopsis* pollen governing the conventional secretory function of the exocyst, analogically to EXO70A1 in the sporophyte. EXO70A2 is essential for efficient pollen maturation, pollen grain germination and pollen tube growth; also its tobacco homologue does localize to the very pollen tube tip. Moreover, EXO70A2 can substitute for the EXO70A1 function in the sporophyte, but *EXO70A1* does not fully complement *EXO70A2 LOF* in pollen indicating specific male gametophyte functions in accordance with deep evolutionary split between EXO70A1 vs. EXO70A2 in Angiosperms. In tobacco EXO70B isoform localizes to the subapical domain of pollen tubes. EXO70C1 and EXO70C2 paralogs acquired a diverged function as negative regulators of the exocytosis functioning probably outside the exocyst complex. The *EXO70C2* (most abundant EXO70 in pollen) loss-of-function resulted in an enhanced growth rate of pollen tubes and a decreased thickness of the tip cell wall, causing tip bursts. Pollen tube growth moderating function of EXO70C2 is regulated by the phosphorylation. SEC15a functions predominantly in pollen with a minor yet non-redundant contribution of major sporophytic paralog SEC15b localized to intracellular compartments. Mutants in *sec15a* show reduced pollen germination efficiency and very short pollen tubes and SEC15a protein localizes at the pollen tube tip. The split between SEC15a vs. SEC15b exocyst subunits correlates with the establishment of seed plants.

Exocyst complex participating in pollen exocytosis has not only different composition than in sporophytic cells, but also several EXO70 isoforms

function in pollen tubes, so that there are possibly several forms of exocyst subcomplexes active within a single pollen tube.

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P210 Effect of heat stress on pollen performance in early maturing soybean varieties

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The cultivation of soybean (*Glycine max* (L.) Merr.) is increasing and becoming more widespread for its desirable high protein content and versatile use. However, high temperatures attributed to climate change continue to present challenges for crop productivity and yield, ultimately impacting global food security. To determine the impact of high temperature stress on the performance of the male gametophyte during soybean reproduction, the pollen of three different cultivars within Maturity Group 00 are exposed to different temperature regimes. The morpho-functional responses of soybean plants during reproduction are largely dependent on the severity of a stress, which is often the result of a combination of its duration and intensity. Here, microscopy is used to observe how different high temperature treatments impact pollen germination and pollen tube growth. In addition to pollen germination rate, pollen tube length, and pollen tube morphology, changes in pollen tube cell wall structure are monitored, specifically callose, pectin and cellulose, to interpret any biochemical changes resulting from high temperature stress. This is achieved by staining pollen samples with aniline blue, propidium iodide and calcofluor white to assess whether temperature affects the biochemical composition of the pollen tube cell wall. Additionally, pollen samples will be stained with dihydrofluorescein diacetate to observe ROS concentrations at different high temperature treatments to quantify the impact of heat stress. The results from these experiments will advance our understanding of how high temperature stress impacts soybean reproduction, and thus, yield, with hopes of improving breeding and seed production for soybean.

P211 The FANCC-FANCE-FANCF complex is evolutionarily conserved and regulates meiotic recombination

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At meiosis programmed meiotic DNA double-strand breaks are repaired via homologous recombination, resulting in crossovers (COs). A large excess of DNA double-strand breaks is formed and only a small proportion get converted into COs because of active mechanisms that restrict CO formation. The Fanconi anaemia (FA) complex proteins AtFANCM, MHF1, and MHF2 were identified in a genetic screen as anti-CO factors that function during meiosis in *Arabidopsis*. Here, pursuing the same screen, we identify *FANCC* as a new anti-CO gene and show, despite low primary sequence conservation, and confirmed it is an conserved FA core complex member which was previously identified only in vertebrates. Further, we show that FANCC, together with its subcomplex partners FANCE and FANCF regulate meiotic recombination: Mutations of any of these three genes partially suppress CO-defective mutants and show synthetic meiotic catastrophe with the pro-CO factor MUS81. They also show elevated recombination, which is particularly marked in female meiosis. This work revealed that FANCC is conserved outside mammals has an anti-CO role during meiosis together with FANCE and FANCF.

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P212 Optimising crop flowers for pollinating insects

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Strawberry (*Fragaria ananassa*), a commercially important fruit crop with worldwide sales in excess of $3.5bn per year, contributes significant levels of micronutrients to the human diet. The flowers are hermaphrodite and self-compatible but do not readily self-pollinate without physical transfer of pollen to stigma. The long-term goals of this study are to contribute to an understanding of how strawberry flowers attract pollinators, and to provide advice on future breeding strategies to maximise pollinator attraction and yield while providing support to pollinator populations.

Fruits which are more even in shape command a higher market price than irregular ones; this evenness is linked to the quality of pollination, as each strawberry requires several hundred separate pollination events (as a strawberry consists of numerous achenes which develop on a swollen receptacle). Field studies of flowers of 20 strawberry cultivars show that there is significant variation in a number of pollinator-relevant traits of strawberry flowers, in terms of both floral morphology and nectar and pollen reward. I am using these results to investigate bee responses to the extremes of this variation in a laboratory bee behavioural facility, identifying which traits make the flowers easier for insects to find.

Crop breeders will be able to use these results to inform their efforts to produce future cultivars which make better use of the insects which visit them, and which are more rewarding to those insects.

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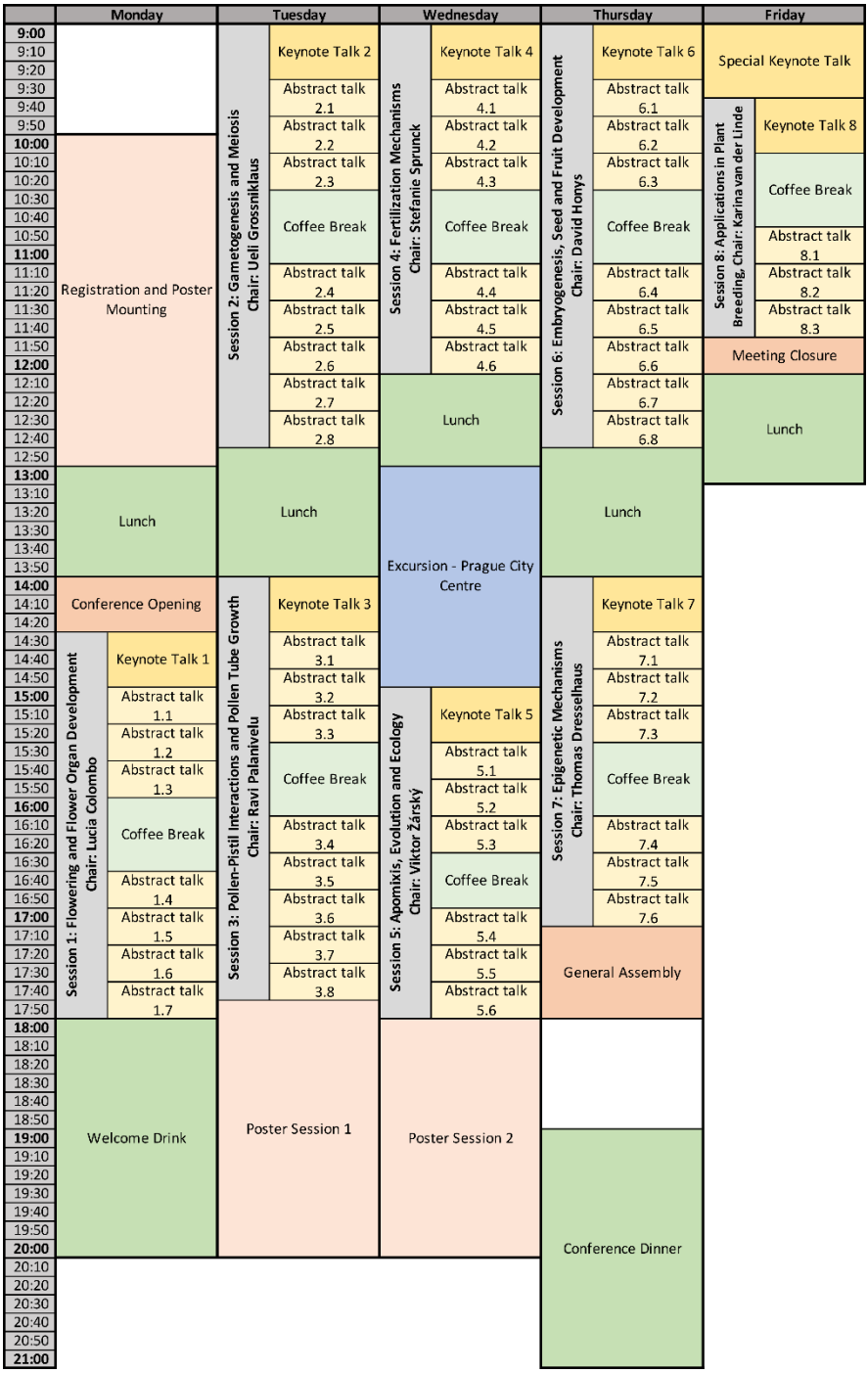
Monday
Tuesday
Wednesday
Thursday
Friday
Registration and Poster Mounting
Lunch
Conference Opening
Session 1: Flowering and Flower Organ Development Chair: Lucia Colombo
Keynote Talk 1
Abstract talk 1.1
Abstract talk 1.2
Abstract talk 1.3
Coffee Break
Abstract talk 1.4
Abstract talk 1.5
Abstract talk 1.6
Abstract talk 1.7
Welcome Drink
Session 2: Gametogenesis and Meiosis Chair: Ueli Grossniklaus
Keynote Talk 2
Abstract talk 2.1
Abstract talk 2.2
Abstract talk 2.3
Coffee Break
Abstract talk 2.4
Abstract talk 2.5
Abstract talk 2.6
Abstract talk 2.7
Abstract talk 2.8
Lunch
Session 3: Pollen-Pistil Interactions and Pollen Tube Growth Chair: Ravi Palanivelu
Keynote Talk 3
Abstract talk 3.1
Abstract talk 3.2
Abstract talk 3.3
Coffee Break
Abstract talk 3.4
Abstract talk 3.5
Abstract talk 3.6
Abstract talk 3.7
Abstract talk 3.8
Poster Session 1
Session 4: Fertilization Mechanisms Chair: Stefanie Sprunck
Keynote Talk 4
Abstract talk 4.1
Abstract talk 4.2
Abstract talk 4.3
Coffee Break
Abstract talk 4.4
Abstract talk 4.5
Abstract talk 4.6
Lunch
Excursion - Prague City Centre
Session 5: Apomixis, Evolution and Ecology Chair: Viktor Žárský
Keynote Talk 5
Abstract talk 5.1
Abstract talk 5.2
Abstract talk 5.3
Coffee Break
Abstract talk 5.4
Abstract talk 5.5
Abstract talk 5.6
Poster Session 2
Session 6: Embryogenesis, Seed and Fruit Development Chair: David Honys
Keynote Talk 6
Abstract talk 6.1
Abstract talk 6.2
Abstract talk 6.3
Coffee Break
Abstract talk 6.4
Abstract talk 6.5
Abstract talk 6.6
Abstract talk 6.7
Abstract talk 6.8
Lunch
Session 7: Epigenetic Mechanisms Chair: Thomas Dresselhaus
Keynote Talk 7
Abstract talk 7.1
Abstract talk 7.2
Abstract talk 7.3
Coffee Break
Abstract talk 7.4
Abstract talk 7.5
Abstract talk 7.6
General Assembly
Conference Dinner
Special Keynote Talk
Session 8: Applications in Plant Breeding, Chair: Karina van der Linde
Keynote Talk 8
Coffee Break
Abstract talk 8.1
Abstract talk 8.2
Abstract talk 8.3
Meeting Closure
Lunch