23rd International Congress on Sexual Plant Reproduction

Seeds for the future

Porto

13th to 18th July 2014

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International Association of
Sexual Plant Reproduction Research
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Welcome to the XXIII International Congress on Sexual Plant Reproduction

The XXIII Sexual Plant Reproduction Meeting invites you to attend the 2014 conference, held at Porto. This is a series of meetings that take place every two years, and are unique opportunities for hundreds of sexual plant reproduction enthusiasts from all over the world to get together. The theme of the congress is: Seeds for the future important when global demand and consumption of agricultural crops for food, feed, and fuel is increasing at a rapid pace. The application of genetic principles to improve cultivated plants is fundamental and new varieties can result only from improvements over the existing varieties in particular characteristics or in combinations of characteristics. The use of model plants to learn the fine points of seed production is essential to improve crop production.

A wide variety of topics are included in the program for the Porto meeting in 2014, flower development, male and female gametophyte development and function, pollination and fertilization mechanisms and self-incompatibility, meiosis, epigenetics, apomixis, seed and fruit development and plant reproduction for species conservation.

Enjoy an excellent scientific program together with the field's foremost experts and the promising newcomers, all while immersed in the warm hospitality of the city.

By the Organizing Committee

SUMMARY

Programme Overview

Session 1	Flowering and flower organ development
Session 2	Meiosis and Apomixis
Session 3	Germline development and function
Session 4	Evolution of reproductive structures and species conservation
Session 5	Self-incompatibility mechanisms
Session 6	Pollen tube growth
Session 7	Fertilization mechanisms
Session 8	Embryogenesis and endosperm development
Session 9	Seed and fruit development

23rd ICSPR Porto 2014 DETAILED PROGRAMME

Monday 14th July		
08:30		**Opening: Sílvia Coimbra, Lucia Colombo and Thomas Dresselhaus**
Session 1 | Flowering and Flower Organ Development		
Chair: Thomas Dresselhaus		
09:00	T1	**Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis** *Doris Wagner, University of Pennsylvania, Philadelphia, USA*
09:30	T2	**Epigenetic regulations of flowering time in rice** *Gynheung An, Kyung Hee University, Yongin, South Korea*
09:40	T3	**Molecular events underlying floral transition at the shoot apical meristem of soybean** *Prem Bhalla, University of Melbourne, Australia*
10:00	T4	**Jasmonic acid is a novel regulator of spikelet development in rice** *Dabing Zhang, School of Life Sciences and Biotechnology, Shanghai, China*
10:15	T5	**Chromatin-level co-regulation of clustered genes in stamen development** *Jens Sundström, Swedish University of Agricultural Sciences, Uppsala, Sweden*
10:30		***Coffee break***
Chair: Lucia Colombo		
11:00	T6	**The interactome of SCI1 (Stigma/style Cell-cycle Inhibitor 1) reveals a potential mechanism for its inhibition of the CDK complex** *Maria Helena Goldman, University of São Paulo/ FFCLRP, Ribeirão Preto, Brasil*
11:15	T7	**Three MYB-like proteins are involved in a subcellular tug-of-war underlying flower asymmetry in Antirrhinum** *Maria Manuela Costa, University of Minho, Braga, Portugal*
11:30	T8	**The genetic pathway in tapetum regulates pollen wall formation in Arabidopsis** *Zhong-Nan Yang, Shanghai Normal University, China*
11:45	T9	**Sorry, reception closed: Age-induced programmed cell death limiting the functional life span of the floral stigma** *Moritz Nowack, VIB/Ghent University, Belgium*
12:00	T10	**Functional analysis of the MADS-box and bHLH transcription factors during ovule development in *Arabidopsis thaliana*** *Irma Roig Villanova, Università degli Studi di Milano, Italy*
12:15		***Lunch***
Session 2 | Meiosis and Apomixis		
Chair: Fred Berger		
14:00	T11	**What limits meiotic recombination?** *Raphael Mercier, Institut Jean-Pierre Bourgin, Versailles, France*
14:30	T12	**Apomixis-like phenomenon occurred in Arabidopsis transformants of ASG-1, an apomixis-specific gene isolated from facultative apomictic guinea grass (*Panicum maximum*)** *Lanzhuang Chen, Minami Kyushu University, Miyakonojo, Japan*

14:45	T13	**JASON maintains cytoplasmic organization during male meiosis** *Lynette Brownfield, University of Otago, Dunedin, New Zealand*
15:00	T14	**The role of germline-specific rice Argonaute MEL1 in meiotic homolog pairing** *Ken-Ichi Nonomura, National institute of Genetics, Shizuoka, Japan*
15:15	T15	**Evidence for accumulation of transposable elements in the parthenogenesis locus of apomictic Taraxacum** *Peter J. van Dijk, Keygene N.V., Wageningen, The Netherlands*
15:30	T16	**Asexual seed formation (apomixis) in Hieracium – a matter of life and death** *Anna Koltunow, CSIRO-Plant Industry, Osmond, Australia*
16:00		**Pecha Kucha I**
16:30		***Poster session I and coffee break***

Tuesday 15th July		
Session 2 | Meiosis and Apomixis (cont.)		
Chair: Tetsuya Higashiyama		
08:30	T17	**CDKA;1 in Arabidopsis regulates the CO-landscape, CO-incidence and is required for WT levels of CO-interference** *Erik Wijnker, University of Strasbourg, France*
09:00	T18	**Molecular basis of meiotic drive in tomato pollen** *Bernard J. Carroll, University of Queensland, Brisbane, Australia*
09:15	T19	**Impact of temperature stress on meiotic cell division in plants – case study in *Arabidopsis thaliana*** *Nicole De Storme, University of Ghent, Belgium*
Session 3 | Germline Development and Function		
09:30	T20	**Distilling the 'Essence of Male' in plant germline development** *David Twell, University of Leicester, U. K.*
10:00	T21	**Tapetum control of pollen development** *Zoe Wilson, University of Nottingham, Loughborough, U. K.*
10:15	T22	**Elucidating the role of signals and cell wall polysaccharides during megaspore specification in Arabidopsis** *Matthew Tucker, University of Adelaide, Urrbrae, Australia*
10:30		***Coffee break***
Chair: Ueli Grossniklaus		
11:00	T23	**Microspore release from the tetrad: Progress towards identifying wall-degrading enzymes and their mechanisms** *Julia Tratt, University of Bath, U. K.*
11:15	T24	**DAZ1 and DAZ2: two novel EAR-dependent zinc finger proteins that promote mitotic transition and sperm fertility in Arabidopsis** *Michael Borg, University of Leicester, U. K.*

11:30	T25	**MTU1, the novel bHLH transcription factor involved in differentiation of inner-anther wall layers during meiosis in rice** *Seijiro Ono, National Institute of Genetics, Shizuoka, Japan*
11:45	T26	**Characterizing new proteins involved in vesicular transport** *Wei-Cai Yang, Chinese Academy of Sciences, Beijing, China*
12:00	T27	**A NOT so simple change of fate: NOT1 as a major regulator of late gametophyte maturation** *Jörg D. Becker, Instituto Gulbenkian de Ciência, Oeiras, Portugal*
12:15		***Lunch***
		Chair: Anna Koltunow
14:00	T28	**Occurrence transposable element-related sequences in transcripts of rice gametes and pollen** *Scott Russell, University of Oklahoma, U. S. A.*
14:15	T29	**Detection of histone methylation marks and expression survey of histone and histone modifying enzymes in sexual reproductive organs of the lower plant *Marchantia polymorpha*** *Martin O'Brien, University of Melbourne, Parkville, Australia*
14:30	T30	**Natural epigenetic variation affects cell specification during megasporogenesis in Arabidopsis** Arnaud Ronceret*, Langebio Cinvestav, Irapuato, Mexico*
		Session 4 | Evolution of Reproductive Structures and Species Conservation ***Organized by: Giovanna Aronne***
15:00	T31	**The implications of deception in pollination on nature conservation policy** *Amots Dafni, Haifa University, Israel*
15:30	T32	**Interspecific hybridization barriers in plants: GWAS reveals a role for glycosylation patterns in gametophyte recognition** *Lena Maria Müller, University of Zürich, Switzerland*
15:45	T33	**The DCL1-miR167-ARF8 pathway in reproductive development and the evolution of seed plants** *Adam Vivian-Smith, Norwegian Forest and Landscape Institute/Bioforsk, Aas, Norway*
16:00		***Coffee break***
		Chair: Giovanna Aronne
16:30	T34	**Repeated evolution of tricellular (and bicellular) pollen** *Joseph Williams, University of Tennessee, Knoxville, U. S. A.*
16:45	T35	**Flower biology of the relict species *Primula palinuri*: adaptation to past climate changes and warning for future scenarios** *Giovanna Aronne, University of Naples Federico II, Portici, Italy*
17:00	T36	**RNA sequencing reveals sexually dimorphic gene expression pre-dating sex organ differentiation in male and female gametophytes of *Marchantia polymorpha*** *Mohan Singh, University of Melbourne, Australia*
17:15	T37	**An evolutionary framework for carpel developmental control genes** *Annette Becker, Justus-Liebig-University, Institute of Botany, Gießen, Germany*

17:30	T38	**Interplay between mating systems, hybridisation and polyploidy drives ongoing speciation in Sorbus** *Simon Hiscock, University of Bristol, U. K.*
17:45	T39	**Monitoring breeding systems for threatened species in disturbance-prone environments — simple steps to unravel infertility in low fecund species** *Caroline Gross, University of New England, Armidale, Australia*
18:15		**IASPR General Assembly**
		Wednesday 16th July
		Session 5 | Self-Incompatibility Mechanisms
		Chair: Sílvia Coimbra
08:30	T40	**Functional analysis of *Papaver rhoeas* stigma and pollen S-determinants, PrsS and PrpS in *Arabidopsis thaliana*** *Zongcheng Lin, University of Birmingham, U. K.*
08:45	T41	**The self-incompatibility fertilization system in the Rosaceae subfamily Prunus** *Martin Goldway, Tel-Hai College, Upper Galilee, Israel*
09:00	T42	**Evolutionary and genetic basis of reduced pollen number in the predominantly selfing species *Arabidopsis thaliana*** *Kentaro K. Shimizu, University of Zürich, Switzerland*
09:15	T43	**Pollen tube cytoskeleton modification by transglutaminase during self-incompatibility in pear** *Stefano Del Duca, University of Bologna, Italy*
09:30	T44	**Variation in the expression of self-incompatibility reaction in *Brassica oleracea* L.** *Houria Hadj-Arab, University of Sciences and Technology Houari Boumediene, Algiers, Algeria*
		Session 6 | Pollen Tube Growth
09:45	T45	**Coordination of pollen tube growth by $Ca2^{+}$: channels and downstream mechanism** *José Feijó, Univ. Maryland (USA) and Instituto Gulbenkian de Ciência (Portugal)*
10:15	T46	**ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases** *Aurélien Boisson-Dernier, University of Cologne, Germany*
10:30		***Coffee break***
		Chair: David Twell
11:00	T47	**Brassinosteroids promote Arabidopsis pollen germination and growth** *Stefanie Sprunck, University of Regensburg, Germany*
11:15	T48	**The role of *Arabidopsis thaliana* phosphatidylinositol and lipid kinases, in pollen tube growth and fertilization: a cellular and molecular analysis** *Rui Malhó, Universidade de Lisboa, Portugal*
11:30	T49	**Translation regulation in tobacco pollen; a proteomic view** *David Honys, Institute of Experimental Botany ASCR, Prague, Czech Republic*

11:45	T50	**Nt-Risap is a TGN associated Nt-Rac5 effector regulating membrane traffic during polar cell growth in tobacco** *Octavian Stephan, University Erlangen-Nuremberg, Erlangen, Germany*
12:00	T51	**Pollen tube tip growth: Chemogenomics approach reveals a new mechanism behind the tip** *Yuan Qin, Fujian Agriculture and Forestry University, Fujian,P.R.China.*
12:30		***Lunch (University House)***
14:30		***Downtown guided tour***
16:30		***River Douro boat trip***
		Thursday 17th July
		Session 7 | Fertilization Mechanisms
		Chair: José Feijó
08:30	T52	**Discovery of AMOR glycan for pollen tube guidance: microfluidics and synthetic chemistry approaches** *Tetsuya Higashiyama, Nagoya University, Japan*
09:00	T53	**F-actin dynamics are essential for Arabidopsis fertilization** *Tomokazu Kawashima, Gregor Mendel Institute, Vienna, Austria*
09:15	T54	**Regulation of LURE-reception ability at the pollen tube tip of *Torenia fournieri*** *Satohiro Okuda, Nagoya University, Japan*
09:30	T55	**Untangling pollen tube and pistil gene expression using SNP-informed deep sequencing** *Alexander Leydon, Brown University, Providence, U. S. A.*
09:45	T56	**Rapid elimination of synergid cells through a cell-to-cell fusion with endosperm** *Daisuke Maruyama, Nagoya University, Japan*
10:00	T57	**MYB97, MYB101 and MYB120 function as male factors that control pollen tube-synergid interaction in *Arabidopsis thaliana* fertilization** *Ze-Min Tan, China Agricultural University, Beijing, China*
10:15	T58	**Cell-to-cell communication in grasses by EA1-like peptides** *Susanne Uebler, University of Regensburg, Germany*
10:30		***Coffee break***
		Chair: José Gutierrez-Marcos
11:00	T59	**A calcium dialog mediated by the FERONIA signal transduction pathway controls plant sperm delivery** *Quy A. Ngo, University of Zürich, Switzerland*
11:15	T60	**The cells of the female gametophyte display specific calcium signatures during double fertilization in *Arabidopsis thaliana*** *Yuki Hamamura, Université de Montréal, Canada*
11:30	T61	**Intercellular interactions within the male germ unit: functional relevance in double fertilization** *Leonor Boavida, Instituto Gulbenkian de Ciência, Oeiras, Portugal*

11:45	T62	**Unravelling the function and expression pattern of AGP4 in *Arabidopsis thaliana* reproduction** *Ana Marta Pereira, Faculdade de Ciências da Universidade do Porto, Portugal*
12:00	T63	**Molecular control of pollen tube reception** *Ueli Grossniklaus, University of Zürich, Switzerland*
12:30		***Lunch***
		Session 8 | Embryogenesis and Endosperm Development
		Chair: Raphael Mercier
14:00	T64	**Reprogramming and zygotic activation in Arabidopsis** *Fred Berger, Temasek Life Science Laboratory, Singapore*
14:30	T65	**Heritable barley genome engineering using TALE-nuclease in haploid cells** *Jochen Kumlehn, IPK, Gatersleben, Germany*
14:45	T66	**NtCYS, a multifunctional player in plant PCD during embryogenesis** *Mengxiang Sun, Wuhan University, China*
15:00	T67	**YODA signaling in the Arabidopsis embryo** *Martin Bayer, Max Planck Institute for Developmental Biology, Tübingen, Germany*
15:15	T68	**Assembly and localization of mRNPs during early embryogenesis in Arabidopsis** *Andrea Bleckmann, University of Regensburg, Germany*
15:30	T69	**Natural variation in the epigenetic control of seed development in Arabidopsis** *Nuno Pires, University of Zürich, Switzerland*
15:45	T70	**Genetic control of identity, growth and shape in the Arabidopsis embryo** *Dolf Weijers, Wageningen University, The Netherlands*
16:15		**Pecha Kucha II**
16:45		***Poster session II and coffee break***
		Congress Dinner
		Friday 18th July
		Session 9 | Seed and Fruit Development
		Chair: Dolf Weijers
08:30	T71	**Auxin dynamics put polarity in the pod** *Lars Østergaard, John Innes Centre, Norwich, U. K.*
09:00	T72	**Signalling mechanisms establishing early seed development in *Arabidopsis thaliana*** *Duarte D. Figueiredo, SLU Inst. för Växtbiologi, Uppsala, Sweden*
09:15	T73	**Investigating the role of transcription factors in fruit development** *Sofia Kourmpetli, University of Leicester, U. K.*
09:30	T74	**Ethylene negatively regulates fruit set and early fruit development** *Manuel Jamilena, University of Almería, Spain*
09:45	T75	**Flavonoid regulation of seed development in Arabidopsis - a role for auxin?** *Maha Aljabri, University of Bath, U. K.*

10:00	T76	**Growing hearts and cylinders: Comparing Arabidopsis and Capsella reveals switching growth patterns after fertilisation** *Tilly Eldridge, John Innes Centre, Norwich, U. K.*
10:15	T77	**TCP14 and TCP15, together with DELLAS, regulate Arabidopsis seed germination** *Simona Masiero, Universitá degli Studi di Milano, Italy*
10:30		***Coffee break***
11:00	T78	**Regulatory mechanisms of plant seed size control** *Rita Groß-Hardt, University of Bremen, Germany*
11:30	T79	**Communication between female gametes modulates early embryo development in flowering plants** *Jose Gutierrez-Marcos, University of Warwick, U. K.*
12:00		**Awards/Meeting Closure**

Oral Presentations

T1

Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis

Nobutoshi Yamaguchi[1], Cara M. Winter[1†],Miin-Feng Wu[1], Yuri Kanno[2], Ayako Yamaguchi[1], Mitsunori Seo[2], and Doris Wagner[1].

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[2] RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, 230-0045, JAPAN.

The switch to reproductive development is biphasic in many plants, a feature important for optimal pollination and yield. We show that dual opposite roles of the phytohormone gibberellin underpin this phenomenon in Arabidopsis. While gibberellin promotes termination of vegetative development, it is inhibitory for floral fate. To overcome this effect, the transcription factor LEAFY induces expression of a gibberellin catabolism gene, consequently increased LEAFY activity causes reduced gibberellin levels. This allows accumulation of gibberellin-sensitive DELLA proteins. The DELLA proteins are recruited by SQUAMOSA PROMOTER BINDING PROTEIN LIKE transcription factors to regulatory regions of the floral commitment gene APETALA1 and promote APETALA1 upregulation and floral fate synergistically with LEAFY. The two opposing functions of gibberellin may facilitate evolutionary and environmental modulation of plant inflorescence architecture.

T2

Epigenetic regulations of flowering time in rice

Gynheung An.
Kyung Hee University, Korea (South).

Molecular genetic studies with Arabidopsis have verified several epigenetic repressors that regulate flowering time. However, the roles of chromatin remodeling factors in developmental processes have not been well explored in rice. We identified a chromatin remodeling factor OsVIL2, that contains a plant homeodomain (PHD) finger, promotes flowering. We demonstrated that OsVIL2 is bound to native histone H3 in vitro. Chromatin immunoprecipitation analyses showed that OsVIL2 was directly associated with OsLFL1 chromatin, indicating that OsVIL2 epigenetically represses OsLFL1 expression. We showed that OsVIL2 physically interacts with OsEMF2b, a component of polycomb repression complex 2. As observed from osvil2, a null mutation of OsEMF2b caused late flowering by increasing OsLFL1 expression and decreasing Ehd1 expression. Thus, we conclude that OsVIL2 functions together with PRC2 to induce flowering by repressing OsLFL1. We also studied a Trithorax group (TrxG) protein that activates target gene expression by antagonistically functioning against the Polycomb group. We observed that suppression of OsTrx1, an ortholog of ATX1, delayed flowering time specifically under long day conditions. In the T-DNA insertion mutant ostrx1, expression of Ghd7 and its downstream genes were altered. We demonstrated that the PHD motif of OsTrx1 binds to native histone H3 and that OsTrx1 binds to Ehd3 through the region between the PHD and SET domains. Finally, we showed that the SET domain at the C-terminal end of OsTrx1 has histone H3 methyltransferase activity. Our results suggest that OsTrx1 plays an important role in regulating flowering time in rice by modulating chromatin structure.

T3

Molecular Events Underlying Floral Transition at The Shoot Apical Meristem of Soybean

Prem L Bhalla, Lim Chee Liew, Chui E. Wong, Chol-Hee Jung, Mohan B Singh
University of Melbourne, Australia.

The control of flowering time is one of the key features governing crop plant adaptation to different environments. Being a key developmental switch in seed production, floral induction can impact key agronomic traits such as yield and stress tolerance. Thus understanding gene expression networks that integrate the developmental and environmental signals that promote or inhibit flowering is crucial for sustainable agriculture under climate challenge conditions. Soybean [Glycine max (L.) Merr.], a major crop legume, is a photoperiod sensitive crop whose floral transition is triggered by exposure to short-day conditions. Our understanding of the molecular control of flower initiation in this agriculturally and economically important legume species is limited. Accordingly we have used an integrated bioinformatic and experimental approach to addresses this gap in our knowledge. Initially, we used comparative genomics tools to reveal complete repertoire of flowering regulatory genes in the soybean genome. Furthermore, a comparison with the, Glycine soja, a wild ancestor of soybean revealed SNPs and structural variants of genes associated with the light-signaling and ambient temperature pathways. Subsequently we used RNA-seq analysis to characterize the transcriptome of soybean leaf and shoot apical meristem at different time points following inductive short-day treatment. A total of 2951 shoot apical meristem and 13,609 leaf sequences with significant profile changes during the time course examined were identified. Most changes in mRNA level occurred within 24 hours of inductive treatment. Transcripts involved in mediating responses to stimulus including hormones or in various metabolic processes represent the top enriched GO functional category for the SAM and leaf dataset, respectively. Our data also revealed that the extensive reprogramming of genes associated with the epigenetic chromatin modifications and RNAi gene silencing in the shoot apical meristem following an exposure to inductive conditions. Our study provides valuable molecular tools for further functional investigations of flowering pathway genes in soybean.

T4

Jasmonic Acid Is a Novel Regulator of Spikelet Development in Rice

Qiang Cai, Zheng Yuan1,Mingjiao Chen, Changsong Yin, Zhijing Luo, Xiangxiang Zhao, Wanqi Liang, Jianping Hu & Dabing Zhang
School of Life Sciences and Biotechnology, Shanghai, China

Spikelet is the basal unit of inflorescence in grasses, and its formation is crucial to reproductive success and cereal yield. Here, we report a previously unknown role of the plant hormone jasmonic acid (JA) in determining rice (Oryza sativa) spikelet morphogenesis. The extra glume 1 (eg1) and eg2 mutants exhibit altered spikelet morphology with changed floral organ identity and number as well as defective floral meristem determinacy. EG1 is a plastid-targeted lipase participating in JA biosynthesis, and EG2/OsJAZ1 is a JA signaling repressor that interacts with a putative JA receptor, OsCOI1b, to trigger OsJAZ1's degradation during spikelet development. OsJAZ1 also interacts with OsMYC2, a transcription factor in the JA signaling pathway, and represses OsMYC2's role in activating OsMADS1, an E-class gene crucial to spikelet development. This work discovers a key regulatory mechanism of grass spikelet development and suggests that JA's role in reproduction has diversified during flowering plant evolution.

T5

Chromatin-level co-regulation of clustered genes in stamen development

Johan Reimegård, Snehangshu Kundu, Ali Pendle, Vivian F. Irish, Peter Shaw, Naomi Nakayama, Olof Emanuelsson, Jens F. Sundstrom
Swedish University of Agricultural Sciences, Sweden

Several lines of evidence suggest that co-expression of physically linked genes arranged in operon-like gene clusters occur much more frequently in eukaryotes than previously anticipated. A bioinformatics pipeline was developed to evaluate large-scale transcriptome datasets for physical clustering tendencies. Independent datasets from male reproductive development in Arabidopsis thaliana showed that genes expressed in differentiating stamens tend to form small chromosomal clusters. Clustering may confer a selective advantage as it enables coordinated gene regulation at the chromatin level. Pollen maturation is controlled by MS1 (MALE STERILITY 1), a transcriptional activator that contains a PHD domain, which is often linked with changes in chromatin structure. qRT-PCR and mRNA in situ hybridization experiments showed that genes in a sub-set of the clusters become upregulated within 48 hours after MS1 induction. DNA fluorescent in situ hybridization (FISH) combined with structured illumination (SIM) super-resolution microscopy further showed that transcriptional activation of the clustered genes was associated with open chromatin conformation. Stamen development seems to involve transcriptional activation of physically clustered genes through chromatin de-condensation.

T6

The interactome of SCI1 (Stigma/style Cell-cycle Inhibitor 1) reveals a potential mechanism for its inhibition of the CDK complex

Edward J. Strini[1], Lígia T. Bertolino[1], Hebréia A. O. Souza[1], Greice Lubini[1], Andréa C. Quiapim[1], Gustavo H. Goldman[2], Maria Helena S. Goldman[1].
[1]FFCLRP/University of São Paulo;
[2]FCFRP/University of São Paulo,Brazil.

SCI1 (Stigma/style Cell-cycle Inhibitor 1) has an important role in stigma/style development, acting as a tissue-specific cell-cycle inhibitor (DePaoli et al., 2011). To study the molecular mechanisms through which SCI1 regulates cell proliferation we have investigated its interaction partners. A Ni-cotiana tabacum stigma/style cDNA library in the yeast two-hybrid (Y2H) system was successfully constructed and screened, using BD-SCI1 as bait. Pull-down assays using recombinant SCI1 and protein extracts from N. tabacum stigmas/styles were also performed. Among the candidates of the pull-down, a novel CDK not yet characterized was identified. The interaction between SCI1 and this CDK was confirmed by Bimolecular Fluorescence Complementation (BiFC) and localized in the nucleolus of interphase cells. The identification of a cyclin that interacts with SCI1 (confirmed by Y2H and BiFC) corroborates SCI1 putative function as an inhibitor of the cyclin-CDK complex. Additionally, the interaction between SCI1 and the 14-3-3D was identified in the pull-down and Y2H screening, and confirmed by co-immunoprecipitation and BiFC. This interaction occurs dis-persedly in the nucleus, despite the fact that 14-3-3D is localized in the cytoplasm of interphase cells. To understand the dynamics of SCI1 in the nucleus, the localization of the fusion protein SCI1-GFP was studied during the different cell-cycle phases. SCI1-GFP was observed in the nucle-olus of BY-2 cells at interphase and prophase, disappeared at metaphase and anaphase, and reap-peared in the nucleolus at the end of telophase, showing that SCI1 is controlled by the cell-cycle. We propose that SCI1 inhibits the cell-cycle sequestering CDK in the nucleolus during interphase. Then, some cell signaling promotes the movement of 14-3-3D to the nucleus, allowing its interaction with SCI1, which dislocates it from the nucleolus at the initial prophase. The interaction between SCI1 and 14-3-3D releases the CDK and allows cell cycle progression.

Financial support: FAPESP, CNPq and CAPES (Brazil).

T7

Three MYB-like proteins are involved in a subcellular tug-of-war underlying flower asymmetry in Antirrhinum

João Raimundo[1$], Rómulo Sobral[1$,] Enrico Coen[2], M. Manuela R. Costa[1].
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[2] Department of Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, UK
[$] These authors contributed equally to this work

The establishment of meristematic domains with different transcriptional activity is essential for many developmental processes. The asymmetry of the *Antirrhinum majus* flower is established by transcription factors with an asymmetric pattern of expression and activity. To understand how this asymmetrical pattern is established, we studied the molecular mechanism through which the dorsal MYB protein RADIALIS (RAD) restricts the activity of the MYB transcription factor DIVARICATA (DIV) to the ventral region of the flower meristem. We show that RAD and DIV interact neither directly by forming heterodimers nor by competing for the same DNA binding site, but rather by competing for MYB like proteins termed DRIFs (DIV and RAD Interacting Factors). DIV and DRIFs are both expressed in all the petals of the flower and can form heterodimer complexes that, in vitro, bind to DNA containing a DIV consensus binding sequence, suggesting that the DRIFs act as co-regulators of DIV transcriptional activity. RAD is able to disrupt the formation of DIV-DRIF heterodimers by competing for the DRIF proteins in vitro. We have also shown that, in vivo, DIV interacts with DRIFs and changes their localization to the nucleoplasm. However, in the presence of RAD, DRIFs are sequestered in the cytoplasm further preventing the formation of DIV-DRIF heterodimers in the nucleus. Therefore, we propose that RAD antagonizes DIV in a subcellular competition for a DRIF protein by inhibiting the interaction between DIV and DRIFs in the dorsal regions of the Antirrhinum flower in order to establish the asymmetric pattern of gene activity in the flower meristem.

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T8

The genetic pathway in tapetum regulates pollen wall formation in Arabidopsis

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The pollen wall, an essential structure for pollen function, consists of two layers, an inner intine and an outer exine. The latter is further divided into sexine and nexine. We will present the results in our lab that a genetic pathway in Arabidopsis regulates pollen exine formation. MS188 encodes a MYB transcription factor MYB103 which was renamed as MYB80. Sexine is absent in ms188 while nexine is similar with wild type. NLC encodes an AHL family protein highly expressed in tapetum during the tetrad stage. Absence of nexine in nlc disrupted the deposition of intine without affecting sexine formation. ChIP and EMSA assays revealed that ABORTED MICROSPORES (AMS) in tapetum directly regulates the expression of NLC and MS188 for the nexine and sexine formation respectively. We also show that NLC directly regulates multiple AGPs including AGP6 for nexine formation. Our data show that a transcriptional cascade in the tapetum specifies the development of pollen wall.

T9

Sorry, reception closed: Age-induced programmed cell death limiting the functional life span of the floral stigma

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Non-pollinated flowers have a species-specific life span terminated by the death of floral organs, which causes the irrevocable loss the flower's potential to produce a fruit and seeds. Though much research has been dedicated to flower senescence, we still know only little of the intricate molecular network that controls this process and finally leads to age-induced programmed cell death.
Using the model plant Arabidopsis thaliana we recently found that ethylene is a central lifespan-regulator of stigmatic papilla cells. The stigma is an epidermal floral organ specialized for pollen reception and crucial for effective pollination and seed set. As the entire flower, the stigma has a specific functional life span that ends in a rapidly executed papilla cell death process. Once cell death sets in, pollination efficiency and seed set are drastically reduced, making the stigma a key determinant of the duration of flower receptivity in Arabidopsis.
On the basis of a recently generated transcriptome profile covering different stages of stigma senescence, and using a webcam-based phenotyping platform, we are unraveling novel nodes and edges in the regulatory network that controls stigma longevity downstream of ethylene signaling and upstream of the actual PCD execution. Our aim is to get to a comprehensive understanding of the molecular network and the key regulatory mechanisms that determine stigma longevity and programmed cell death in flowering plants.

T10

Functional analysis of the MADS-box and bHLH transcription factors during ovule development in Arabidopsis thaliana

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SEEDSTICK (STK) is a MADS-box transcription factor that together with its closely related genes SHATTERPROOF1 (SHP1) and SHP2 controls ovule identity in Arabidopsis thaliana. In order to deepen into the roles of STK, we developed a bioinformatics analysis based of co-expression patterns to find genes correlated with STK. One of the identified genes is named CESTA (CES), and encodes a basic Helix-Loop-Helix (bHLH) protein recently reported to be related to the BR positive signaling factors BRASSINOSTEROID ENHANCED EXPRESSION (BEE)1, BEE2 and BEE3.
Differently from the stk and ces single mutants, that do not show any ovule phenotype, the stk ces double mutant presents a high percentage of ovule abortions. The detailed characterization of this double mutant pointed out that STK and CES might be acting together in the regulation of the correct formation of ovules, regulating integument development. Moreover, we have observed by BiFC experiments that STK and CES are able to interact. A similar ovule abortion phenotype was observed in the triple mutant shp1 shp2 ces-2 or the quadruple mutant stk bee1 bee2 bee3, indicating that closely related MADs-box transcription factors play a role together with BR-related bHLH proteins in the control of ovule development. The genetic interaction of these factors, and the possible connection of these factors with hormonal pathways, is currently being investigated.

T11

What limits meiotic recombination?

Raphael Mercier

Meiotic crossovers generate genetic diversity by creating new allelic combinations. Intriguingly the meiotic crossover rates are restricted within a very narrow range. A minimum of one crossover is formed per pair of homologous chromosomes per meiosis. This "obligate" crossover ensures correct segregation of the homologs at the first division leading to balanced gametes and ultimately favors fertility of an organism. Crossover frequency does not typically vary above of the range of one to four crossovers per homologous pair. A great deal has been discovered about how meiotic crossovers are formed. However, very little is known about what limits their formation despite an abundance of molecular precursors. Using a forward genetic screen specifically designed to identify mutants with increased meiotic crossover frequency, we revealed that several pathways limit crossover formation. Crossover frequency can be largely increased without affecting meiotic chromosome segregation and fertility. This opens the possibility of manipulating recombination for plant breeding and raises the question of the selection pressure that maintains recombination at a low level in most eukaryotes.

T12

Apomixis-like phenomenon occurred in *Arabidopsis* transformants of *ASG-1*, an apomixis-specific gene isolated from facultative apomictic guinea grass (*Panicum maximum*)

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In order to clarify the function of *ASG-1*, an apomixis-specific gene isolated from facultative apomictic guinea grass (*Panicum maximum*), we have used the model plant of *Arabidopsis thaliana*, and constructed plasmids of pActnos/Hm2::ASG-1 and hsp::ASG-1::GFP to establish realizable transformation system. For detection of *ASG-1*, DNAs of T3 plants were used for PCR, using the primers designed according to *ASG-1*, and GFP was observed, respectively. For *ASG-1* expression in cytology and embryology, the young buds and flowers were treated and observed with Nomarski differential interference-contrast microscopy. The results obtained in this study are the following. 1) The PCR products gave *ASG-1* specific bands in different primer combinations. 2) The GFP was expressed in different parts of transformants. 3) When comparing with the un-transformant of *Arabidopsis*, T3 plants showed apomixis-like phenomenon with three types. (1) Two-embryo sac formation in the same ovule appeared simultaneously in micropylar end with equal size and same direction of the sac toward to chalazal end, and equal embryo development; (2) Formation of embryo and embryoid occurred from the same place in micropylar end of the same embryo sac; (3) Formation of one embryo and one embryoid occurred in the same sac, but derived from the different sides of horseshoe-shaped embryo sac, respectively. From the above results, it could be concluded that apomixis-like phenomenon occurred in *Arabidopsis* transformants of *ASG-1*, indicating the *ASG-1* gene may play a main role in both of formation of multiple embryo sac and multiple embryogenesis.

T13

JASON maintains cytoplasmic organization during male meiosis

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Accurate positioning of spindles is a critical aspect of cell division as it ensures that each daughter cell is the appropriate size and contains a single nucleus. In plant male meiosis with simultaneous cytokinesis, two chromosome separations occur without intervening cytokinesis. Thus, disturbance of spindle position during meiosis II can result in the interaction of chromosome groups and their subsequent incorporation into a single cell, giving rise to unreduced male gametes. The production of unreduced gametes is believed to be the main route to polyploid plant formation.
Loss of the JASON protein in Arabidopsis results in disturbances in spindle position at meiosis II and the formation of a high frequency of unreduced male gametes. Here we will show that the primary role of JASON is to maintain cytoplasmic organization in meiocytes. The position of organelles is altered in jason meiocytes, including the loss of an organelle band that forms between the two chromosome groups after the first division, and provides a physical barrier between the two spindles. JASON is located in plasma-membrane derived vesicles within this organelle band. We propose that endocytosis of vesicles containing JASON occurs during meiosis and that these JASON-containing vesicles are required to provide a scaffold that supports the organelle band. Our work uncovers a new role for vesicles during meiosis and highlights the importance of cytoplasmic events that occur during the meiotic chromosome divisions and impact upon spindle position.

T14

The role of germline-specific rice Argonaute MEL1 in meiotic homolog pairing

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Meiotic homolog pairing and recombination are central events to create new haplotypes, and known to be achieved by complicated and highly organized mechanisms in eukaryotes. Recently, the importance of noncoding RNAs in germline development and meiosis has been uncovered in animals, insects and microbes, while little is known in plants. We previously identified germline-specific Argonaute protein, MEL1, in rice. The *mel1* mutation resulted in no elongation of the central elements of the synaptonemal complex, indicating the essential role of MEL1 in homolog pairing. To further examine the molecular function of MEL1, we performed RNA-immunoprecipitation using anti-MEL1 antibody and deepseqencing of coprecipitated small RNAs. 75% of small RNAs isolated were 21-nucleotides (nt) long. We found that these 21-nt RNAs were processed from >700 long intergenic noncoding RNAs (lincRNAs). The lincRNAs were transcribed from >1,000 intergenic loci, in which the 21-nt RNAs were mapped on both strands, and arranged in 21-nt phased intervals. Cytoplasmic localization of MEL1 protein suggests that MEL1 is involved in translational control of unknown target genes in germline cells prior to or during meiosis. A current hypothesis of MEL1 function will be discussed.

T15

Evidence for accumulation of transposable elements in the Parthenogenesis locus of apomictic Taraxacum

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Apomixis is clonal reproduction through seeds. In the common dandelion, Taraxacum officinale, apomixis can be dissected into three developmental elements: 1. avoidance of meiosis I reduction (diplospory), 2. parthenogenesis of the unreduced egg cell, and 3. autonomous endosperm development. Since apomictic dandelions often produce pollen, they can be crossed with sexual con-specifics and apomixis-loci for the developmental elements can be genetically mapped. Genetic mapping indicates that parthenogenesis in dandelions is controlled by a single dominant locus PAR exhibiting suppression of recombination. Furthermore, the dominant PAR haplotype can only be transmitted by diploid pollen grains, hence apomicts are always polyploids. Similar features have been observed in apomicts in other genera and are consistent with evolutionary theory, which predicts that deleterious mutations will accumulate especially in the vicinity of apomixis-genes, since here the mutation load can accumulate over multiple clonal generations. New apomictic clones will be formed when pollen from apomicts fertilizes egg cells of sexual con-specifics. New apomictic clones inherit a clean purged genome derived from the sexual gene pool. However, new apomictic clones will also inherit a genome with a mutation load from their paternal clones. During pollen meiosis the genomes with the low and the high mutation load will recombine. This effectively leads to an accumulation of the mutation load around the apomixis-genes over multiple clonal generations. From an applied point of view this would mean that the negative features associated with natural apomixis-loci (like suppression of recombination, hemizygosity, polyploidy) are secondary and could be separated from the valuable apomixis-genes themselves (“a ruby in the rubbish”, Peck 1994).To test this hypothesis we compared the density of transposable elements in PAR and par haplotypes.

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T16

Asexual seed formation (apomixis) in Hieracium - a matter of life and death

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Apomixis (asexual seed formation) is the result of a plant gaining the ability to bypass the most fundamental aspects of sexual reproduction: meiosis and fertilization. Without the need for male fertilization, the resulting seed germinates a plant that develops as a maternal clone. This dramatic shift in reproductive process has been documented in many flowering plant species, although no major seed crops have been shown to be capable of apomixis. The ability to generate maternal clones via seed, rapidly fix desirable genotypes and potentially heterosis in crop species has the potential to accelerate agricultural breeding strategies. The potential of apomixis as a next-generation breeding technology has contributed to increasing interest in the mechanisms controlling apomixis in species where it naturally occurs. We have been studying the genetic and molecular control of apomixis in the genus Hieracium (Asteraceae) where a diploid female gametophyte is formed without meiosis (termed meiotic avoidance) and both formation of the embryo and endosperm is fertilization-independent. Two different modes of meiotic avoidance are observed in two distinct subgenera Hieracium and Pilosella. Apomixis in Pilosella is a dominant trait and independent loci confer the developmental components of meiotic avoidance, fertilization-independent embryogenesis and also fertilization-independent endosperm formation. Loss of these loci by deletion mutagenesis leads to a reversion to sexual reproduction in apomicts indicating sexual reproduction is the default mode. Genomic regions have been identified for some of these loci and the chromosome carrying meiotic avoidance is repeat rich in key genotypes under investigation. Analyses of gene expression profiles and small RNAs in ovules of apomicts and mutants indicate changes in specific small RNA populations presage the formation of the cell that initiates apomixis in subgenus Pilosella. Laser capture microdissection and transcriptome analyses of the initiating cell and other ovule cell types is providing indications of the types of changes required to switch reproductive development in Hieracium ovules from a sexual to an asexual pathway and also to inhibit development of sexually programmed cells.

T17

CDKA;1 in Arabidopsis regulates the CO-landscape, CO-incidence and is required for WT levels of CO-interference

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The main cell-cycle regulator in of Arabidopsis thaliana is CYCLIN DEPENDENT KINASE A;1 (CDKA;1). Its kinase activity is –among others- mediated by the known meiotic specific cyclins SDS and TAM1. We investigated the role of CDKA;1 in the meiotic cell division program using a series of CDKA;1 hypomorphic alleles, in which CDKA;1 activity is lowered as compared to WT. Very low CDKA;1 activity leads to complete sterility due to an asynaptic prophase and arrest after meiosis I. When CDKA;1 activity is at intermediate levels, chromosome pairing and crossover (CO-) formation do occur, but some univalents appear at metaphase I (desynapsis). Because at this activity level viable gametes are formed, we could study meiosis through backcrosses of Col-Ler heterozygotes with reduced CDKA;1 activity. CO-numbers are indeed reduced, corroborating our cytological data. Surprisingly, CO-interference is also reduced in these plants while COs tend to localize more to the distal ends of chromosomes. We tentatively conclude that WT levels of CDKA;1 activity are essential for maintaining CO-interference and a stable recombination landscape.

T18

Molecular basis of meiotic drive in tomato pollen

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The tomato DEFECTIVE EMBRYO AND MERISTEMS (DEM) gene is highly expressed in meristems, and homozygous dem mutants lack functional meristems (1). Consequently, homozygous tomato dem mutants fail to develop beyond the seedling stage, and mutated lines need to be maintained as DEM/dem heterozygotes. Surprisingly, in DEM/dem plants, frameshift dem alleles confer a meiotic drive phenotype in pollen.

Meiotic drive is defined as preferential transmission of one allele due to a disproportionate representation of one member of a chromosome pair in the gametes. In DEM/dem heterozygotes, dem pollen develops normally, whereas DEM pollen is arrested at the monocellular stage and aborts prior to gamete formation and pollen maturity.

DEM is a protein of unknown biochemical function, but we have evidence that it interacts with Ras-related nuclear protein (RAN) and is required for accumulation of small regulatory RNAs (sRNAs). RAN is known to play crucial roles in control of the cell cycle and nucleo-cytoplasmic transport of proteins and RNAs, including nucleo-cytoplasmic transport of sRNAs.

Meiotic drive has been previously described through the female germline of maize (2) and the male germline of Drosophila (3). In both cases, repetitive DNA is involved, suggesting an epigenetic mechanism. The Drosophila meiotic drive system has been characterized further at the molecular level, and remarkably, involves another interacting partner of RAN.

We have recently used Fluorescence-Activated Cell Sorting (FACS) to sort monocellular DEM pollen and bicellular dem pollen from DEM/dem plants, and are currently using small RNA sequencing to investigate a possible epigenetic basis for dem-conferred meiotic drive in tomato pollen.

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T19

Impact of temperature stress on meiotic cell division in plants - case study in *Arabidopsis thaliana*

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In flowering plants, the reproductive cell lineage is extremely sensitive to adverse environmental stress conditions, with temperature stress constituting the predominant stress factor. Upon exposure to heat or cold, both male and female gametophyte development typically show cytological alterations that eventually lead to pre-mature spore abortion and gametophytic lethality. These alterations are highly variable and include irregularities in tapetal development, defects in sugar metabolism, altered auxin metabolism and oxidative stress response. However, despite these well-known effects on gametogenesis, little is known about the impact of adverse temperature conditions on the process of sporogenesis. In this study, we therefore aim to characterize the effect of both high and low temperature stress on meiotic cell division, thereby focusing on homologous pairing and recombination, reductional chromosome segregation and cytoskeletal figure dynamics.
Cytological analysis of Arabidopsis male sporogenesis revealed that short periods of low temperature stress (1-40 hrs, 4-5°C) do not affect meiotic chromosome dynamics and segregation, but instead have a detrimental effect on the process of post-meiotic cytokinesis. More specifically, short periods of cold affects the formation of radial microtubule arrays (RMAs) which normally function as internuclear phragmoplast-like structures required for the formation of the cell plate. As a result, cold induces the formation of syncytial bi- and polynuclear microspores that show subsequent nuclear fusion before PMI to yield di- and polyploid pollen grains. Since these gametes may form a basis for sexual polyploidization, yielding highly adaptive polyploid genomes, we here postulate that, in contrast to the general detrimental effect on plant sporogenesis, environmental stress may alter the reproductive pathway in such a way that it drives plant evolution and speciation. In this perspective, state-of-the art findings on the impact of high temperature stress on Arabidopsis male sporogenesis will be presented.

T20

Distilling the 'Essence of Male' in Plant Germline Development

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There have been exciting advances in our understanding of the ontogeny of the male gametophyte generation of flowering plants, which delivers the two sperm cells needed for double fertilisation1,4,5. A central outstanding question in this example of 'micro-developmental patterning' in plants is how polarity and asymmetric division of the microspore are linked to the differential fate of the germline and its 'companion' vegetative cell. Mutants that disturb microspore division, together with new cell-fate and chromatin markers have allowed this issue to be re-examined. A key finding is that male germ cell fate does not strictly depend on cell isolation, supporting a 'germ-plasm' model for germ cell specification. Once the male germline is segregated following asymmetric microspore division, gamete specification depends on the germline-specific transcription factor DUO1 and its downstream target genes2. These include two ERF-associated amphiphilic repression (EAR) motif-containing zinc finger proteins (DAZ1 and DAZ2) that interact with the Gro/Tup1-related co-repressor TOPLESS3, highlighting the contribution of repressive mechanisms to germline development and fertility. We have started to explore the scale and specificity of the DUO1-DAZ1/DAZ2-dependent germline network by exploiting differentially expressed markers to isolate mutant germ cells by fluorescence-activated cell sorting. Our results link large-scale changes in gene expression with deregulated chromatin-associated pathways in mutant germ cells. Finally, our data provides compelling insight into the phylogenetic significance of the DUO1-DAZ1/DAZ2 regulatory module for the coordination of cell proliferation and gamete specification in angiosperm male germline development.

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T21

Tapetum control of pollen development

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Anther and pollen development is critical for plant breeding and the production of seed, and therefore has a direct impact on crop yield. The tapetum is the cell layer closest to the developing pollen, which is needed for meiotic progression and provides energy and resources for pollen wall formation. Communication between the tapetum and developing pollen, and the timing of tapetal activities are critical, as indicated by many male sterile mutants showing tapetum aberrations. The tapetum undergoes a defined development, forming before meiosis and then going through Programmed Cell Death (PCD) prior to pollen maturation. A number of transcription factors have been identified in Arabidopsis as critically involved in tapetal development, for example DYSFUNCTIONAL TAPETUM1 (DYT1), ABORTED MICROSPORE (AMS) and MALE STERILITY1: these have been shown to be highly conserved in monocots, for example in rice (1) and temperate cereals (2).
These tapetum transcription factors show different regulatory roles and expression characteristics, ranging from prolonged expression, for example of AMS, to extremely transient expression of MS1. In recent work in collaboration with the Zhang lab, SJTU, we have shown the functional role of AMS in the regulation of pollen wall development (3; 4). However we have recently identified an additional role for AMS, during the early stages of tapetum development around Pollen Microspore Meiosis. We have been developing tools to enable the analysis of this and to establish the function of AMS during meiosis and pollen development. This work will be put into context with changes occurring during tapetum development and PCD.

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T22

Elucidating the role of signals and cell wall polysaccharides during megaspore specification in Arabidopsis

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The composition of the plant cell wall varies greatly during development. Dynamic alterations in the amount and structure of plant cell wall polysaccharides influence wall flexibility and strength, particularly in rapidly dividing organs. In both female and male organs, β-1,3-glucan (callose) is heavily deposited around immature reproductive cells and degraded by β-1,3-glucanases prior to the initiation of gametogenesis. Premature or delayed dissolution of callose in male organs leads to defects in pollen development, but a role in megaspore development has proved difficult to address. We previously identified a Hieracium β-1,3-glucanase (GLUC) expressed in female reproductive tissues during early ovule development. Biochemical assays confirmed the substrate specificity of GLUC and transient localization assays revealed its subcellular localization. Cell-specific transgenic expression of GLUC in or around Arabidopsis female reproductive cells led to defects in gametogenesis, somewhat surprisingly independent of large-scale changes in callose deposition. Notably, the abortion phenotypes were remarkably similar to those seen in transgenic lines expressing viral suppressors of small RNA function. Our results suggest that ectopic GLUC expression may disrupt intercellular communication between reproductive and somatic cell types, possibly dependent on specific callose deposits in symplastic channels, which is required for correct megaspore function. We are currently using this system to investigate how female cell types differentiate and communicate within a complex tissue.

T23

Microspore release from the tetrad: Progress towards identifying wall-degrading enzymes and their mechanisms

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Dissolution of the pollen tetrad is essential for successful pollen development. The thick wall of the tetrad consists of callose (beta-1,3-glucan), with a primary cellulosic wall surrounding the whole structure. Despite the central importance of this process to plant fertility, the cell-wall degrading enzymes responsible for microspore release have not yet been identified. Using a combination of in silico and experimental approaches, a total of 21 beta-1,3-glucanase genes were identified that are expressed in the young bud of Arabidopsis thaliana at microspore release. Three genes are expressed exclusively in the tapetum, and a further two are expressed exclusively at microspore release, although are not tapetum-specific. Single, double and triple T-DNA insertion lines for the genes of interest showed no detectible phenotypic effect, indicating possible gene redundancy. A quadruple knockout line is currently undergoing phenotypic analysis.

No beta-1,4-glucanase genes have yet been reported that would be capable of degrading the cellulosic outer tetrad wall. Knockout lines for endo-beta-1,4-glucanase genes expressed in the young bud have no detectible effect on the reproductive phenotype, and no secreted exo-beta-1,4-glucanases have been identified. This suggests that enzymatic degradation of the cellulose component of the outer tetrad wall may not be necessary for microspore release.

A clue to an alternative mechanism comes from mutational analysis of the three QUARTET genes, where pollen tetrads fail to separate due to an impaired ability to degrade pectin (presumably in the outer cellulosic wall). However, their precise role has not been established. Double and triple QUARTET mutants have been produced for phenotypic analysis and the biochemical role of pectin and pectin degrading enzymes in tetrad wall dissolution is being assessed further in vitro. A new model for microspore release that integrates our new findings will be presented.

T24

DAZ1 and DAZ2: two novel EAR-dependent zinc finger proteins that promote mitotic transition and sperm fertility in Arabidopsis

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The male gametes of flowering plants arise from haploid progenitor germ cells that become functionally specialised to ensure fertilisation. In Arabidopsis, sperm cell development is controlled by the male germline-specific transcription factor DUO1, which integrates division of the germ cell with sperm cell specification (1,2,3). DUO1 promotes germ cell division by regulating the G2 accumulation of the mitotic cyclin CYCB1;1 (3) and is required for the expression of several sperm cell genes, including those essential for fertilisation (3,4). However, the regulatory hierarchy in which DUO1 operates to promote mitotic transition and regulate sperm cell development is unknown.

We present the essential role of two germline-specific DUO1 target genes, DAZ1 and DAZ2, which encode ERF-associated amphiphilic repression (EAR)-motif containing C2H2-type zinc finger proteins (5). We will show that DAZ1 and DAZ2 are required for germ cell division and for the proper accumulation of mitotic cyclins. Intriguingly, DAZ1/DAZ2 are sufficient to promote division of duo1 mutant germ cells, demonstrating that DAZ1/DAZ2 occupy a regulatory node in the DUO1 network that mediates G2/M transition. DAZ1/DAZ2 are also involved in the expression of DUO1 target genes and are essential for gamete fusion. We propose that DAZ1/DAZ2 regulate these developmental processes through transcriptional repression and show that the two EAR motifs in DAZ1 are important for germ cell division, gene repression and for interaction with the co-repressor TOPLESS. Our findings uncover an essential two-component regulatory module in which DUO1 and DAZ1/DAZ2 drive mitotic transition and implicate gene repression pathways in sperm cell formation and fertility.

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T25

MTU1, the novel bHLH transcription factor involved in differentiation of inner-anther wall layers during meiosis in rice

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Male meiocytes are surrounded with four-layered anther wall cells (tapetum, middle layer, endocecium and epidermis, from inside to outside) during meiosis in rice (Oryza sativa L.). Especially, development of tapetum has been well studied, because of their crucial roles in post-meiotic pollen development in angiosperm species. In contrast, little is known about genetic regulations of earlier development of anther wall layers and its significance on male reproduction. Here, we report a novel gene encoding bHLH-type transcription factor, named MTU1 (MIDDLE LAYER AND TAPETUM UNDIFFERENTIATED1), have an important role for differentiation of middle layer and tapetum in rice. Analyses of gene expression of MTU1 and cellular localization of YFP-fused MTU1 protein revealed that MTU1 was predominantly expressed in middle layer and tapetum at early meiotic stages. In wild-type anthers, middle layer cells dramatically thinned and degenerated during meiosis, and subsequently tapetal cells also degenerated following the production and the supply of pollen coat materials to microspores. In mtu1 mutant, no degenerative morphological changes occurred in middle layer and tapetum. The expression analysis of genes involved in development of anther wall layers revealed that MTU1 functions to promote differentiation of middle layer and tapetum from parietal cells, the primordial cells of inner anther walls, and that in mtu1, middle layer and tapetum stayed in parietal cell-like, undifferentiated state even after the cell division, and did not degenerate. Furthermore, meiosis progression was defective in mtu1 male meiocytes. Inner three layers of the anther wall and meiocytes have a common origin in archesporial cells, the primordial germ cells in plants. From this viewpoint, our results may suggest that proper differentiation of middle layer and tapetum is an essential event not only for anther wall development, but also for completion of male meiosis.

T26

Characterizing new proteins involved in vesicular transport

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Eukaryotic cells constantly refresh their repertoire of plasma membrane proteins by endocytosis to adapt to the extracellular environment. Evidence of the compartment and molecular machinery of endocytotic trafficking is accumulating, mainly on the trafficking between plasma membrane and early endosome (EE), EE to late endosome (LE), and LE to latic vacuole. Different from mammalian cells and yeast, the trans-Golgi network (TGN) in plant has been identified as equivalent to early endosome as an independent organelle dynamically associated with the Golgi. The bilateral trafficking between TGN/EE and PM, the unilateral trafficking direction from EE to LE and then to vacuole has been identified in mammalian cells, yeast and plants. At least some secretory anterograde trafficking from Golgi to PM pass through TGN/EE. The fluorescent membrane selective dye FM4-64 stains endocytic intermediates (END), Golgi and vacuole membrane, which indicate direct trafficking between END and these compartment. During these processes, multiple proteins, like GTPase or sorting receptors have been identified. However, understanding the molecular machinery of trafficking between END and other compartments is still a long way to go. Through forward genetics and cell-based fluorescence microscopy, we isolate two proteins TGG1 and TGG2, both involved in trafficking between END and other compartment. And the mutation of these two genes cause retrograde and anterograde transport defect. Endocytosis tracking by FM4-64 staining indicates that the two proteins take part in different cellular processes. Further analysis will demonstrate the molecular function and mechanism of these proteins.

T27

A NOT so simple change of fate: NOT1 as a major regulator of late gametophyte maturation

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Male gametogenesis initiates with an asymmetric division of haploid uninucleated microspores, giving rise to a vegetative cell enclosing a smaller generative cell that subsequently divides to originate two sperm cells. Gene expression profiling has shown that the last steps of gametophyte differentiation coincide with an extensive reduction of transcript diversity of the pollen transcriptome. We show that the conserved CCR4-NOT complex plays a major role in this reprogramming process leading to the final steps of gametophyte maturation. The CCR4-NOT complex is known to have a broad impact on the life cycle of mRNA and proteins, from transcriptional activation over RNA export to translational repression, mRNA decay and protein degradation. NOT1 is the major scaffolding protein of this complex and analysis of NOT1 T-DNA insertion lines revealed a severe segregation distortion and failure to recover homozygotes, consistent with defects in gametophyte transmission. Genetic analysis through reciprocal backcrosses with wild type confirmed a reduced transmission through the female and absence of transmission through the male gametophyte. In accordance, a subset of ovules showed developmental defects causing abnormal seed set, while not1 pollen grains showed defects in MGU organization and were largely impaired in pollen tube growth. Most importantly, comparative transcriptional profiling of wild type versus not1/+ pollen revealed that missing NOT1 expression adversely affects transcriptome reprogramming. Transcripts strongly down-regulated from the bi- and tricellular to mature stage in wild type pollen were among the most up-regulated in mutant pollen, indicative of a failure of CCR4-NOT driven mRNA decay. In addition, a number of transcripts that ought to increase in abundance during this phase failed to do so in mutant pollen. The broad implications of this misregulation for maturation and cell fate determination of the developing male gametophyte will be discussed.

T28

Occurrence transposable element-related sequences in transcripts of rice gametes and pollen

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Sexual fertilization involves a genomic level reorganization of the male and female genomic complements into a new founding cell lineage, combining genomes into a new sporophyte generation. Thus, transposable elements (TEs) are regarded as a challenge to eukaryotic organisms, as any such reorganization in the germ lineage has unique access to heritability in future generations. Effective TE suppression has therefore been the subject of considerable attention in the germ lineage. ESTs and microarrays, however, have shown high levels of TEs in the germ lineages of grasses—especially rice and maize. These TEs could potentially represent sequences that could gain expression in future generations, there is also a possibility that these encode suppression sequences that may in fact entrain chromatin modification, dicer-like or argonaute-like repression are alternative possibilities. TE signatures in pollen and sperm cells of rice appear particularly abundant, so we interrogated the genomic sequence for potential evidence of prior or historic incorporation of signature sequences that may have been inserted into the genome. Several of these TE signature regions recognized by RepeatMasker and RetrOryza appear be highly expressed in both cell types, but differentially in the sperm cells, whereas less were evident in pollen and in far lower intensity in female lineage cells. The vast majority of these sequences do not appear complete enough to encode functional transposons or retrotransposons, reflecting instead sporadic historical incorporation of TEs in the rice genome.

T29

Detection of Histone Methylation Marks and Expression Survey of Histone and Histone Modifying Enzymes in Sexual Reproductive Organs of the Lower Plant *Marchantia polymorpha*

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As the earliest descendant to land plant, *Marchantia polymorpha* is a species of choice to study evolutionary conserved mechanisms in plants. As such, we have verified that epigenetic marks on histone are part of a conserved code in the production of gametes. We conducted a histone methylation survey by immunolocalization in antheridiophores and archegoniophore of *Marchantia*, the male and female reproductive structures in *Hepaticaceae*. We have also used a large scale RNA sequencing project from immature and mature antheridiophores and archegoniophores to monitor the expression of histones and histone modifying enzymes. Here, we described the *in situ* degradation of Histone H3 during spermatogenesis, a change known to leads to protamine-based DNA compaction. A result support by RNAseq showing that a specific histone H3 gene expression is downregulated in mature antheridiophore. We have also demonstrated that histone H4 is sequencially removed after histone H3, a scenario reminiscent of animal histone removal during spermatogenesis. We also described the presence of abundant methylation marks on H3K4 and H3K36 in both types of gametes. Both of this histone H3 methylation marks have been associated with important signalling purposes in *Arabidopsis thaliana* gamete development. We have surveyed the pattern and presence of other methylation marks throughout *Marchantia* gametogenesis and recorded the very high abundance of H4R3me2s mark in every tissue tested. We have also surveyed the expression of small basic proteins specific to the antheridiophore and generated a list of potential candidates for the role of a protamine type protein in the flagellate sperm cell of *Marchantia*.

T30

Natural epigenetic variation affects cell specification during megasporogenesis in *Arabidopsis*

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The transition to the gametophytic phase relies in the specification of gametic precursors from sporophytic cells in the ovule. Natural developmental variations in the process of cell specification are reported to frequently occur in flowering plants, but the mechanisms and prevalence of these variations remain unknown. We systematically analyzed megasporogenesis in selected ecotypes of Arabidopsis thaliana and found variable number of cells specified as gametic precursors. Intra-specific hybridization and ploidy variation produced phenotypes similar to those reported for epigenetic mutants affected in the small RNA-dependent pathways. Phenotypic variability is correlated with subtle differences in the spatial and temporal pattern of localization of ARGONAUTE proteins, suggesting that natural variation affects megasporogenesis through epigenetic regulatory mechanisms that depend on the action of small RNAs. Our results suggest that the evolution of developmental alternatives occurring in the ovule acts through epigenetic mechanisms controlling cell specification in the ovule.

T31

The implications of deception in pollination on nature conservation policy

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Food deception occurs when rewardless flowers imitate the signals (colour, shape and size) of rewarding ones, in the same habitat and season, and "borrow" their pollinators. It could be specific (Batesian mimicry") or a "general food deception". (e.g. Orchis s.l. spp.)In "sexual deception" the flowers produce a blend of chemicals that imitate the pheromones of a specific solitary bee (mainly) species, than attract males of this species. These males pollinate the flowers while trying to copulate with it ("pseucopulation", e.g. *Ophrys spp*.).
In "shelter imitation" the flowers imitate a night shelter for various male of solitary bees which sleep in the flowers and carried out the pollination (Iris spp. sect. *Oncocylus, Serapias* spp. and *one Ophrys sp*.).
Pollinators: In general food deception, several bee species are participating in the system in accordance with the available "model flower species" occurring in the habitat and may vary at different locations. In Baetsian mimicry the deceptive species is completely dependent on the pollinators of the model, their abundance, phenology, distribution and learning ability to avoid the fraud.
Conservation: While planning any conservation scheme for a specific endangered or rare species (in the Mediterranean area many of the deceptive species are rare or endemic) there is a need to consider the following aspects:
1. There is an evidence for a partial overlap in nectar sources exploited by male bees, they exploit the same resources as the females. So it is important to conserve the food plants (pollen + nectar) of wild bees, particularly when they have evolved pollen specialization (oligolecty). The protection of these plants is an essential prerequisite to safeguard the pollination service of wild bees that are key to the reproductive success (and therefore survival) of the “deceptive” species.
2. The diversity of the rewarding plant species that indirectly contribute to the pollination of the deceptive species, may vary in abundance and phenology from one year to the next. Thus a given deceptive species may be dependent upon a varying network of different rewarding species at different years.
3. The nesting resources are also key to the maintenance of large populations of wild bees, both males and females, to ensure sufficient visitation rates by the pollinators and to avoid the local extinction of the deceptive plant species.
While considering all these factors, we point the risk that very small nature reserves may not supply the requested needs of all the partners of the deceptive pollination systems. It is recommended not to protect only a certain "desirable" rare or endangered deceptive species, but to consider all the components and resources which sustain the pollination system, that is the network of interactions sustaining the population plant species targeted by the conservation action plans. Very low-cost but highly efficient techniques are available to estimate the extent to which the deceptive plant species are dependent upon pollination services and field observations by trained field biologists are essential to uncover the links between rewarding and deceptive plant species. These approaches will help develop a more scientifically-sound set of guidelines for the development of efficient conservation practices.

T32

Interspecific hybridization barriers in plants: GWAS reveals a role for glycosylation patterns in gametophyte recognition

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Species are maintained by a variety of reproductive barriers that prevent interspecific hybridization. In contrast to pre-pollination barriers, which can be spatial or temporal patterns that prevent plants from being pollinated by pollen of a different species, post-pollination barriers come into play after an interspecific pollination event occurred. In our studies, we focus on pre-zygotic, post-pollination barriers acting at the last stage of pollination: the fertilization event itself.

When Arabidopsis thaliana (Col-0) flowers are pollinated with interspecific pollen from A. lyrata, more than half of the ovules in a silique remain unfertilized (Escobar-Restrepo et al., 2007). In these unfertilized ovules, the pollen tube (PT) is not recognized by the synergid cells of the embryo sac and, therefore, does not stop its growth in order to rupture and release its sperm cells, thus leading to an PT overgrowth phenotype.

In order to identify key players mediating the recognition of interspecific PTs by the embryo sacs we conducted a genome-wide association study (GWAS) using 86 A. thaliana accessions that were pollinated with A. lyrata pollen. We found a striking variation in interspecific PT reception ranging from 10-90% of ovules with PT overgrowth per silique, depending on which A. thaliana accession was used as a mother plant. Association mapping pointed us to a gene encoding a specific subunit of the oligosaccharyltransferase complex, which we confirmed to be involved in this process by an independent mutant analysis. The mutants display a strongly increased proportion of ovules with PT overgrowth per silique compared to the wild type when pollinated with A. lyrata, but not with A. thaliana, indicating that differential glycosylation patterns of putative recognition receptors contribute to the specificity of the interaction and are responsible for the interspecific incompatibility during PT reception.

T33

The DCL1-miR167-ARF8 pathway in reproductive development and the evolution of seed plants

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DICER-related genes encode multifunctional RNase proteins that are essential for viability in multicellular organisms. In plants DICER-LIKE1 (DCL1) is required for miRNA biogenesis, and the mature miRNAs it produces perform essential functions in regulating translation and guiding RNA cleavage. Mutations in DCL1 render Arabidopsis sterile. Nullomorphs are characterized by embryo lethality, while hypomorphic DCL1 alleles display wide ranging pleiotropic phenotypes that include maternal effect sterility, unfused carpels and ovules with short integuments. We show that each of these phenotypes are rescued in dcl1-7 and dcl1-9 through a dosage dependent manner by mutations in the miR167 target gene FRUIT WITHOUT FERTILIZATION/AUXIN RESPONSE FACTOR8 (FWF) that normally governs auxin responses, vascular development, integument morphogenesis and fruit initiation in wild type plants. The fact that the loss of this specific transcription factor rescues dcl1 hypomorphic mutants, along with sterility associated with mutations in other miRNA biogenesis pathway members, such as HEN1, HYL1, and HST, without restoring miRNA biogenesis, indicates a very central and special role for the DCL1-miR167-FWF/ARF8 pathway in the regulation of reproductive development, pleiotropy and phenotypic plasticity. Conservation of miR167, and its cognate transcription factor targets, FWF/ARF8 and ARF6 (particularly within the miR167 binding site), amongst orthologs in all seed plants, indicates remarkable purifying selection pressure, conserved action, and an ancient evolutionary origin coincidently associated with a suite of character innovations contained in seed plants and angiosperms. The inception and original ancestral function of miR167 is discussed.

T34

Repeated Evolution of Tricellular (and Bicellular) pollen

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In most seed plants, pollen is dispersed in a sexually immature state, but approximately 30% of flowering plants have evolved pollen that contains two fully formed sperm at anthesis. A classic paper by Brewbaker (1967 American Journal of Botany 54: 1069-1083) found that such "tricellular pollen" had many parallel, but irreversible, origins from an ancestrally immature and bicellular dispersal state. We modeled the evolution of pollen cell number for 2511 species on a time-calibrated angiosperm phylogeny. We used modern comparative phylogenetic methods that accounted for the effect of species diversification rates on character transition rates (BiSSE method), or that allowed variation in transition rates across the phylogeny (HRM method). Seventy-percent of species had bicellular pollen, including 21 early-divergent angiosperms that we studied. BiSSE found a 1.9-fold higher bicellular to tricellular transition rate than in the tricellular to bicellular direction, and bicellular lineages had a 1.8-fold higher diversification rate than tricellular lineages. HRM found heterogeneity in evolutionary rates, with bidirectional transition rates in three of four rate classes. These results show that the tricellular condition is not irreversible. However, tricellular lineages have slower rates of evolution than bicellular lineages, since they both diversified slowly and gave rise to bicellular lineages slowly. The slow evolutionary rates of tricellular lineages reflect a linkage between the evolution of sporophyte lifestyles and the developmental lability of male gametophytes.

T35

Flower biology of the relict species Primula palinuri: adaptation to past climate changes and warning for future scenarios

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Primula palinuri Petagn. is reported as a Pleistocene relict species with a severely fragmented geographical range. It is endemic to a narrow Tyrrhenian coastal area of Southern Italy and most of the plants live confined on vertical, north-facing limestone cliffs. Although it is phylogenetically linked to alpine species, it is the only Mediterranean and maritime species in the whole Primula genus. It is on the Red List of threatened species compiled by the International Union for the Conservation of Nature (IUCN) and is classified as endangered because of its small range and declining populations, possibly due to human impact in coastal areas. Because of its rarity and threatened status, this species deserve concern for conservation.
Basic consideration of our work was that in rare species, natural and anthropogenic selective pressures, including global warming, often result in demographic problems through the disruption of the reproductive processes or the failure of seedling establishment. The first aim of our work was to verify possible occurrence of bottleneck processes preventing successful accomplishment of the whole reproductive cycle. The second aim was to analyse the effect of different temperatures on pollen viability and germination.
Results showed that a full distyly syndrome occurs in *P. palinuri*: long-styled and short-styled floral morphs, two pollen morphological types and intra-morph incompatibility. Reproductive success, in terms of fruit- and seed-set, was high only as a result of inter-morph crossings. In both morphs, we have ascertained with specific experiments that pollen viability was affected by flower age, temperature and air humidity. Pollen germination was even more sensitive to temperature than viability and resulted significantly different between the two morphs.
Within a scenario of global warming, differences in male function between long-styled and short-styled individuals might alter their proportion in the few relict populations and prime local extinction vortex.

T36

RNA sequencing reveals sexually dimorphic gene expression pre-dating sex organ differentiation in male and female gametophytes *of Marchantia polymorpha*

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Dioecy where male and female reproductive organs are carried on separate male and female plants is relatively rare in plants. *Marchantia polymorpha* is a dioecious liverwort possessing many ancestral characteristics that lend it as a great model for tracking the missing pieces of sex determination in evolution. In *M. polymorpha*, the haploid set of chromosomes comprises eight autosomes and a single sex chromosome, X chromosome in females and a Y chromosome in males. To obtain novel insights into the molecular basis of sex differentiation in liverworts, we used RNA-seq to generate a comprehensive profile of gene expression in male and female thalli prior to and during reproductive development. Detailed analysis of gene expression in *Marchantia* gametophytes reveals sexually dimorphic gene expression well prior to antheridiophore and archegoniophore initiation, involving both the sex chromosomes and autosomes. Interestingly most of the genes expressed in male and female specific manner are located on the autosomes. Further our differential expression analysis revealed genes differentially expressed between male and female reproductive organs. Mature reproductive stages exhibited highest number of differentially expressed genes as compared to vegetative and immature male and female reproductive stages highlighting greater diversity and abundance of transcripts. We also identified several genes expressed in a stage-specific manner. Moreover, our study indicates that the *Marchantia* gametophyte commence sexual differentiation at the molecular level at the vegetative stage, through sexually dimorphic gene expression from both sex chromosomes and autosomes.

T37

An evolutionary framework for carpel developmental control genes

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Carpels are the female reproductive organs of flowering plants (angiosperms), enclose the ovules, and develop into fruits. The presence of carpels unites angiosperms and distinguishes them from all other plant groups suggesting that carpels are the most important autapomorphy of the angiosperms. Many transcriptional regulators and co-regulators essential for carpel development are encoded by diverse gene families and well characterized in Arabidopsis thaliana. Among these regulators are AGAMOUS (AG), CRABS CLAW (CRC), ETTIN (ETT), HECATE (HEC), LEUNIG (LUG), NGATHA (NGA), SEUSS (SEU), SHORT INTERNODE/STYLISH (SHI/STY), and SPATULA (SPT). Homologs of these carpel developmental regulators were sampled from the sequenced genomes of phylogenetically informative species, such as Physcomitrella patens, Selaginella moellendorfii, Picea glauca, and several angiosperms. We review current knowledge on the gene family and function in carpel development of the selected genes. Careful phylogenetic analyses were carried out that provide a phylogenetic background for the different gene families and provide minimal estimates for the ages of these families. Our analyses show that LUG-, SEU-, and SHI/STY-like genes were already present in the Most Recent Common Ancestor (MRCA) of all land plants, AG- and SPT-like genes were present in the MRCA of seed plants and their origin coincides with the ξ Whole Genome Duplication (WGD) and CRC-, HEC, ETT-, and NGA-like genes are angiosperm specific, most likely resulting from the ε WGD. Our work shows that the carpel development regulatory network was not recruited from a preexisting network that has been modified to serve in carpel development. Rather, the network directing carpel development was patched together by recruiting very old modules and combining them with comparatively novel, angiosperm-specific genes.

T38

Interplay between mating systems, hybridisation and polyploidy drives ongoing speciation in Sorbus

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Interspecific hybridisation and polyploidy are key processes in plant evolution and are responsible for ongoing genetic diversification 'in action' in the genus Sorbus (Rosaceae). The Avon Gorge, Bristol, UK, is a world 'hotspot' for Sorbus diversity and home to diploid sexual species and polyploid apomictic species. Diploid Sorbus are outcrossing and self-incompatible (SI), while triploid taxa are apomictic and pseudogamous, but also SI, so require pollen from other Sorbus taxa for seed set, which offers direct opportunities for hybridisation. Tetraploid taxa are pseudogamous and self-compatible so do not have the same obligate requirement for inter-taxon pollination. The mating interrelationships among Avon Gorge Sorbus taxa are complex and are the driving force for hybridisation and on-going genetic diversification of Sorbus. In particular, the presence of SI in triploid pseudogamous apomicts imposes a requirement for inter-specific cross pollination, thereby facilitating continuing diversification and evolution through rare sexual hybridisation events. This is the first known report of naturally-occurring pseudogamous apomictic SI plant populations, and we suggest that inter-specific pollination, in combination with a relaxed endosperm balance requirement, is the most likely route to the persistence of these populations. We propose that Avon Gorge Sorbus represent a model system for studying the establishment and persistence of SI apomicts in natural populations.

T39

Monitoring breeding systems for threatened species in disturbance-prone environments simple steps to unravel infertility in low fecund species

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Maintaining processes that allow natural selection to operate in plant populations is crucial for the conservation of species and for the production of offspring that will harbour evolutionary potential. Pollen flow, a key process, is moderated by a plant's breeding system, population size, the efficiency of pollinators and by habitat quality. All of these factors are indirectly or directly impacted by disturbance for which an unnaturally high-frequency (often by human activities) may prevent flower production— delivering knock-on effects to pollen flow, pollination, seed-set and the evolutionary potential of individuals and populations. Testing for plant breeding systems and pollination effi-cacy in these environments presents several challenges as populations may be small, threatened and poorly fecund. Here I show, by using breeding systems and molecular data from the fire-prone and threatened, Grevillea rhizoma-tosa (Proteaceae), that (1), an ultimate consequence of disrupted flowering in re-sprouters may be a build-up of so-matic mutations, which may induce inbreeding depression and sterility in populations and (2) that inbreeding de-pression may shelter as a self-incompatibility mechanism which could give an incorrect conservation evaluation for a population. A further consequence of this cascade of problems is that genetic diversity in populations can appear high although the diversity is somatic in origin with untested evolutionary benefits. Early detection of inbreeding and fertility issues can be facilitated by measuring pollen viability and by using inter-population crosses in breeding sys-tem trials. Disturbances that regularly prevent a re-sprouting species from flowering (e.g., frequent fires, frost dam-age to buds from premature snow melt, constant herbivory) may cause somatic mutations to accrue rather than be-ing purged via sexual reproduction and careful elucidation of the breeding system is necessary to unravel the conse-quences that limited successful reproduction has on plant fertility and population demography.

T40

Functional analysis of *Papaver rhoeas stigma and pollen S-determinants*, *PrsS* and *PrpS* in *Arabidopsis thaliana*

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Self-incompatiblity (SI) is a key mechanism used by many flowering plants to promote outcrossing. In the *Papaver rhoeas* (field poppy) SI system, a multi-allelic *S* locus allows discrimination between "self" (incompatible) pollen from "non-self" pollen on the stigma. Interaction of cognate pollen and pistil *S*-determinants triggers rejection of incompatible pollen. The poppy *S*-determinants are *PrpS* and *PrsS*. PrsS is a small novel protein that acts as a signalling ligand, and PrpS is a small novel transmembrane protein. Interaction between cognate PrpS and PrsS triggers programmed cell death (PCD) in incompatible pollen.

We recently introduced PrpS into self-compatible *A. thaliana* and demonstrated that *Papaver* PrpS is functional. When transgenic pollen expressing PrpS was grown *in vitro* with recombinant PrsS protein, a remarkably similar response to that triggered in incompatible poppy pollen was elicited. Whether *PrsS* can be expressed and functional in *A. thaliana* remains to be established. Current work aims to establish this and ultimately to establish if the *Papaver* SI system functions *in vivo* in transgenic *A. thaliana*. To investigate this we have introduced *PrsS-GFP* into *A. thaliana* driven by the *Brassica* stigma-specific promoter, *SLR1*. Here we will provide evidence demonstrating that *SLR1* can drive *PrsS-GFP* expression specifically in *A. thaliana* stigmas. We will also present recent preliminary *in vivo* pollinations between transgenic *A. thaliana* plants expressing PrsS and PrpS. Our data suggest that PrsS is functional and can interact with cognate PrpS *in vivo* to inhibit "self" pollen tubes in *A. thaliana*.

T41

The self-incompatibility fertilization system in the Rosaceae subfamily Prunus

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Rosaceae are Gametophytic Self-Incompatible (GSI). The GSI system is governed by the multi allelic S-locus which holds a specific pistil ribonuclease (S-RNase) and pollen specific genes (SLF/SFBs). The SLF/SFBs are F-Box genes which are components of the SCF complex that leads to poly-ubiquitination of selected proteins followed by their degradation by the 26S proteasome. Solanaceae also carry an RNase mediated GSI. Kubo et al. (2010) based on their findings in Petunia inflate, suggested the "collaborative non-self-recognition" model. Current data suggests that in the Rosaceae subfamily Ryrus (apple, pear) the systems might be analogous to petunia. However, there are indications that in the subfamily Prunus (plum, cherry, almond, apricot) the system is somewhat different. For example in Pyrus self-compatibility (SC) is due to mutations in the S-RNase gene but not in the F-Box genes whereas in Prunus SC can be due mutations in both S-RNase and SLF/SFB gene.

We are using apricot as a model system. The system is examined molecularly, by in vitro protein interaction methods, by cross pollination between species and bioinformatics tools. Our major findings are: 1) self RNase and SLF/SFBs proteins interact. 2) Pollen tube growth is not ceased in cross pollination between Rosaceae species 3) bioinformatic analysis shows that the level of divergence in Prunus SLF/SFBs is relatively higher than in Pyrus and Solanaceae. 4) an F-Box gene located outside the S-locus, termed parFB, also interact with the S-RNase in vitro.

Based on these findings and more, we hypothesize that in Prunus self-pollination a specific interaction between the SFB and its self S-RNase leads to protection of the S-RNase allowing it to destroy the pollen RNA and prevent self-fertilization. And in cross fertilization a universal interaction between other F-Box gene and the S-RNase leads to its ubiquitination and degradation, thus enabling fertilization.

T42

Evolutionary and genetic basis of reduced pollen number in the predominantly selfing species *Arabidopsis thaliana*

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The number of male gametes is a critical trait for reproductive success. Evolutionary and ecological studies has focused on selective forces favoring increased or reduced number of sperms in animals and of pollen grains in plants. The emergence of the selfing syndrome is a frequent evolutionary transition in flowering plants, and its major hallmark is the reduced number of pollen grains relative to ovules, namely a low pollen/ovule (P/O) ratio. Reduced pollen number would be advantageous in (predominantly) selfing species because the production of extra pollen grains is a waste of resource when outcrossing opportunities are limited. So far, in spite of the adaptive importance of pollen number, very little is known about the genes responsible for this quantitative trait and thus about the action of natural selection on these genes. We conducted a genome-wide association study to identify segregating polymorphisms in the model plant Arabidopsis thaliana. We found large variation in pollen number among accessions, and identified a number of loci underlying variation in pollen number confirmed by knockout mutants. Most notably, newly characterized genes associated with pollen number tend to show high levels of extended haplotype sharing. In addition, these loci overlap significantly with genomic regions with recent selective sweeps. This result is consistent with a scenario that the evolution of the selfing syndrome has occurred recently in A. thaliana.

T43

Pollen tube cytoskeleton modification by transglutaminase during self-incompatibility in pear

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The cytoskeleton of pollen tubes is a network of single and polymer-forming proteins that are involved in many aspects of pollen germination and growth. In the self-incompatibility response, changes to both actin filaments and microtubules are likely to be triggered by specific proteins, resulting in either the de-polymerization of cytoskeleton filaments, or the formation of aberrant structures. Transglutaminases are enzymes that catalyze the post-translational conjugation of polyamines to different protein targets among which the cytoskeleton ones as well as the cross-linking among protein substrates giving rise to protein aggregates. The binding of cytosolic TGase to actin filaments was shown to be Ca^{2+} dependent.
In the self-pollinated style of Abbè Fétel (AxA, incompatible system), the activity of TGase increased when the pollen tube stopped its growth inside the style leading to the formation of high molecular mass cross-linked products, including aggregates of tubulin and actin, as it is also shown by the in vitro post-translational modification of actin and tubulin catalyzed by purified pollen TGase. On the contrary in Abbè Fétel styles pollinated with Williams pollen (AxW, compatible system), TGase activity decreased during pollen germination.
Immunolocalization of TGase showed that the enzyme was present in the growing region of pollen tubes in the cytoplasm and in membranes; moreover, the enzyme colocalizes with cell wall markers; TGase is likely to be secreted by a mechanism involving both membrane dynamics and the cytoskeleton. Since actin filaments are perturbed during the self-incompatibility response, it is likely that the distribution and the activity of extracellular TGase is also affected, leading to the arrest of pollen tube growth. This enzyme was expressed during pollen germination inside the style to a similar extent in both systems (AxA and AxW) showing that it is only the activity to have been stimulated by some factors dependent on SI.

T44

Variation in the expression of self-incompatibility reaction in Brassica oleracea L.

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Self-incompatibility (SI) plays a key role in genetic diversity and evolution of species as it pro-motes outcrossing. In Brassicaceae, SI is under the control of a complex multiallelic S locus which contains at least three genes that co-segregate with the SI phenotype. This recognition system is associated with quantitative variation of the strength of the SI reaction. We analyzed the variability of the SI response in different lines of Brassica oleracea and in homozygous plants for three class-II haplotypes (S15S15, S5S5 and S2S2). We found a continuous phenotypic variation for SI response in plants with heterogeneous and plants with homogenous genetic backgrounds, from the strict SI reaction to self-compatibility. The self-incompatible response varies in each line but the variation is much greater in wild cabbage compared to cultivated groups. Molecular analysis revealed that partial-self-compatibility (PSC) was associated with decreased SRK or SCR/SP11 expression. A significant reduction of the SRK gene expression in old flowers characterizes the PSC plants, resulting in reduction of the SI phenotype during the life of the flower. The plants described in this study, constitute a material of choice to identify new mechanisms that could contribute to clarify the extent of phenotypic plasticity in SI.

Key words: Brassica oleracea, Partial-self-compatibility, S-locus, Class-II haplotypes.

T45

Coordination of pollen tube growth by Ca2+: channels and downstream mechanims

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Pollen transcriptomics revealed the expression of of about 7.000 genes in pollen, but theoretical modelling suggests that the cooperation of all of these into the processes of wall surface and cytoplasmic volume production is a minimal condition to explain most of the morphogenic events that characterize these cells. Spatial and temporal integration of extended biochemical and biophysical processes is mandatory, and in the past we have proposed that ion dynamics can be a common regulator of fundamental growth processes. I will report on advances on the biology of Glutamate- Receptor Like Ca2+-channels. These channels are hypothesized to participate on the generation of the Ca2+ focused gradient characteristic of functional pollen tubes, and eventually regulate Ca2+ fluxes into other compartments as well. I will also describe a new regulatory loop downstream of the Ca2+ signal, based on the activation of specific Ca2+ dependent kinases (CPK) and the regulation of the anion channel SLAH2. We have developed novel chloride (Cl-) sensing genetic probes, and imaged for the first time the dynamics of the cytosolic concentration of this ion. I will present data that allows the proposition of a feed-back between Cl- and Ca2+ as underlying the regulation of pollen tube growth

T46

ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases

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It has become increasingly apparent that the plant cell wall can influence intracellular activities in ways that go far beyond its supposedly passive mechanical support. Plant growing cells use mechanisms sensing cell wall integrity to coordinate cell wall performance with the internal growth machinery to avoid growth cessation or loss of integrity. How this coordination precisely works is unknown. Previously, we reported that in the tip-growing pollen tube the ANXUR receptor-like kinases (RLKs) of the Catharanthus roseus CrRLK1L subfamily are essential to sustain growth without loss of cell wall integrity [Boisson-Dernier et al., 2009]. Here, we show that over-expression of the ANXUR RLKs leads to over-activation of exocytosis and the over-accumulation of secreted membrane and cell wall material that subsequently triggers growth arrest. Moreover, the characterization of mutations in two partially redundant pollen-expressed NADPH oxidases coupled with genetic interaction studies demonstrate that the ANXUR RLKs function upstream of these NADPH oxidases. Using the H2O2-sensitive HyPer and the Ca2+-sensitive YC3.60 sensors in NADPH oxidase-deficient mutants, we reveal that NADPH oxidases generate tip-localized, pulsating H2O2 production that functions, possibly through Ca2+ channel activation, to maintain a steady tip-focused Ca2+ gradient during growth. Our findings support a model where cell wall-sensing receptors regulate ROS production, Ca2+ homeostasis and exocytosis to coordinate cell wall performance with the internal growth machinery [Boisson-Dernier et al., 2013]. Moreover, to identify new players of this largely unexplored pathway, a anx1 anx2 sterility EMS-induced suppressor screen was performed and led to the identification of suppressors with improved fertility due to rescue of anx1 anx2 PT growth. For the strongest suppressor, SNP-ratio mapping by next-generation sequencing identified a non-synonymous mutation in a pollen-preferentially expressed uncharacterized receptor-like cytoplasmic kinase (RLCK), opening the door to unravel the function of a unique RLK-RLCK receptor complex that coordinate cell wall integrity and polar growth.

T47

Brassinosteroids promote Arabidopsis pollen germination and growth

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Pollen tubes are fast growing tip-polarized plant cells, which, besides their fundamental role in double fertilization, represent excellent experimental systems for studying the dynamics and spatiotemporal control of polarized cell growth. Nevertheless, investigating pollen tube tip growth in the model plant Arabidopsis thaliana remains difficult because in vitro pollen germination and pollen tube growth rates are highly variable and differ from the in vivo situation. The reproductive tract of the pistil, especially the morphology of the transmitting tract (TT) and its nutrient-rich extracellular matrix (ECM), are considered to facilitate and to accelerate pollen tube growth over a long distance. However, growth-promoting ECM components are largely unknown.

We tested the potential influence of brassinosteroid (BR) on Arabidopsis pollen germination and growth in vitro and in vivo, and investigated the promoter activity of CYP90A1/CPD, encoding one of the key enzymes in BR biosynthesis, within the pistil of Arabidopsis. Here we will present our data, showing that BR acts on Arabidopsis pollen germination and pollen tube tip-growth in a dose-dependent manner, with growth kinetics more similar to the *in planta* situation. Furthermore, pollination experiments with mutant pistils revealed that BR synthesis is necessary to achieve efficient tube growth through the TT. Our results suggest that BR, or BR intermediates, are provided by the cells of the reproductive tract to promote pollen germination on the stigmatic papillae, and to accelerate tube growth through the TT.

T48

The role of Arabidopsis thaliana phosphatidylinositol and lipid kinases, in pollen tube growth and fertilization: a cellular and molecular analysis

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Polarized growth depends on an intricate and dynamic link between membrane secretion and signalling pathways. Phosphoinositides (PPIs) are minor membrane lipids which play and important role in this link. The immediate precursor of all PPIs is phosphatidylinositol and phosphorylation of the lipid head group by the action of phosphoinositide kinases results in the generation of several PPIs species.
Following recent work on this topic, here we adopted a reverse genetics approach coupled to molecular and cellular analysis to investigate the function of the Arabidopsis thaliana pollen-expressed gene encoding FAB1 phosphatidylinositol-3-monophosphate 5-kinases and DGK diacylglycerol kinases. FAB1 produce phosphatidylinositol (3,5)-bisphosphate [PtdIns(3,5)P2] and have been implicated in endomembrane trafficking control and pH control in the vacuole. Diacylglycerol kinases (DGKs) phosphorylate diacylglycerol (DAG) to form phosphatidic acid (PA).
Data shows that pollen germination, tube growth, and polarity were not significantly impaired in homozygous mutant plants lacking FAB1 or DGK proteins. In vivo, mutant pollen is able to fertilize ovules leading to normal seed set. Analysis of growth rates, endocytic events, vacuolar pH and labelling of ROS revealed discrete differences between wild-type and mutant genotypes which can account for a function of these genes. These results are correlated with protein localization characterized in elongating tobacco pollen tubes transiently transformed with GFP fusion constructs.
For both FAB1 and DGKs, the functional data obtained suggests the role of these proteins extends the simple catalytic activity of phosphoinositide and lipid production.

T49

Translation regulation in tobacco pollen; a proteomic view

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Male gametophyte, highly organized haploid flower organ, offers an unique chance to analyze development and differentiation of single haploid cell, cell-cell interactions and recognition, cellular polarity and pollen tube tip growth. Posttranscriptional control of gene expression plays a vital role during tobacco pollen maturation and tube growth. The need for high rate of translation during pollen tube growth suggests a demand for a robust storage system that could withstand a long-term storage and transport, ongoing cellular morphogenesis, and yet to deliver the message efficiently accompanied with an instant translation. Number of pollen genes showed apparent expression discrepancy at mRNA and protein levels and their respective transcripts were shown to be associated with long-term stored ribonucleoprotein particles annotated as EPP complexes. Similarly to the role played in growing mammalian neurons, EPP particles represent pre-loaded complex machinery devoted to mRNA processing, transport, subcellular localization and protein synthesis. Here we performed a detailed functional, transcriptomic and proteomic characterisation of pollen storage RNP particles in tobacco (Nicotiana tabacum L.). In particular, we aimed to integrate our knowledge on the categorization of translationally regulated transcripts in developing pollen and to identify the mode of action of the translational repression and derepression of mRNAs stored in developing pollen and gradually activated during progamic phase.

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T50

Nt-Risap is a TGN associated Nt-Rac5 effector regulating membrane traffic during polar cell growth in tobacco

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Rac/Rop GTPases, the Rho GTPases of plants, coordinate actin dynamics and membrane traffic during polar plant cell expansion. Tobacco (Nicotiana tabacum) pollen tube tip growth is controlled by the Rac/Rop GTPase Nt-Rac5, which specifically accumulates at the apical plasma membrane. Here, we describe the functional characterization of Nt-Risap, a novel Nt-Rac5 effector identified by yeast (Saccharomyces cerevisiae) two-hybrid screening. Nt-Risap belongs to a family of putative myosin receptors containing a “domain of unknown function 593” (DUF593), interacts with F-actin and is associated with a sub-apical TGN compartment, whose cytoplasmic position at the pollen tube tip is maintained by the actin cytoskeleton. At this TGN compartment, apical secretion and endocytic membrane recycling required for tip growth appear to converge. Nt-Risap overexpression interferes with apical membrane traffic and blocks tip growth. Nt-Rac5 constitutively binds to the N-terminus of Nt-Risap, and interacts in an activation dependent manner with the C-terminal half of this protein. In living pollen tubes, interaction between Nt-Rac5 and Nt-Risap is detectable at the sub-apical TGN compartment. A model of Nt-Risap regulation and function is presented, which integrates all these findings

T51

Pollen tube tip growth: Chemogenomics approach reveals a new mechanism behind the tip

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Rapid tip growth efficiently generates highly elongated cells for to exploe their environment or to penetrate tissues, e.g., fungal hyphae invade animal and plant host tissues or explore nutrients in the environment, and pollen tubes penetrate female tissues to deliver sperms to the ovule for fertilization. Such rapid polarized growth relies on massive targeting and fusion of vesicles to the apical region of the plasma membrane, termed tip growth domain. Our previous studies suggest that the growth domain is defined and maintained by a self-organizing mechanism that is localized to the tip growth domain and involves the ROP1 Rho GTPase signaling network composed of an interlinking positive and negative feedback loops. However it is unclear whether subapical regions of pollen tubes are also involved in the regulation of pollen tube tip growth. To uncover new mechanisms for the regulation of pollen tube tip growth, we use a chemogenomics approach, i.e., isolating T-DNA insertional mutants that exhibit a tip growth phenotype in the presence of a low dosage of chemical that targets the ROP1 signaling network. We have isolated a large number of mutants that show tip growth defects only in the presence of low dosage of Latrunculin B. Detailed characterization of one mutant led tot the discovery of a subapical mechanism that regulates the elongation rate and the growth direction of pollen tubes in Arabidopsis.

T52

Discovery of AMOR glycan for pollen tube guidance: microfluidics and synthetic chemistry approaches

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Sexual plant reproduction involves complex cell-to-cell communication. It is, however, difficult to study in the living material due to an embedded structure of female reproductive cells. Our group has been working on pollen tube guidance, double fertilization, and early embryogenesis in the living material by using two model plant species, Torenia fournieri and Arabidopsis thaliana (e.g., for review, Kurihara et al., 2013, Cell Growth Differ.). New interdisciplinary approaches with nano-engineering and synthetic chemistry are now in progress (e.g., for review, Arata and Higashiyama, 2014, Biochem. Soc. Trans.). In my own presentation, as one of typical examples, I will specially focus on the discovery of a novel key molecule in pollen tube guidance, AMOR glycan. We have reported previously that defensin-like peptide LUREs are pollen tube attractants working at the final step, which are secreted by two synergid cells on the side of the egg cell (Higashiyama et al., 2001, Science; Okuda et al., 2009, Nature; Takeuchi and Higashiyama, 2012, PLoS Biol.). However, LURE peptides are not sufficient for pollen tube attraction. We found that in Torenia, some ovular factor was critical to make pollen tubes competent to be attracted by LURE. This factor, named AMOR after activation molecule for response-capability, was purified by using a bio-assay method using manipulated ovules. Finally specific structure of arabinogalactan sugar chain was shown to be responsible for AMOR activity. Discovery of AMOR will open up a new filed of plant glycobiology especially in plant reproduction.

T53

F-actin dynamics are essential for Arabidopsis fertilization

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In most animals, microtubules organised from the centrosome enable migration of the gamete nuclei after gamete fusion. This process is essential to conclude fertilisation with the fusion of the two parental genomes. Flowering plants, however, do not possess centrosomes, questioning what type of cytoskeleton is essential for double fertilisation, a plant-specific process that requires fusion of two sperm cells with the two female gametes. Here, we report that F-actin but not microtubules is essential for fertilisation in Arabidopsis. Fertilisation is successful in female gametes defective in microtubule assembly. By contrast, disruption of F-actin dynamics prevents sperm nucleus migration in both female gametes. Supporting this essential role of F-actin dynamics, actin cables associate with the male nucleus during migration toward the female gamete nucleus. This dynamic F-actin movement required for sperm cell nucleus migration depends on myosin and a female gamete specific Rho GTPase. We demonstrate that in flowering plants F-actin assists migration of the male gamete nucleus and enables karyogamy, a role generally played by microtubules in most animals. This dependence on actin is probably linked to the loss of centrosomes, an event that occurred not only in plants but also in some animal species during evolution of eukaryotes.

T54

Regulation of LURE-Reception Ability at the Pollen Tube Tip of Torenia fournieri

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During fertilization in flowering plants, chemo-attractants from the synergid cells have been thought to be key molecules in pollen tube guidance. We have identified defensin-like peptide LUREs as attractant peptides (Okuda, Tsutsui et al., Nature, 2009). Here, we show that pollen tubes of Torenia fournieri are regulated by a stylar tissue in a length-dependent manner to receive and respond to attractant LURE peptides (Okuda et al., Molecular Plant, 2013). We found that LURE peptides bound specifically to the tip of pollen tubes growing through a cut style, depending on the elongation time. The peptides also bound to pollen tubes growing through a shorter style, which were not competent to respond to these peptides. It was demonstrated that an appropriate length of the style is critical for pollen tubes to become competent in being attracted to LURE peptides. These results suggested a possibility that acquisition of the LURE peptide reception ability and acquisition of full competency are separable processes.

T55

Untangling pollen tube and pistil gene expression using SNP-informed deep sequencing

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Pollen tubes deliver sperm to female gametes through complex pistil tissue with high efficiency and species specificity. During pollen tube growth in the pistil many cell:cell contacts are made preparing it for successful interaction with the ovule. Recent advances have described the transcriptional regulation of pollen tube differentiation during tube growth, including the identification of three R2R3-MYB transcription factors required for pollen tube reception (MYB97, MYB101, MYB120). While RNA analysis by microarray technology identified both MYB-dependent and independent transcriptomes, the available arrays have incomplete coverage of the genome. Furthermore, cell type of RNA origin (ie pistil or pollen) is not discernable from RNA samples that come from mixed cell types of a homogenous genetic background. We therefore utilized natural genetic variation in Arabidopsis thaliana populations in conjunction with Next Generation sequencing to perform in silico dissection of male and female transcripts. The Cvi-0 ecotype is highly polymorphic to the reference strain Col-0 containing on average one SNP per 180 base pairs. Therefore Cvi-0 was utilized as a pistil donor in crosses to wild-type and myb97, myb101, myb120-3 Col-0 strains, which were subjected to RNA deep sequencing and analysis. This analysis has identified both the full repertoire of MYB-regulated pollen tube genes, and pistil expressed genes that are induced by wild-type pollen tube growth and guidance. Interestingly, we also identified pistil expressed transcripts which are induced by wild-type pollen tubes, but not mutant pollen tubes. These findings underscore the rich communication between pollen tubes and the pistil, which cooperate to ensure reproductive success.

T56

Rapid elimination of synergid cells through a cell-to-cell fusion with endosperm

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The synergid cell is an entrance of female gametophyte, which attracts pollen tube and receives two sperm cells for fertilizations of the egg cell and the central cell. Synergid cells also function as gatekeepers that allow minimum times of pollen tube discharge through its degeneration. One of the two synergid cells degenerates by accepting a pollen tube, while degeneration or inactivation of the other synergid cell, termed persistent synergid cell, depends on double fertilization. If double fertilization did not occur, the persistent synergid cell keeps attracting second pollen tube and receives the contents to recover the failure of first pollen tube. The persistent synergid cell inactivated within a few hours after successful double fertilization. The rapid inactivation would be important for reducing fruitless male approaches and preventing polyspermy. To examine the inactivation mechanism, a synergid cell-specific pMYB98::GFP marker line was fertilized and time-lapse imaging of the ovules were performed. Surprisingly, we observed rapid mixing of the cytoplasm between the persistent synergid cell and the endosperm, suggestive of the cell-to-cell fusion event that was observed other than double fertilization. The fusion not only diluted AtLURE1, a pollen tube attractant peptide in the synergid cell, but also induced the synrgid nucleus disorganization associated with the endosperm division. The sequence of events revealed the origin of rapid inactivation of the persistent synergid cell. We propose that cell-to-cell fusion is an efficient strategy to eliminate specific cell functions.

T57

MYB97, MYB101 and MYB120 Function as Male Factors That Control Pollen Tube-Synergid Interaction in Arabidopsis thaliana Fertilization

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Pollen tube reception involves a pollen tube-synergid interaction that controls the discharge of sperm cells into the embryo sac during plant fertilization. Components of both the pollen tube and synergid are believed to be involved in the process. Several proteins associated with this process have been identified in synergid cells. However, very little is known about the components contributed by the pollen tube. Here we report that the pollen-expressed transcription factors MYB97, MYB101 and MYB120 probably control genes whose encoded proteins play important roles in Arabidopsis thaliana pollen tube reception (Liang et al., 2013, PLoS Genet 9(11): e1003933). They share a high amino acid sequence identity and are expressed mainly in mature pollen grains and pollen tubes. None of the single or double mutants of these three genes exhibited any visible defective phenotype. Although the myb97 myb101 myb120 triple mutant was not defective in pollen development, pollen germination, pollen tube growth or tube guidance, the pollen tubes of the triple mutants exhibited uncontrolled growth and failed to discharge their sperm cells after entering the embryo sac. The myb97 myb101 myb120 triple mutation significantly affected the expression of a group of pollen-expressed genes in mature pollen grains, including those genes that encode cysteine-rich proteins (CRPs). Furthermore, MYB101 could bind to the promoters of these down-regulated genes. All these results indicate that MYB97, MYB101 and MYB120 participate in pollen tube reception, possibly through controlling the expression of downstream genes whose products are involved in pollen tube-synergid signaling process.

T58

Cell-to-cell communication in grasses by EA1-like peptides

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Small secreted peptides mediate numerous cell-cell-communication events in reproductive and developmental processes in plants. Although computational analyses of plant genomes have revealed the existence of hundreds of genes encoding for putative secreted peptide ligands, only little is known about their possible roles. In maize, the signaling peptide ZmEA1 generated by the egg apparatus was shown to represent the sole female gametophytederived pollen tube attractant (Márton et al. 2005, Science). Recently, ZmEA1 has been shown to interact with a yet unidentified interaction partner at the surface of maize pollen tube tips and becomes internalized, likely by receptormediated endocytosis (Márton et al. 2012, Curr Biol; Uebler et al. 2013, Plant Signal Behav). An update will be presented on the molecular identification of the ZmEA1 receptor.

Moreover, by searching for homologous peptides, we have discovered a novel class of hydrophobic and polymorphic small proteins in grasses, named as EA1-like peptides (EALs). ZmEAL1 was the first additional members of this family, which was studied in more detail. It was shown that this egg cell-secreted peptide is required for germ cell identity. (Krohn et al. 2012, Dev Cell). Besides maize, EALs were also found in other grasses such as rice and Sorghum bicolor. As a common feature they all share a C-terminal EA1-box, short P-and A-boxes as well as N-terminal located signal sequences. Here we will present a molecular and cellular survey of several EA1-box containing proteins that enter the secretory pathway.

A homolog of particular interest is ZmEAL2 representing the closest relative to ZmEA1. Quantitative expression analysis of ZmEAL2 showed strong expression during embryogenesis, indicating that this candidate signaling peptide contains a role unrelated to that of ZmEA1 and ZmEAL1, respectively.

T59

A calcium dialog mediated by the FERONIA signal transduction pathway controls plant sperm delivery

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Sperm delivery for double fertilization of flowering plants relies on interactions between the pollen tube (PT) and two synergids, leading to programmed cell death (PCD) of the PT and one synergid. The mechanisms underlying the communication among these cells during PT reception is unknown. We discovered that the synergids control this process by coordinating their distinct calcium signatures in response to the calcium dynamics and growth behavior of the PT. Induced and spontaneous aberrant calcium responses in the synergids abolish the two coordinated PCD events. Components of the FERONIA signaling pathway are required for initiating and modulating these calcium responses and for coupling the PCD events. Intriguingly, the calcium signatures are interchangeable between the two synergids, implying that their fates of death and survival are determined by reversible interactions with the PT. Thus, complex intercellular interactions involving a receptor kinase pathway and calcium-mediated signaling control sperm delivery in plants.

T60

The cells of the female gametophyte display specific calcium signatures during double fertilization in Arabidopsis thaliana

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Calcium waves and oscillations are key signaling elements during the fertilization process in animals, and are involved for example in egg activation. In flowering plants, the fertilization process is unique as not only the egg cell but also the neighboring central cell fuses with a sperm cell during double fertilization. During in vitro fertilization in maize sperm cell fusion to the isolated egg cell causes Ca2+ increases (Digonneto et, al., 1997, Antoine et, al., 2000). However, whether this phenomenon occurs in vivo or in other cells of the female gametophyte has not been demonstrated. By using a semi-in vivo fertilization assay based on Arabidopsis thaliana, we investigated the Ca2+ dynamics in the cytoplasm of the egg cell, the central cell and the synergid cells during double fertilization. YC3.60 was expressed in the cells of the female gametophyte to visualize Ca2+ dynamics and HTR10-mRFP was used for the labelling of sperm cell nuclei. We detected two Ca2+ peaks in the egg cell during the fertilization process. The first peak correlated with the timing of pollen tube discharge whereas the second occurred during the fertilization (plasmogamy) of the egg cell. In the central cell on the other hand, typically only one Ca2+ peak was observed and it coincided with the timing of pollen tube discharge. Occasionally, a second peak was observed, but its timing did not always correlate with fertilization. The synergid cells displayed calcium oscillations upon pollen tube arrival, leadingup to a maximum of the cytosolic Ca2+ concentration in these cells when the pollen tube discharged. In the persistent synergid continued oscillations were observed after double fertilization. These calcium dynamics in the cells of the female gametophyte seem to be highly specific signatures that may be involved in coordinating successful double fertilization in the flowering plants.

T61

Intercellular Interactions within the Male Germ Unit: Functional Relevance in Double Fertilization

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Cell-cell interactions and intercellular communication are undoubtedly fundamental processes for germ line specification and the success of double fertilization. The “cell within a cell” structural organization of the male gametophyte in angiosperms reveals an intimate association of the male gametes (sperm cells) with the vegetative nucleus. An endomembrane of vegetative origin encloses both sperm cells and extends from one of the sperm cells to the vegetative nucleus through a cytoplasmic projection forming the male germ unit (MGU). This cellular structure assures that both sperm cells are transported as a unit by the leading vegetative nucleus until pollen tubes deliver sperm cells in the vicinity of female gametes. However, recent evidences indicate that this membrane organization may play additional functions by establishing a communication link between the vegetative nucleus and sperm cells. We have recently found that two sperm-enriched tetraspanins (TETs), TET11 and TET12 localize in a polarized way in a TET-enriched microdomain (TEM) at the interface of sperm cells, raising the hypothesis that this membrane microdomain may create a molecular scaffold essential for the stabilization of sperm-sperm cell adhesion or act by facilitating sperm-sperm cell communication. Could these physical connections restrict or facilitate the flow of information between sperm cells and influence sperm cell behavior and the outcome of double fertilization? We will report our more recent approaches to address these questions at the molecular and cellular level, focusing on the relevance of TETs and potential binding partners in MGU organization, intercellular communication and their potential impact on double fertilization.

T62

Unravelling the function and expression pattern of AGP4 in Arabidopsis thaliana reproduction

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Arabinogalactan Proteins (AGPs) undergo several post-translation modifications. The cDNAs encoding the protein backbones of AGPs show a characteristic domain structure consisting of an N-terminal secretion signal, absent from the mature protein, a central domain rich in proline/hydroxyproline, alanine, serine and threonine, followed by a C-terminal signal sequence for GPI-anchor attachment. Mature AGPs are therefore extensively glycosylated and predicted to be attached by a GPI anchor to the plasma membrane. The GPI anchor can be cleaved by specific phospholipases, releasing the polypeptide into the extracellular matrix in a regulated manner, suggesting that they might play signaling roles. Coimbra et al. in 2007 have shown that AGPs can be used as molecular markers for reproductive development, showing that they are present in pistil tissues, especially along the pathway followed by the pollen tube during its guidance to reach the egg cell inside the embryo sac of the female gametophyte. One of the purposes of this work is to study the AGPs present in these tissues, as well as to unravel their roles along this path. AGP4 was one of the AGPs selected for further analysis and functional characterization based on microarray data available (Genevestigator, Arabidopsis eFP Browser, Wuest, S.E. et al., 2010). In the present work we show some of our first results regarding the study of the agp4 mutant and discuss its possible involvement in multiple pollen tube block.

Coimbra, S. et al. (2007) J. Exp. Bot., 58-16, 4027-4035.

T63

Molecular Control of Pollen Tube Reception

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Research in our laboratory focuses on the developmental genetics of plant reproduction, with an emphasis on cellular interactions during double fertilization. Preceeding fertilization, sperm cell release depends on the proper reception of the pollen tube by the synergid cells. We have isolated and characterized female gametophytic mutants that disrupt pollen tube reception. Pollen tubes that encounter such mutant female gametophytes are unable to rupture and release the sperm cells (Huck et al., Development 130:2149; Kessler et al., Science 330:968). These phenotypes suggest that the female gametophyte controls the behavior of the male gametophyte in this process. One of the mutants, feronia (fer), was shown to disrupt a receptor-like kinase of the CrRLK1L family (Escobar-Restrepo et al., Science 317:656), while another, nortia, affects a seven-transmembrane-domain-protein similar to the powdery mildew resistance protein Mlo (Kessler et al., Science 330:968). We will present structure-function studies of FER and related kinases that shed light onto the conservation and diversification of signaling processes mediated by the CrRLK1L kinases. Furthermore, we identified additional components mediating pollen tube reception, which suggest the involvement of glycosylation in the recognition process between male and female gametophyte in a way that resembles sperm-egg interactions in mammals.

T64

Reprogramming and zygotic activation in Arabidopsis

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In plants fertilization marks simultaneously two transitions. The transition between the gametophytic and sporophytic life phases is specific to plants. The transition between the gametes and the zygote of the next generation, which is common to all eukaryotes. These impose the requirement to reprogram the transcriptional activity of the genome. Transcriptional activity depends largely on the chromatin-associated factors, DNA methylation, Histone modification and histone variants. The presentation will report evidence for DNA methylation reprogramming and its impact on transcriptional repression in the zygote. Reprogramming also affects histone variants and the extent and impact of this step on zygotic activation will be discussed.

T65

Heritable barley genome engineering using TALE-nuclease in haploid cells

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Site-specific genome engineering is a breakthrough technology that greatly facilitates the functional validation of genes and offers versatile novel possibilities of crop improvement. Aiming to establish genome engineering as a viable tool in cereals, we generated and expressed GFP-specific transcription activator-like effector nuclease (TALEN) in embryogenic pollen of barley lines harboring a single copy of the GFP gene used as experimental target sequence. When Agrobacterium-mediated transfer of TALEN-coding sequences was achieved during pollen embryogenic development and followed by spontaneous or artificially triggered duplication of the haploid genome, regenererated plants proved to be non-chimeric and instantly homozygous with regards to the mutations obtained, as was unambiguously shown by non-segregating mutant progeny. In an alternative setup also using embryogenic pollen cultures for stable transformation, the two TALEN units required for site-directed cleavage activity were separately used to retransform GFP-transgenic barley. Regenerants were selected for homozygosity and expression of the single-unit TALEN coding sequences prior to cross-combination of pairs carrying the two different TALEN units, which entailed endonuclease activation via TALEN heterodimerization upon fusion of egg and sperm cells. This approach resulted in unprecedented efficiency of site-directed mutagenesis, as virtually all generated F1-plants were mutated in the target sequence, with the wild-type GFP being only rarely detectable in some multi-allelic individuals. Besides the highly efficient gene knock-out achieved, the sequence analysis of hundreds of independent mutants revealed DNA-repair patterns that might be utilized to generate functional allelic derivatives of endogenes of choice. Our results may also pave the way for even more sophisticated procedures aiming to precisely edit plant genomes based upon double strand break repair via homologous recombination using customizable DNA-repair templates.

T66

NtCYS, a multifunctional player in plant PCD during embryogenesis

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Zygote first division gives rise to a larger basal cell and a smaller apical cell with a distinct developmental fate. The small apical cell will develop into the main body of the embryo proper, whereas the larger basal cell will mainly grow into a suspensor that goes through predetermined degeneration at late stages of embryo development. Critical roles of suspensor degeneration at certain time point and molecular mechanism underlying it are two core questions in the field of plant embryogenesis.We have previously proved that the suspensor degeneration is a typical process of programmed cell death (PCD) and discovered a basal cell exclusively located cysteine protease inhibitor, NtCYS, which exert its anti-cell death effect by directly inhibiting cathepsin H-like protease NtCP14 to protect the basal cell lineage from precocious activation of PCD in early embryogenesis. Thus, NtCYS-NtCP14 works as a molecular switch to control suspensor cell fate. Further study revealed that NtCYS has multifunctional role in embryogenesis and even in whole process of sexual plant reproduction. These works suggest that NtCYS is a key regulator for PCD during both developmental and stress-induced cell deaths.

T67

YODA Signaling in the Arabidopsis embryo

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In Arabidopsis thaliana, the fertilized egg cell or zygote elongates approximately three-fold before it divides asymmetrically. The two daughter cells differ in size and follow different developmental routes.
While the smaller apical cells forms the spherical pro-embryo, the cells of the basal lineage continue to divide horizontally to form the stalk-like suspensor.
Zygote elongation and suspensor formation is regulated by a MAP kinase pathway including the MAPKK kinase YODA (YDA).
YDA activation depends on the membrane-associated receptor-like cytoplasmic kinase SHORT SUSPENSOR (SSP) which accumulates transiently in the zygote after fertilization. SSP transcripts on the other hand accumulate specifically in sperm cells prior to fertilization, suggesting an intriguing mechanism of YDA activation: SSP transcripts seem to be inherited during the fertilization events and subsequent SSP translation in the zygote might link fertilization with YDA activation.
We will present our latest findings on the regulation and function of the YDA pathway in the Arabidopsis embryo.

T68

Assembly and localization of mRNPs during early embryogenesis in Arabidopsis

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The temporal and spatial control of mRNA translation is a precise mechanism to control the availability of gene products. This mechanism is found in all kingdoms of life and appears essential for asymmetric cell division, cell fate determination and many other developmental processes. In contrast to animals and fungi, to date little is known about the localization and translational control of mRNPs in plants. Based on egg cell and pro-embryonic transcriptome, we are analyzing several potential polar localized mRNAs in the egg cell and during the first cell divisions of the zygote. For this purpose we use a two-component system that takes advantage of the sequence-specific binding of a viral RNA-binding protein to its respective target RNA hairpin loop. By this method, we are able to analyze mRNA distribution of specific targets in vivo. We found so far, for example that the mRNA of one candidate localizes in globular structures, which are moving through the egg cell. These globular structures indicate the formation of specific mRNPs, which might be essential for localization and translational control. We are currently following up on the hypothesis that the preferential distribution of mRNP granules in the egg cell is an essential mechanism to control cell development as it was previously shown for the oskar mRNA in the Drosophila embryo. Additionally, we have identified a plant specific RNA binding protein essential for embryo development, which is currently studied for its role in mRNA localization and/or translational control.

T69.

Natural variation in the epigenetic control of seed development in Arabidopsis

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Seed development requires the correct integration of maternal and paternal genetic information. The balancing of parental genomes is largely controlled by FIS-PRC2, a maternal-specific chromatin modification complex. In Arabidopsis thaliana, mutations in the FIS-PRC2 subunit MEDEA (MEA) cause maternally controlled defects in endosperm and embryo proliferation that lead to seed abortion. However, mea seeds have been known to be partly rescued paternally with pollen from the Cvi-0 and C24 accessions. To investigate the genetic basis of mea seed rescue, we crossed mea plants with 167 A. thaliana accessions. We found that the penetrance of mea is highly dependent on the genotype of the pollen donor. The rescue of mea seeds is paternal specific but does not require the presence of a functional paternal MEA allele. Using a combination of genome wide association studies (GWAS), QTL mapping and bulk segregation sequencing, we identified four loci that are responsible for the rescue of mea seeds. In addition, we profiled the transcriptome of mea rescued seeds at 4 days after pollination and found that it is more similar to the transcriptome of mea inviable seeds than to the transcriptome of wild-type seeds. This suggests that mea seeds can develop normally despite large alterations in their epigenetic landscape. Taken together, our results indicate that the epigenetic regulation of seed development is unexpectedly diverse in accessions of A. thaliana.

T70

Genetic control of identity, growth and shape in the Arabidopsis embryo

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Both growth and tissue patterning are processes that occur continuously during plant life. A key question is how these are coordinated in space and time to generate plant shape and function. We use the early Arabidopsis embryo as a simple and highly predictable model in which cell identity specification, growth and patterning are intricately coordinated. I will discuss our recent work aimed at understanding the cellular basis for the establishment of multicellular patterns in 3D. Furthermore, I will present our progress towards understanding the definition of embryonic and tissue identity through transcriptional control.

T71

Auxin dynamics put polarity in the pod

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Multicellular organisms including plants and animals develop specialised organs, which are composed of different types of tissues. The structure – or pattern – of organs is determined by the polarity within tissues along axes of symmetry. In order to coordinate polarity across a tissue or organ, multicellular organisms use mobile substances such as hormones.

In plants, auxin plays an essential role in initiating organ formation and in patterning the organs in specific tissue types, including for example lateral roots, young leaves and those of the female reproductive organ, the gynoecium. Auxin signalling is achieved through interactions between the auxin molecule and specific proteins thereby causing the degradation of repressors of gene expression. It has also been established that auxin can influence the direction of its own transport via inhibiting internalisation of PIN auxin transporters.

Interactions among key regulators of Arabidopsis gynoecium development have revealed a network of transcription factor activities required for dividing this organ into discrete domains. Regulation of auxin dynamics is emerging as an immediate downstream output from these activities. We show that a set of transcription factors ensure precise auxin distribution to facilitate key events of polarity establishment in gynoecium development. Moreover, recent observations in the lab have revealed a potentially powerful feedback regulatory mechanism for gynoecium development involving a physical interaction between auxin and an ARF-bHLH transcription factor complex. Rather than directing protein degradation, a transcriptomic analysis indicates that this auxin-controlled interaction mechanism determines the identity of downstream targets of the ARF-bHLH complex.

In conclusion, our work is aimed at reaching a mechanistic and developmental understanding of how auxin facilitates precise switches in polarity during plant organ development.

T72

Signalling mechanisms establishing early seed development in Arabidopsis thaliana

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The seeds of angiosperms are composed of three main structures: the embryo, the endosperm and the seed coat. Upon entering the female ovule, the paternal pollen tube releases two sperm cells that will fertilize the egg and central cells. The fertilization of the haploid egg cell will give rise to the diploid embryo, which is the only component of the seed that will remain after germination to form the next generation. The second fertilization event, of the homodiploid central cell, will trigger its division, resulting in the development of the triploid endosperm. Surrounding the female gametophyte are the ovule integuments which, after the double fertilization event, grow and differentiate to form the sporophytic seed coat. Unlike the other two structures, embryo and endosperm, the seed coat originates solely from the maternal tissues.

The sexual endosperm, rather than the embryo, is necessary and sufficient to drive seed coat formation after fertilization in Arabidopsis thaliana. Moreover, the development of the seed coat is also known to impact positively on the proliferation of the endosperm. Nevertheless, the signalling pathways that promote both endosperm development and seed coat growth, as well as those that occur between these two structures, are yet to be understood. One of the key regulators of early endosperm and seed coat development in Arabidopsis is AGL62, a type I MADS-box transcription factor. Seeds that are mutant for AGL62 fail to form a seed coat, which we found leads to a premature endosperm cellularization. Our data suggests that this transcription factor is upstream of signaling mechanisms in the endosperm that work to trigger seed coat development after fertilization.

Our most recent findings on the signaling mechanisms that ensure proper communication and coordinated development between the endosperm and seed coat will be presented.

T73

Investigating the role of transcription factors in fruit development

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Fruits have always been considered as a major source of food and pharmaceuticals. They come in an array of types, shapes and sizes and their main function is to support and nourish the developing seeds and ensure their dispersal once they have matured, in order to secure the survival of the next generation. The majority of work on gene regulatory networks involved in fruit development has been done in core eudicots, such as Arabidopsis and tomato, and in monocots, such as rice and maize. Our work is focused on two morphologically distinct and evolutionary distant fruits; the capsule of Papaver somniferum (opium poppy) and the caryopsis of Brachypodium distachyon. Opium poppy belongs to the Ranunculales order, which represents the earliest divergent eudicot clade. It is globally renowned for its pharmaceutical value, little however has been reported on the genetic control of its capsule development. Brachypodium on the other hand, has been emerging as a model species for the temperate grasses and its fruit is being studied in order to shed more light in the grain development of its close relatives, such as wheat, barley and oats. The Brachypodium genome is already publicly available and T-DNA insertion lines have been generated for several genes involved in grain development. Using an RNA-seq approach we have identified a number of transcription factors involved in poppy fruit development. Gene expression and protein interaction analyses in both species are underway in order to understand and compare key regulatory networks that are involved in fruit development in an attempt to shed more light in the evolution of fruits in the angiosperms.

T74

Ethylene negatively regulates fruit set and early fruit development

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1SM and CM have contributed equally to this work

The production of ethylene and the expression of ethylene genes were studied in pollinated and non-pollinated ovaries and fruits of different cucurbit and solanaceous species (zucchini, melon, watermelon and tomato) during the days immediately after anthesis (DPA). Results indicate that fruit set and early fruit development requires a low level of ethylene in the ovary/fruit. In fact, the absence of pollination/fertilization is associated with a peak of ethylene production at 3-5 DPA, concomitantly with fruit abortion. The time between anthesis and the ethylene peak defines a pollination window which is characteristic for each species and cultivar. In zucchini, genetic and auxin-induced parthenocarpy also inhibits ethylene production and the expression of genes involved in the biosynthesis and signaling of the hormone, demonstrating that fruit set and early fruit development requires low levels of ethylene. Furthermore, since the ethylene inhibitors AVG and STS were able to induce the parthenocarpic development of the zucchini fruit we conclude that ethylene is directly and negatively involved in these development processes.

T75

Flavonoid regulation of seed development in Arabidopsis - a role for auxin?

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Many plants, including agronomically important species, exhibit post-zygotic barriers to hybridisation. These barriers can also extend to interploidy crosses within a species. For example, crosses between diploid (maternal) and tetraploid (paternal) plants cause a triploidblock where severe endosperm over-proliferation kills the developing triploid embryo. Whilst most ecotypes of the model species Arabidopsis thaliana tolerate 2xX4x crosses to produce viable seed, one ecotype, Columbia (Col), exhibits a triploid block when the paternal parent is tetraploid. Loss-of-function mutants affecting the flavonoid biosynthesis pathway (FBP) in the Arabidopsis Landsberg erecta (Ler) ecotype have been identified as powerful maternal suppressors of Col4x-mediated triploid block. The likely rescue mechanism involves communication between the maternal tissues of the seed coat and the zygotically-derived endosperm. The current hypothesis is that a maternal factor regulating the timing of endosperm cellularisation (promoting earlier endosperm cellularisation) is blocked by a functional FBP in the endothelium of the seed coat; therefore loss of the pathway by mutation promotes earlier cellularisation and rescue of paternally-induced lethality. However the same FBP mutants in triploid plants derived from the Col ecotype fail to rescue, suggesting the existence of ecotypic variation in sensitivity to the cellularisation factor. Flavonoids are potent auxin transport regulators. Hence, auxin may be the cellularisation factor. We are attempting to identify the cellularisation factor, understand the role of the FBP in its regulation and to further explore the effect that different ecotypes have on the severity of the triploid block and the underlying mechanism behind this effect.

T76

Growing Hearts and Cylinders: comparing Arabidopsis and Capsella reveals switching growth patterns after fertilization

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Members of the Brassicaceae represent excellent models for investigating different fruit shapes due to the vast variations of form within the family. In this study the long cylindrical siliques of Arabidopsis thaliana were compared to the heart-shaped silicles of Capsella rubella. Despite the differences in shape, these two fruit forms share a similar tissue patterning with two valves (fruit walls) attached to a central replum. Clonal analysis has highlighted the anisotropic growth of the Arabidopsis gynoecium, extending much more along the longitudinal axis, which is unsurprising given the long form of the fruit. Capsella's gynoecium in the early stages of development matches Arabidopsis until flower stage 8 where growth clearly becomes isotropic leading to a round form by anthesis. Following fertilisation a change in growth patterns transforms the rounded gynoecium to the characteristic heart form of Capsella fruit. Computational modelling indicates that differential growth of specific regions is essential for generating this elaborate shape. The genetic patterning of Arabidopsis fruit has been thoroughly described. A potential candidate for controlling fruit forms is FRUITFULL (FUL) which plays a key role in valve expansion post fertilisation. We have identified ful mutant alleles in Capsella and propose that a mutation in this gene underlies the phenotype observed in 1914 by one of the founders of genetics George Harrison Shull, where he described Capsella variants with cylindrically shaped fruits. Together with Shull's classical genetics work, our analyses demonstrate that FUL plays a key role in generating both heart-shaped and cylindrical fruits.

T77

TCP14 AND TCP15, TOGETHER WITH DELLAS, REGULATE ARABIDOPSIS SEED GERMINATION

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The TCP transcription factors are characterised by a 59 amino acid basix-helix-loop-helix (bHLH) DNA binding domain. The acronyms "TCP" derives from TEOSINTE BRANCHED (TB1, Zea mays), CYCLOIDEA (CYC, *Antirrhinum majus*), and from the rice PROLIFERATING CELL FACTORS 1 and 2 (PCF1 and PCF2).

Here we describe the role TCP14 and TCP15 as key regulators of Arabidopsis seed germination. The tcp14 and tcp15 single mutants and the tcp14/tcp15 double mutant are characterised by a strong delay in germination. However such phenotypes can be partially rescued by either adding gibberellins or by prolonged vernalisation, suggesting a possible role of these two transcription factors in gibberellin homeostasis.

DELLA proteins are negative regulators of gibberellin signalling and they act immediately downstream of the GA receptor. Either TCP14 and TCP15 are able to heterodimerise with DELLA proteins. All together our data indicate that the joint regulation of germination, by gibberellin and TCPs, occurs through physical interactions with DELLA. Moreover our data indicate that TCPs/DELLA complex participate in cell cycle gene regulation.

T78

Regulatory mechanisms of plant seed size control

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The formation of gametes and the subsequent development of seeds are key steps in the life cycle of any sexually reproducing organism. In higher plants, gametes develop along with non-gametic cells within a few-celled haploid structure, termed gametophyte. A characteristic feature of angiosperms is the formation of two distinct female gametes, egg and central cell, which both get fertilized to form the main components of the seed. In a screen for mutants with altered egg cell marker gene expression we have identified two mutants, which express an egg cell marker at reduced levels. Here, we present our analysis of the lagg mutant, which is defective in a putative component of a large ribonucleoprotein involved in rRNA maturation and hence ribosome biogenesis. Ribosomes are essential for protein synthesis and previous studies by several groups have provided compelling evidence for an essential role of RNA biogenesis and processing during female gametophyte maturation and seeds development. Comprehensive loss- and gain-of-function analysis of LAGG now suggests an unprecedented and tissue-specific role of RNA biogenesis in the control of seed size.

T79

Communication between female gametes modulates early embryo development in flowering plants

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A key question in the reproductive biology of flowering plants is; how do the three components of the seed co-ordinate their growth and development to ensure success to the next generation. We have developed a molecular screen to identify factors that regulate this process. Our analysis has uncovered a novel group of secreted peptides that are specifically expressed in the central cell and in endosperm cells surrounding the developing embryo. Altering the expression of these peptides in the endosperm affects embryo patterning only; thus we named them Embryo Surrounding Factors (ESFs). We found that in several plant species maternally derived ESFs are required during early stages of embryo development. Genetic analysis in Arabidopsis indicates that ESFs act synergistically with paternally-derived SSP2 to regulate early embryo patterning in Arabidopsis. In addition, our analysis has revealed that maternal factors play a critical role after fertilization by regulating of early embryo patterning in plants.

Poster

Presentations

PS1-1

Jasmonic Acid Regulates Spikelet Development in Rice

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The spikelet is the basal unit of inflorescence in grasses, and its formation is crucial to reproductive success and cereal yield. Here, we report a previously unknown role of the plant hormone jasmonic acid (JA) in determining rice (Oryza sativa) spikelet morphogenesis. The extra glume 1 (eg1) and eg2 mutants exhibit altered spikelet morphology with changed floral organ identity and number as well as defective floral meristem determinacy. EG1 is a plastid-targeted lipase participating in JA biosynthesis, and EG2/OsJAZ1 is a JA signaling repressor that interacts with a putative JA receptor, OsCOI1b, to trigger OsJAZ1's degradation during spikelet development. OsJAZ1 also interacts with OsMYC2, a transcription factor in the JA signaling pathway, and represses OsMYC2's role in activating OsMADS1, an E-class gene crucial to spikelet development. This work discovers a key regulatory mechanism of grass spikelet development and suggests that JA's role in reproduction has diversified during flowering plant evolution.

PS1-2

ABORTED MICROSPORES Acts as a Master Regulator of Pollen Wall Formation in *Arabidopsis Thaliana*

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Mature pollen is covered by durable cell walls, principally composed of sporopollenin; an evolutionary conserved, highly resilient, but not fully characterized, biopolymer of aliphatic and aromatic components. Here we report that ABORTED MICROSPORES (AMS)acts as a master regulator coordinating pollen wall development and sporopollenin biosynthesis in *Arabidopsis*. Genome-wide co-expression analysis revealed 98 candidate genes with specific expression in the anther, and 70 that show reduced expression in *ams*. Among these 70 members, we showed that AMS candirectly regulate 23 genes implicated in callose dissociation, fatty acids elongation, formation of phenolic compounds, lipidic transport putatively involved insporopollenin precursors-synthesis. Consistently, *ams* mutants showed defective microspore release, a lack of sporopollenindeposition, and a dramatic reduction in total phenolic compounds and cutin monomers. The functional importance of the AMS pathway was furtherdemonstrated by the observation of impaired pollen wallarchitectureinplant lines with reducedexpression of severalAMS targets: the abundant pollen coat protein extracellularlipases (EXL 5 and EXL 6), and CYP98A8 and CYP98A9,which are enzymesrequired for production of phenolic precursors.These findings demonstratethe central role of AMS in coordinating the sporopolleninbiosynthesis and secretion of materials for pollen wall patterning.

PS1-3

The *Primula S* locus: genetics, genomics and gene identification

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Floral heteromorphy is an out-breeding mechanism characteristic of the Primulaceae in which plants develop distinct forms of flower, known as pin and thrum. Pin flowers posses long styles with the stigma at the mouth of the flower, and anthers attached half way down the inner corolla wall. Thrum flowers have a short style, and anthers attached to the inner corolla at the mouth of the flower. The reciprocal positioning of male and female reproductive structures promotes insect-mediated cross pollination. These developmental characteristics, and other features of floral heteromorphy, including differential pollens size, and a sporophytic self-incompatibility system, are controlled by a co-adapted linkage group known as the S locus. Pin plants are homozygous recessive s/s; thrum pants are heterozygous S/s.
To identify genes at the S locus that control style length, anther height and pollen size, we have taken a multidisciplinary approach involving classical genetics, molecular genetics, transcriptomics and genomics. We have identified and characterised several genes and polymorphic markers, as well as developmental mutations linked to the S locus. Using these sequences and mutant phenotypes we developed a linkage map of the S locus region in Primula vulgaris delineated by flanking markers. Construction and screening of BAC libraries has enabled us to generate a contig spanning the S locus and to identify pin and thrum specific sequences.
Transcriptomic analysis of pin and thrum plants has identified morph specific floral transcripts, and transcriptomic and genomic analyses of linked mutations have provided new insight into the S locus. We have also undertaken de novo genome sequencing of Primula vulgaris and closely related species and comparative genomic analyses are underway. The development of a transformation system and large mutant population screens have contributed to the available resources that are facilitating the characterisation of S locus associated genes.

PS1-4

Analysis of sex determination in *Silene dioica* by transposon tagging

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Silene dioica is a dioecious species where sex is determined by a heteromorphic XY sex chromosome system. Dioecy has evolved independently in several different genera as an out-breeding mechanism which results in single sex male and female flowers occurring on separate plants. Males (XY) are the heterogametic sex and females (XX) are the homogametic sex. The male determining Y chromosome causes the promotion of stamen development, pollen development and the suppression of carpel development. In the absence of a Y chromosome, female flowers develop functional carpels and contain arrested stamen primorida that fail to develop.

Our studies involve use of a naturally occurring endogenous transposon in S. dioica for a transposon tagging strategy to identify key sex determination genes. Genetic analysis over several generations revealed that the floral pigment instability locus is sex linked and on the X chromosome. Isolation of genes from the anthocyanin biosynthesis pathway, together with mass spectrometry analysis of the anthocyanin pathway intermediates enabled us to define the floral pigment instability locus as the gene encoding Flavone 3 hydroxylase.

We will present our characterisation of this locus, and progress towards identifying the transposon, as well as the identification of floral mutants, including stamenless and hermaphrodite flowers from large scale mutant screens of plants carrying the active transposon.

In addition to the wild type dioecious form of S. dioica, there are four horticultural varieties which produce double flowers. These flowers contain a greatly increased number of petals compared to the wild type and lack both stamens and carpels. Our work has involved the characterisation and comparison of these varieties and the identification of the mutations to the MADS box C function gene SLM1 which gives rise to the double flower phenotype.

PS1-5

Expression of two duplicated polygalacturonase genes Bra011440 and Bra037005 in *Brassica campestris* ssp. *chinensis*

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Polygalacturanase (PG), a hydrolase and loosening enzyme involved in pectin metabolism, plays significant roles in a lot of important development processes including pollen development. In the present study, Bra011440 and Bra037005, two polygalacturonase (PG) genes, were isolated from Brassica campestris L. ssp. chinensis Makino. They were both homologues of Arabidopsis pollen-specific PG gene At4g33440. Sequence and syntenic analysis further showed these two genes were duplicated genes. However, expression analysis in different organs, floral parts, and five developmental stages of flower buds indicated the two genes have each its own expression pattern. Results from qRT-PCR analysis showed the highest expression level of Bra037005 appeared in the tender pods, while Bra011440 was mainly expressed in inflorescences, though they were both expressed in root, stem and leaf.β-glucuronidase (GUS) assay of the two genes' promoter-GUS constructs respectively in transgenic Arabidopsis showed that the promoter of Bra037005 could drive gene expression in cotyledon, radicle, stem, leaf and inflorescence. While the promoter of Bra011440 drove gene expression mainly in flower buds at later stages of development, but not in flower buds at earlier stages of development and open flowers.

Key words Brassica campestris, polygalacturonase, duplicated gene, expression pattern

PS1-6

Flower development and dormancy in *prunus avium*

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In many woody perennials flower development lasts for several months, and at winter this development is halted as the flower buds enter a dormant period. In this way, flower development supports the low winter temperatures, and adapts the subsequent reproductive process to the suitable conditions of spring and summer. Still dormancy is not just a survival strategy, but also a requisite for proper flowering and is one of the main drawbacks for the cultivation of temperate fruit trees to warmer latitudes. In spite of its agricultural implications, what occurs during dormancy remains elusive. Chilling requirements are genetically controlled for they vary among genotypes, and have been traditionally calculated in an empirical way with different mathematical models. Recently there is a search for the genetic control of this process, but this search is hindered by the absence of a biological frame where to fit results. In this work, flower development is characterized in sweet cherry (Prunus avium) with microscope image analysis combined with cytochemistry. Results show at what stage flower buds enter dormancy and what are the first events at waking up time. These events mark a sporophytic gametophytic boundary and the onset of the new male gametophyte generation, with meiosis taking place in the anther. But also the observation of reproductive structures, along the dormant period, surprisingly shows that important changes in carbohydrate accumulation occur during this apparently flower bud dormant stage.

PS1-7

Comparison of Triticeae and Brassicaceae Mature Stigma Proteomes

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A proteomic analysis of the mature stigma of triticale (x Triticosecale Wittmack) and Brassica napus was performed using three different gel-based approaches 1D LC-MS/MS, 2D LC-MS/MS and OFFGEL Electrophoresis (OGE) LC-MS/MS. More than 2,000 proteins were identified for each proteome with the majority being described to be expressed in the stigma for the first time. The Triticeae and Brassicaceae stigma proteomes displayed both conserved and divergent classes of proteins involved in stigma function and development.

PS1-8

Phytohormone-regulated changes in the cell wall composition of *Lupinus luteus* during flower abscission

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Exessive abscission of generative organs is a major cause of yield decrease in Lupinus luteus, which is a widely cultivated species in Poland, Australia and Mediterranean countries, thus representing important economical drawbacks for cultivators. Detachment of flowers and fruits occurs in the specialized group of cells forming the abscission zone (AZ). During plant growth, these cells become competent to respond on specified signals such as phytohormones thus initiating separation events, and consequently leading to breakdown in cell adhesion.
The aim of this work was to provide a comprehensive analysis of the changes taking place in the pectin composition of the cell walls of this plant through abscission. The study was based on the analysis of the immunofluorescence localization carried out using four anti-pectin (JIM5, JIM7, LM5, LM6) antibodies.
After AZ activation, it was noteworthy the presence of a high rate of cells divisions, accompanied by the dissolution of the AZ cell walls. During floral development, such AZ activation was also followed by an increase in the levels of acid esterified pectins in comparison to de-esterified pectins. Moreover, significant increase of the galactose- and arabinose-rich pectins pools was detected at that time. Ethylene, acting as an activator of abscission by enhancing the expression of cell wall degrading enzymes, highly led to an increase of the content of esterified and de-esterified pectins. In contrast, auxin treatment resulted in decreased level of the latter ones and in the increased content of galactose- and arabinose-rich pectins in the AZ.
The results of this study confirm that floral abscission occurs through concomitant changes in cell wall composition, and that cell wall loosening and degradation during the abscission process are regulated by ethylene and auxin.
This work was supported by ERDF-cofunded projects P2010-AGR6274, P2011-CVI7487 and BFU2011-22779. A. Kućko and J.D. Alché thank the eidA3-ceiA3 consortium for funding throughout the program for Ph.D. co-supervision for foreign students.

PS1-9

Diversity of flowering time for the expanding rice areas

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In 2050, world population will surpass 9 billion. Plant biology contributes for the food supply for the increasing human demands. For the stable food production, the shaping of the adaptability is the most important objectives in plant breeding programs. In the last decade, the molecular networks of flowering time control have been identified in plants. Rice is a major crop in the world. Rice is originated from tropical regions and short-day plant. Due to the large efforts, now rice has been grown over the world from 53N to 40S. Flowering time is a major factor in regional adaptability. Many flowering time control genes have been identified. However, it is unclear which genes have roles for the adaptability to diverse environmental conditions. Previously, we identified QTLs controlling extremely early flowering time using rice varieties adapted to one of northern-limits of rice cultivation in the world for the understanding this considerable variation. Two loss-of-functional alleles in *GHD7* and *OsPRR37* genes have important roles of extremely early flowering time behavior. The understanding of diversification of flowering time can shape the adaptability for the marginal regions of rice cultivation over the world. In this study, we collected 60 rice varieties exhibiting extremely early flowering time among 20,000 accessions over the world in Japanese Genebank. We evaluated the diversity of phenotype as flowering time under the environmental conditions in northern-limits of rice cultivation, naturally long-daylength. Also, we evaluated the genetic diversity of the whole genome. Based on the results, the differentiation of such unique phenotype among cultivated rice was discussed.

PS1-10

Transcriptomic analysis of Vitis flower development and sex specification

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Vitis vinifera is one of the most important cultivated grapevines in the world. Vitis vinifera subsp. vinifera produces hermaphroditic flowers but the wild grapevine form (Vitis vinifera subsp. sylvestris) is dioecious. The V. sylvetris male plant produces flowers with a reduced pistil without style or stigma, whereas female flowers present reflexed stamens and produce infertile pollen. The mechanisms behind sex determination and sex specification in grapevine are still unknown.
A full transcriptomic analysis of four developmental stages from male, female and hermaphrodite flowers was performed using Illumina RNA-Seq technology. We showed that sex determination must occur very early during flower development possibly involving two closely linked genes. We also found that the expression of genes from the ABCDE model seems to be not directly correlated with the establishment of sexual determination in grapevine. A set of cluster of genes with expression related to just one of the flowers sex types was found, and may contain putative key players in Vitis sex determination. We also detected some transcripts originated from genomic regions annotated as non-coding and that were exclusively expressed in one type of flower but not in the others. It is possible that this kind of transcripts may be essential for flowering sex determination. Our findings enabled us to create a comprehensive catalogue of transcribed genes and pseudo-genes across flower developmental stages, and genders, that will contribute for future work in sex determination and specification in seed plants. A temporal comprehensive model of two mutations in two linked genes, to specify the gender and sex determination, is proposed, and may be provide helpful insights into the Vitis domestication process.

PS1-11

Metabolomics Of Tomato Pollen Development

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Pollen play a key role in plant pollination. The success of fertilization is supported by a dynamic regulation of components during pollen development. Metabolic activity plays an essential role in pollen nutrition to ensure fertility and also to provide protection against environmental stresses. Alterations in metabolism can lead to a decrease of pollen viability and, in consequence, to a decrease of fruit set. Several metabolites have already been shown to be important for pollen viability, such as carbohydrates, amino acid proline, hormones, polyamines and flavonoids. This study aimed to explore pollen metabolome of tomato cultivar Micro-Tom (*Solanum lycopersicum* L.) at different developmental stages of pollen. Two untargeted metabolomics platforms were used, gas chromatography - mass spectrometry (GC-MS) and liquid chromatography - mass spectrometry (LC-MS). GC-MS was used to detect trimethylsilylated derivatives of primary metabolites, such as carbohydrates, organic acids and amino acids. LC-MS was used to detect secondary metabolites, such as flavonoids, alkaloids, phenolic acids and polyamines. Three pollen developmental stages have been analyzed by metabolomics platforms: polarised microspore, early bi-cellular microspore and mature pollen. Dramatic changes in metabolic constitution of developing tomato pollen were observed. At mature pollen stage, sucrose, amino acid asparagine, flavonoids and specific conjugated polyamines were accumulated whereas hexoses, organic acids, amino acids and phenolic acid were decreasing during pollen development. The regulation of those metabolites could contribute to the development of fertile pollen. Therefore, the putative role of identified metabolites will be discussed.

PS1-12

The ABCDE model in *Quercus suber* flower type specification

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Quercus suber is one of the most important forest species in Portugal, being the dominant tree of the oak woodlands. This monoecious wind--pollinated species has a protandrous system and several seasons of flowering. Staminate flowers occur in early spring and autumn, whereas pistillate flowering buds usually appear only in spring. Despite its ecological and socio--economic importance, very little is known regarding the genetic mechanisms involved in cork oak reproductive development. Non--normalized libraries of different developmental stages of male and female flowers were generated using 454 GS--FLX Titanium massive parallel pyrosequencing technology. In order to identify genes involved in flower development, the amino acid sequences of genes homologous to the regulatory floral homeotic genes (ABCDE model) APETALA1 (AP1), APETALA3 (AP3) PISTILLATA (PI), SEPALLATA1/2/3 (SEP), AGAMOUS (AG), SHATTERPROOF (SHP), APETALA2 (AP2) and an AP2/Ethylene--responsive element binding protein (EREBP) were obtained and their phylogenetic relationships were inferred, confirming the presence of potential orthologues in the Cork Oak EST database. The temporal expression of these genes was analysed using qRT--PCR analysis. Interestingly, A--class transcripts (QsAP1 and QsAP2) were more abundant in female flowers, whereas B--class genes were predominantly expressed in male flowers throughout their development. Yeast--two--hybrid analyses showed that QsPI and QsAP3 are able to interact in agreement to what was observed in other species. According to the ABCDE model, the expression of SEPALLATA--like genes was similar in both types of flowers. However, we identified a SHP--like transcript that is present in both male and female organs, which might suggest a different role in flower development other than ovule development. Our results provide the first insights into the molecular mechanisms involved in the flower type specification in *Quercus suber*.

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PS1-13

Study of Ehd1, a major regulator of flowering signal pathway in rice

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Flowering is one of the most important developmental processes in plants to ensure successful reproduction. The floral transition depends on accurate measurement of environmental changes such as photoperiod and temperature. Early heading date 1 (Ehd1) is a major regulator of the flowering signal by inducing expression of Hd3a and RFT1, florigens in rice. However, the molecular mechanism how this protein regulates downstream genes is poorly understood. To study the molecular function of Ehd1, we generated *Ehd1* RNAi plants. All RNAi lines flowered late under both short day and long day conditions. In contrast, transgenic rice plants overexpressing *Ehd1* flowered extremely early. Transcript levels of *Hd3a* and *RFT1*were dramatically increased in the *Ehd1* overexpressing plants. To elucidate the tissue specific expression pattern of *Ehd1*, transgenic plants harboring a chimaeric molecule between the *Ehd1* promoter and *GUS* reporter were created. The GUS was expressed preferentially in phloem, similar to the *Hd3a* and *RFT1* expression patterns. Taken together these results suggest that *Ehd1*directly regulates *Hd3a* and *RFT1* in phloem tissues. Ehd1 is a member of B-type response regulators that carry N-terminal receiver domain and C-terminal DNA binding domain. The N-terminal receiver domain has the conserved DDK motif, which can be phosphoryled. We generated point mutation forms of the DDK motif to verify the importance of phosphorylation. Overexpression of Ehd1 D63E, which mimics the constitutive phosphorylation state, caused extremely early flowering, suggesting that phosphorylation activates Ehd1. We are investigating Ehd1 by elucidating functional roles of each domain.

PS1-14

Writers and erasers of chromatin marks during reproductive development in *Quercus suber*

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DNA and chromatin can be chemically modified with different marks giving rise to distinct chromatin functional states, and consequent different gene expression patterns. Proteins belonging to different families, represented by numerous members, can write or erase these modifications. The writers - DNA and Histone methyltransferases (DNMT and HMT), and histone acetyltransferase (HAT), and the erasers - (Histone demethylases and deacetylases) (HDMT and HDAC) are enzymes belonging to different classes known to be involved in the control of several steps of plant reproductive development such as: flowering time, gametophyte development, fertilization and flower and seed development assuring the reproductive success of the species.

Quercus suber is a monoecious tree species with a long progamic phase and great importance to Portuguese economy and ecological sustainability. Bioinformatics resources are now available for cork oak such as the EST database (Corkoak DB) that was used to mine genes encoding proteins responsible for writing and erasing the chromatin marks. Gene sequences for all the families involved in the epigenetic regulation were identified: four QsDNMTs; 20 QsHMTs, 7 Qs (HAT), 15 QsHDMT, and 13 QsHDAC. Their phylogenetic relationship was determined with homologous sequences, confirming the presence of all enzymes families and classes in Q. suber.

Taking advantage of Quercus suber is a monoecious species we studied the gene expression of these epigenetic players in male and female flowers, during their development. Four ESTs libraries representing early and late stages of female and male Q. suber flowers were used to find genes with differential expression during these two stages.

Our results give the first overall description and potential network of the epigenetic players involved in flowering development in Quercus suber.

PS1-15

Immunolocalization of pectins and arabinogalactan proteins in the ovule and obturator of Billbergia nutans (Bromeliaceae, Poales): a comparative view of flowering plants

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Pistil plays a key role in plant reproduction. However, the pollen-pistil interactions within the ovary remain poorly studied. Pectins and arabinogalactan proteins (AGPs) have been identified as important components of cell wall during reproductive processes. This study aims to identify pectins and AGPs in the ovule and obturator of *Billbergia nutans* (Bromeliaceae, Poales) before and after fertilization, in order to increase the information about the glycan dynamics within the ovary, and to verify the distribution of these molecules in a commelinid monocotyledon in a comparative perspective. For immunolocalization analyzes, flower buds close to its opening and flowers at post-anthesis were collected and labelled with monoclonal antibodies recognizing pectins and AGPs epitopes. At pre-pollination stage of *B. nutans,* partially methylesterified HGs (JIM7), arabinans (LM6), galactans (LM5) and AGPs (JIM8, JIM13) are present in the ovules, and partially methylesterified HGs (JIM7) and galactans (LM5) occur in the obturator. After fertilization, a few modifications occur in these tissues, especially the disappearance of the JIM8-labelling. The spatial modulation found between arabinans, galactans and AGPs in the ovary of *B. nutans* delineates the porogamic pathway of the pollen tube to the female gametophyte. The presence of these molecules in unpollinated flowers demonstrates that its appearance is developmentally regulated. The chemical detection of this pathway has been observed in ovules of other flowering plants. However, each species possess its own set of epitopes, apparently unrelated to the phylogeny. The occurrence of galactans and the particular distribution of arabinans in the ovule, as well as pectin labelling and the absence of AGPs in the obturator of *B. nutans,* stand out as new results for literature. We show evidence for possible roles of pectins and AGPs in the pollen-pistil interactions within the ovary, which may involve successive directional cues for the pollen tube attraction.

PS1-16

Ontogeny of the proliferous spikelet in *Eleocharis*

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The family Cyperaceae presents unusual asexual reproductive strategies when compared to the other groups of angiosperms. One of these is the presence of pseudovivipary, which consists of the formation of new individuals from somatic tissues of floral structures. In *Eleocharis* R. Br the pseudovivipary appears as an important process of reproduction in *Tenuissimae* series, which results in a structure called proliferous spikelet. The aim of this study was to describe the ontogenetic processes of the pseudovivipary in *E. viridans*. For this purpose, proliferous spikelets at different developmental stages were analyzed by light and scanning electron microscopy. The proliferous spikelet develops from a meristem located in the axil of the basal glume in the culm of the parental plant. This glume is homologous to bracts of other Cyperaceae groups. Each proliferous spikelet is formed by sympodial units, which consists of an addorsed prophyll, an outer and an inner sheath and a culm, which develops a primordium of floriferous spikelet at the terminal region, that can be aborted, and may develop a proliferous spikelet laterally in the axil of the basal glume. The second internode of each sympodial unit contains a root primordium and an intercalary meristem at the culm base. Our results indicate that pseudovivipary can coexist with sexual reproduction, as an alternative reproductive strategy that allows the rapid spread of populations.

PS1-17

Reproductive development in *Aechmea gamosepala*

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Many Bromeliaceae are endemic to the Atlantic Forest in Brazil and classified as vulnerable or under risk, in part due to human impacts. Information on their reproduction is lacking and useful for a more precise characterization of the species, with applications in conservation programs and genetic breeding for ornamental purposes. This study is part of a project on Bromeliaceae reproduction aiming to describe floral development in Aechmea gamosepala Wittm, as well as pollen viability and germination and stigma receptivity. Flower buds and flowers at different stages of development were processed for light and scanning electron microscopy. Pollen grains collected at anthesis were germinated in vitro in BK medium, the viability evaluated by staining in Alexander solution (Alexander1969, Stain Technol 44:117-22) and stigma receptivity assessed throughout the day. The flower is trimerous, the perianth heterochlamydeous, surrounded by a bract. The floral organs develop centripetally, three sepals, three petals in which bases petal appendages develop. The androceum is composed of six stamens, three free and three epipetalous. The anthers are tetrasporangiate and bithecal, the tetrad of the androspore is formed by successive meiosis with cleavage of the centrifugal type, forming isobilateral tetrads.

The conduplicate-spiral stigma differentiates early in flower development and the petal appendages develop in an intermediate phase. The ovary is inferior, gamocarpelar and trilocular, presenting interlocular septal nectary. The ovule is anatropousand bitegmic, with no appendage. The pollen grains are biporate, oblate shape with reticulate exine and presenting starch grains inside. Pollen viability and germination were greater than 90% and the stigma was receptive throughout the period of flower opening. Detailed reproductive information is presented to characterize this vulnerable Bromeliad species. (Acknowledgements: CNPq, NAP/MEPA-ESALQ/USP).

PS1-18

Analysis of the regulation and function of kiwifruit FLOWERING LOCUS T (FT)

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FLOWERING LOCUS T (FT) genes are key regulators of flowering time and can perform additional roles during the plant life. Three kiwifruit Actinidia chinensis FT genes were identified, designated AcFT, AcFTa and AcFTb. Recently we demonstrated conservation of AcFT function in Arabidopsis, functionality from the vasculature and interaction of AcFT with Arabidopsis FD and Actinidia FD-like proteins, suggesting a conserved role as florigen. Expression in source leaves and in response to winter chilling further supported a role for AcFT in regulation of kiwifruit flowering. However, AcFT expression was not always correlated with the flowering process and AcFT failed to promote flowering upon ectopic expression in kiwifruit, suggesting that it may confer meristem termination, but is not sufficient to promote floral fate. To further understand the regulation and function of AcFT, a sequence 3.5 kb upstream of its translation start site was isolated and characterized using transcriptional fusions to reporter genes and translational fusions to AcFT cDNA. This promoter region contained all the regulatory elements required to mediate vascular expression in diverse plant species, which was strictly confined to a specific phloem tissue. Photoperiodic responses of the promoter and the capacity of AcFT cDNA to perform as florigen when expressed at physiological levels from AcFT promoter varied between species. Increased availability of functional AcFT protein in cotyledons resulted in abnormal polarity of the first leaf, suggesting disrupted hormonal signaling. The promoter activity was under control of a kiwifruit DOF transcription factor, which also affected growth and flowering time upon ectopic expression in transgenic Arabidopsis. Overall, this work adds to our understanding of the regulation and function of the FT family in diverse plants.

PS1-19

A mutation in the CpACS27A gene is responsible for monoecy instability in Cucurbita pepo

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Sex expression in species of the Cucurbitaceae family is controlled by ethylene. The arrest of stamens during the development of female flowers in melon, cucumber and zucchini depends on the specific expression of CmACS7, CsACS2 and CpACS27A homologues in the carpel primordial of female flowers at very early stages of development. Mutations in CmACS7 and CsACS2 lead to andromonoecy in melons and cucumbers, but the implication of this gene in monoecy instability is unknown. We have identified a number of Cucurbita pepo genotypes showing an instable monoecy or a partial andromonoecy, i.e. an incomplete conversion of female into bisexual flowers when grown under high-temperature conditions. Moreover we have found that the F2 generation of the cross Bolognesse (Bog) x Vegetable Spaghetti (Veg), two stable monoecious inbred lines, segregates for monoecy instability. By cloning and sequencing CpACS27A gene in contrasting genotypes, we have detected a missense mutation in Bog and other genotypes that resulted in a substitution of serine by alanine at position 176. The co-segregation analysis between the mutation and the monoecious instable phenotype in various F2 populations derived from Bog x Veg clearly demonstrates that the mutation is necessary but not sufficient to confer monoecy instability in C. pepo. The A176 was detected in no monoecious stable cultivars, but was found in 5 of the cultivars showing a partial andromonoecious phenotype when grown under high-temperature conditions The mutation is however not associated with other sexual expression traits, including the transition to female flowering and the percentage of female flowers per plant, suggesting that monoecy instability did not co-segregate with a higher number of female or male flowers per plant.

PS2-1

Cytomixis: specific cellular features and prevalence in higher plants

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Cytomixis is migration of the nuclei from one plant cell to another through intercellular channels of a special type (cytomictic channels). This unique phenomenon was discovered over a century ago, which has been followed by numerous attempts to clarify the essence of this process as well as to determine its causes and consequences. Most attention of researchers has been paid to cytomixis in microsporogenesis, since the transfer of part of genetic material between microsporocytes may influence the ploidy level of the produced pollen and, presumably, have an evolutionary significance. To date, cytomixis was found in the microsporogenesis of over 400 plant species belonging to 84 families.
Using tobacco lines with different ploidy levels for light and electron microscopic analysis, we found that the frequency of cytomixis in tobacco microsporogenesis dramatically increases in triploid and tetrapolid plats compared to diploid ones, haploid plants have almost the same cytomixis frequency as diploids do. On ultrastructural level it was shown that chromatin migrated between cells within the nuclear envelope, and its disintegration was unobservable. No signs of pyknosis were observable in the chromatin after cytomixis. The dynamics of changes in the nucleoli during cytomixis was monitored. It was found that cytomictic channels can be formed on the base of preexisting plasmodesmata or de novo. Telomere distribution in meiotic nuclei of tobacco during cytomixis was studied by FISH.
Our data support the hypothesis that cytomixis in the plant microsporogenesis is the mechanism that increases genetic diversity of the formed gametes.

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PS2-02

Shugohin and patronous are required for chromosome cohesion in meiosis

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In Arabidopsis, sister chromatid cohesion is maintained by the Shugoshin AtSGO1 in meiosis I, whereas AtSGO2 is not required for protection of cohesion in meiosis and mitosis (Zamariola et al., 2013). Here, we demonstrate that both AtSGOs act redundantly to protect sister chromatid cohesion in meiosis I, and SISHO, a gene with unknown function, is necessary for protection at a later stage, in conjunction with AtSGOs. Furthermore, SISHO is required for appropriate microtubule organization at the end of meiosis I and in meiosis II. This additional role does not seem to be linked to premature loss of sister chromatid cohesion since atsgo1/atsgo2 mutants do not display similar microtubules defects as sisho.
In addition to a function in meiosis, we show that SISHO plays a role in the regulation of mitotic cell division, unlike AtSGOs proteins that seem not required for mitosis. Indeed, we detect growth defects in sisho plants and aneuploidy in sisho somatic cells, suggesting that SISHO mediates sister chromatid cohesion also in mitosis."

PS2-03

Challenging to establish the rice apomixis system by analyzing and manipulating mechanisms of rice reproductive molecules

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Rice has long been the Japanese principal food but even now, apomixis rice has not been created although the apomixis lines are extremely important in agriculture. Apomixis is a term that a plant creates new plants without fertilization and in wheat, apomictic lines have been created for over decades. To create apomictic plants, there should be 3 steps. The first step is that the line needs to produce diploid female gametophyte. In this step, the line needs to fail meiosis. Since these kinds of mutants and genetic factors are widely known in Arabidopsis, for example DYAD and MIME, gene modification is most likely applicable on these factors. The second step is that the line needs to fail fertilization. In this step, since restricted numbers of genetic factors are identified so far in Arabidopsis, additional screening is required. The last step is that the line needs to develop seeds by parthenogenesis. Although factors in parthenogenetic endosperm are widely known (medea, fie, fis2 and so on), only one mutant, msi1 showing parthenogenetic embryo phenotype has been identified so far. In this step, additional screening is required as mentioned in the second step. My research goal is to create new apomictic lines in rice by efficient screening of my own method in Arabidopsis and by modifing rice genome in a new method. To establish gene modifications in rice, I started isolation of fertilized egg cell first and will try to do TALEN or CRISPR. In this meeting, I mainly discuss about progress in cellular manipulation in rice. My ultimate goal is to provide bio-resource from CO2 resource by modifing genes and fixing the important traits in rice apomictic lines.

PS2-04

Sex and apomixis shape chloroplast DNA variation patterns in diploid and tetraploid Limonium spp

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The genus Limonium Mill. (Plumbaginaceae) has long been recognized to have sexual and apomictic (asexual seed formation) modes of reproduction. In this study, reproduction modes and genetic cpDNA variation patterns in populations of three putative sexual diploid species (L. nydeggeri, L. ovalifolium, L. lanceolatum), and three apomict tetraploid species thought to be related (L. binervosum, L. dodartii, L. multiflorum) were investigated. Inter- and intraspecific levels of cpDNA variation were analyzed investigated using two chloroplast sequence regions (trnL intron and trnL-trnF intergenic spacer). Furthermore, cytoembryological analyses were performed in representative species of each ploidy group in ovules collected in plants from greenhouse collections. Our findings reveal higher cpDNA haplotype variation in diploid than tetraploid species and no correlation between cpDNA haplotype and geographic distributions. Nevertheless, cpDNA haplotype sharing within and among species with distinct ploidy levels is detected. Moreover, our results provide first direct evidence of facultative apomixis in diploid plants and obligatory apomixis in tetraploid ones. Altogether, these results support that gene flow via pollen between populations through hybridization and/or facultative sexuality could be the most plausible mechanisms for generating new apomictic lineages.

PS2-05

Transcriptome vs proteome in the diploid apogamous fern Dryopteris affinis ssp. Affinis

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Apogamy in ferns involves the formation of sporophyte from gametophyte without the intervention of sexual cells. In D.affinis ssp. affinis both transcriptome and proteome analyses were done using a combination of next-generation sequencing (Illumina HiSeq), and shotgun proteomics (LTQ-Orbitrap tandem mass spectrometry). For protein identification we used the publicly available viridi plantae database to identify orthologue proteins from other plant specis as well as the transcriptomics data directly to generate a “species specific transcriptome database”. Putative transcripts (Trinity algorithm) were six-frame translated into amino acids (six-pack algorithm). Reads with the length of 60 amino acids or longer were retained and annotated with the swissprot database using BLASTp. The mass spectrometry data was searched and validated with Mascot 2.4 and Scaffold using the target-decoy approach. In total 943 protein clusters with 6552 unique peptide sequences were identified (protFDR <0.02). The effect of no genome annotation but searching against an orthologue database concatenated to an organism specific transcriptome database revealed that, of all unique identified peptide sequences, more than 73% were exclusively matched in the transcriptome database, while only about 18% were exclusively matched to viridi. The intersection of peptides identified in both databases is about 9%. Also on protein cluster level, more than 73% (696 clusters) were exclusively identified in the transcriptome database while only 247 (26.2%) in total would be identified if one would only do the search against the viridi database. The identified proteins are involved in general biological processes of living organisms, being especial relevant the presence of proteins related to reproduction.

This study represents the first transcriptome and proteome data reported from an apogamous species until present, valuable for leading further research on apogamy. We demonstrate that working with non-model organism species the benefit of a species specific database is tremendous. About 4 times more peptide sequences and also proteins are identified with very high confidence.

PS2-06

Evolving Reproduction: From Sexual Genomic Recombination to Asexual Genome Stability

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The natural genetic variation that makes evolution possible can be attributed to the recombination events during gametogene-sis and fusion of maternal and paternal genomes after fertilization. Thus, sexual reproduction produces offspring that are genetically diverse from their parents and between each other. The allelic combination of a plant that is better suited to the particular environment it is living in, is the basis of agricultural selection, i.e. the crossing of individuals with desirable traits to produce superior hybrids that will thrive under specific conditions and, thus, result in crops of higher fitness and yield.
But sexual reproduction of these hybrids introduces new recombination and genomic variation, and ultimately the loss of the hybrid phenotypical traits. Apomixis, the asexual reproduction through the formation of clonal seeds, circumvents recombination (apomeiosis) and forms an embryo without fertilization (parthenogenesis). Apomeiotic unreduced embryo sacs can be derived from a developmentally altered megaspore mother cell (MMC; diplospory) or from a cell other than the MMC (apospory). Thus apomixis can be viewed as a deregulation of sexual development in both space and time.
In Arabidopsis thaliana several mutants have been identified that show aspects of diplosporic or aposporic megagametogenesis, which is the first step towards the production of clonal offspring. This project aims to compare the sequence, expression patterns and levels of these genes with Arabidopsis' closest naturally apomictic relative, Boechera. Evolutionary analysis of these genes between Arabidopsis and sexual and apomictic Boechera species will tell us whether they are indeed involved in the deregulation of the sexual pathway that leads to naturally occurring apomixis; and if confirmed to be so, whether they can be manipulated to induce apomictic development in agriculturally relevant crops, thus preserving hybrid vigour through generations.

PS2-07

Transgenerational Inheritance and Epigenetic Reprogramming in clonal offspring of Arabidopsis thaliana

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The dynamic changes and resetting of epigenetic marks on the genome between generations is still a field with many open questions. In mammals, the resetting of epigenetic marks within each new generation is crucial for the establishment of totipotency and normal development. Consequently, only a few, mostly controversial, examples of transgenerational epigenetic inheritance are known in mice (Youngson, NA; Whitelaw E 2008; Annu Rev Genomics Hum Genet. 9: 233-257).
In plants, however, the stable inheritance of epigenetic information has been firmly established (Henderson, IR; Jacobsen, SE 2007; Nature 447: 418-424). Nevertheless, there is growing evidence that epigenetic reprogramming is also happing in plants. Epigenetic modifications occurring throughout the life cycle of a plant are considered to play an important role in adaptation to different environments. Analysing to what extent epigenetic information is transmitted or erased between generations is of great importance for further applications, e.g. in agriculture where the fixation of vigorous hybrid phenotypes through apomixis is a major goal.
Within this research project we will analyse the extent of epigenetic information that is inherited from one generation to the next by using the model organism Arabidopsis thaliana. We plan to create two genetically identical hybrid populations that are either produced sexually or clonally. The sexually produced hybrid population will undergo normal development and reprogramming events during gametogenesis, while the clonal population will short-circuit sporogenesis and, thus, developmental stages thought to be important for reprogramming are skipped. By comparing these two populations on a phenotypic as well as on a genomic level (methylome and transcriptome analysis), we will be able to identify the parts of the genome that are subject to reprogramming and, moreover, assess the relevance of transgenerational epigenetic inheritance for phenotypic variation.

PS2-08

Apomeiosis and the oxidative damage initiation hypothesis for meiosis

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The maintenance of sexual reproduction in eukaryotes is still a major enigma in evolutionary biology. Meiosis represents the only common feature of sex in all eukaryotic kingdoms. Almost all asexuality modes maintain meiosis either in a modified form or as an alternative pathway. The DNA restoration hypothesis (Hörandl, 2009 in Heredity 103: 445-457) suggests that the major function of meiosis is 1) DNA repair of oxidative lesions at prophase I in germline cells; and 2) elimination of defect mutants in haploid stages (gametophytes or gametes) in which deleterious mutations are being exposed to purifying selection. Microscopic studies on early generation hybrids corroborate previous hypotheses that emergence of apospory is correlated to disturbance of sporogenesis, but sexuality persists as a facultative pathway. Apomictic plants tend to increase frequencies of sexuality relative to apomixis after abiotic stress. The recently proposed oxidative damage initiation hypothesis (Hörandl & Hadacek 2013, Plant Reproduction 26:351–367) proposes as in integrative model for explaining these phenomena. The meiosis-specific spo11 protein acts like an antioxidant reducing the oxidized DNA target, thereby removing oxidative lesions in germline cells but also causing double strand breaks that are afterwards repaired during meiosis I. In hybrids aposporous initials emerge as a surrogate for inefficient sporogenesis, and frequencies increase when the stress trigger for meiosis remains low. Unreduced gametophytes can mask deleterious mutations, while reduced embryo sacs are under purifying selection against deleterious mutations. Our hypothesis may contribute to explaining various enigmatic phenomena of meiosis and apomeiosis: first, DSB formation outnumbers crossovers and, thus, effective recombination events by far because the main target of meiosis would be the removal of oxidative lesions; second, it offers an argument for why expression of sexuality is responsive to stress in many eukaryotes; and third, DNA restoration turns meiosis into an essential functional component of eukaryotic reproduction.

PS2-09

Quantitative and in situ analyses of BbrizGID1 gene expression in sexual and apomictic plants

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Brachiaria brizantha is an important forage grass of the Poaceae family. The occurrence of both apomictic and sexual reproduction within Brachiaria makes it an interesting system for studying the molecular pathways involved in both modes of reproduction. Apomictic and sexual plants have differentiated structure of embryo sac, Panicum and Polygonum type, respectively. There is evidence that phytohormones are involved in structuring embryo sacs in sexual plants of other species, but no study in apomictic plants is described. Results obtained from RNA-seq indicated that some genes involved in the biosynthesis of phytohormones are differentially expressed in sexual and apomictic ovaries, including a homolog of the Arabidopsis GID1 gene, encoding a gibberellin receptor. The objective of this study was to characterize and analyze the expression of this GID1-like gene, herein called BbrizGID1. Two accessions were analyzed, BRA 002747, a diploid (2n=2x=18) sexual and BRA 00591, a tetraploid (2n=4x=36) facultative apomictic. RT-qPCR was performed in ovaries at different stages of development of sexual and apomictic. Southern blot was performed using DNA from both genotypes and a 300 bp fragment of BbrizGID1. To observe the pattern of gene expression, mRNA in situ hybridization was performed in semi thin sections of ovaries and anthers at megasporogenesis. The results of RT-qPCR validated the RNA-seq for BbrizGID1 data showing higher expression in early stages of development of the embryo sacs of apomicts, compared with expression in sexuals. Southern blot results suggested that BbrizGID1 is present in a single copy in the genome of both plants. First results of in situ hybridization revealed a strong signal in the nucellar cells, including the meiocyte, and in the microspore mother cell, only in the apomictic plant. The results will be shown and discussed according to the possible involvement of BbrizGID1 during the reproductive development of *Brachiaria*.

PS2-10

Homoeologous chromosome sorting and progression of meiotic recombination in *Brassica napus*: ploidy does matter!

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Meiosis is a two-round cell division which produces balanced gametes and generates diversity within species. Crossovers (one of the products of meiotic recombination) between maternal and paternal homologous chromosomes are required for proper segregation of chromosomes at meiosis and thus genome stability and plant fertility. This condition is more difficult to fulfill in allopolyploid species, which have more than two sets of related chromosomes (called homoeologues) still able to recombine together. In this study we examined meiosis of *Brassica napus* (AACC), a young polyphyletic allotetraploid crop species with closely related homoeologous chromosomes. We combined a set of cytogenetic approaches to investigate the formation, progression, and completion of several key hallmarks of meiosis, including sister chromatid cohesion, chromosome axes, the synaptonemal complex and meiotic recombination. Altogether, our results demonstrate a precocious and efficient sorting of homologous versus homoeologous chromosomes during early prophase I in two representative B. napus accessions that otherwise show a genotypic difference in the progression of homologous recombination. More strikingly, our detailed comparison of meiosis in near isogenic allohaploid (AC) and euploid plants showed that the mechanism(s) promoting efficient chromosome sorting in euploids is adjusted to promote crossover formation between homoeologs in allohaploids. This suggests that, in contrast to other polyploid species, chromosome sorting is context dependent in *B. napus*.

PS3-1

The role of paralogous bHLH proteins in rice anther development

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Male reproductive development is an essential event in the alternation between diploid sporophyte and haploid gametophyte in the life cycle of higher plants. Through cell divisions and differentiation, precursor cells possessing a transient stem-cell-like feature produce the anther, male reproductive organ that contains four distinct layers of wall (epidermis, endothecium, middle layer and tapetum) and centrally positioned microsporocytes. We report here that the rice (Oryza sativa) basic helix-loop-helix protein TDR INTERACTING PROTEIN2 (TIP2) functions as a crucial switch in meristemoid transition and differentiation during early anther development. tip2 displays un-differentiated inner three anther wall layers and abort tapetal programmed cell death (PCD), causing complete male sterility. TIP2 has two paralogs in rice, TDR and EAT1, which are key regulators of tapetal PCD. We revealed that TIP2 acts upstream of TDR and EAT1 and directly regulates the expression of TDR and EAT1. In addition, TIP2 can interact with TDR, indicating a role of TIP2 in later anther development. Our findings suggest that the bHLH proteins TIP2, TDR and EAT1 play a central role in regulating differentiation, morphogenesis and degradation of anther somatic cell layers, highlighting the role of paralogous bHLH proteins in regulating distinct steps of plant cell type determination.

PS3-2

The role of *RPL10A* during female gametophyte development

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In a screen for mutants with reduced egg cell marker gene expression, we have isolated the *nogg* mutant. *NOGG* codes for the *Arabidopsis RIBOSOMAL PROTEIN of the LARGE SUBUNIT 10 (RPL10)* gene family. We confirm previous data, according to which RPL10A but not its paralogue RPL10C, is essential for the development of the sporophyte. We additionally demonstrate that RPL10A plays a critical role for pollen development as male transmission of the mutant allele is strongly reduced. Furthermore, the developmental program of female gametophytic cells is perturbed with nuclei fusion and programmed cell death (PCD) being not correctly initiated. We discuss the potential role of RPL10A and its paralogue RPL10C for the specification of haploid cells.

PS3-3

TOPLESS: Undressing a mechanism for the function of Arabidopsis DAZ1 and DAZ2 proteins in the male germline

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Development of the male germline in Arabidopsis thaliana culminates in the production of a pair of sperm cells inside a pollen grain that is shed at dehiscence. These sperm cells result from a mitotic division of the germ cell, and previous work has described duo1 in which germ cells fail to divide (1). DUO1 is a male germline-specific R2R3 MYB transcription factor and among its many targets for activation are two paralogous C2H2 zinc finger proteins, DAZ1 and DAZ2 (2). We have now shown that, like DUO1, DAZ1 and DAZ2 are specific to the male germline and when both genes are mutated germ cell cycle progression fails (3). Further, daz1-1 daz2-1 germ cells are rescued by a transgene coding for DAZ1 using in planta complementation assays. DAZ1 and DAZ2 possess two ERF-associated amphiphilic repression (EAR) motifs at their C-termini. The EAR motif is a transcription repression motif responsible for the recruitment of the Gro/Tup1-related co-repressor TOPLESS (TPL) and its family members TOPLESS RELATED (TPR) 1-4 (4). Using deletion and mutation constructs of the DAZ1 CDS in complementation assays in planta we are exploring a role for EAR motifs in the function of DAZ1 (3). We are also investigating the wider role of TPL/TPRs in the male gametophyte.

1. (1) Durbarry et al. 2005 Plant Physiol 137:297-307
2. (2) Borg et al 2011 Plant Cell 23:534-49
3. (3) Borg et al 2014 Plant Cell in press
4. (4) Kagale and Rozwadowski 2011 Epigenetics 6:141-146

PS3-4

Aperture formation on Arabidopsis pollen surface

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Cells rely on a regulated production of extracellular materials to control their morphology, growth, and motility, to promote tissue formation, and to protect themselves from harmful influences. Despite the importance of extracellular structures in development and disease, the question of how cells decide when, where, and in which manner these materials should be produced, deposited, and specifically assembled or modified is far from being understood in any system. Pollen presents a unique and powerful model for studying how controlled formation of extracellular structures is achieved. Pollen grains are surrounded by a complex extracellular structure, exine, which assembles into intricate 3D patterns of enormous morphological diversity among species, yet very conserved within a species. In most plant species, the pollen surface has characteristic areas called apertures, which lack exine and which are species-specific in their number, location, and morphology. This indicates that exine deposition machinery in a given species reliably recognizes particular areas on pollen surface as different from others and does not deposit exine onto these areas. In a forward genetic screen in Arabidopsis I have recovered multiple mutants defective in exine development, including those with abnormal aperture formation. Here I will describe what we have learned about this process based on the analysis of the inp1 mutants that either lack apertures or have abnormally short apertures and the lsq mutants that have ectopic apertures. INP1 protein exhibits a very distinct tripartite localization in the developing pollen, consistent with its direct involvement in specification of aperture position and controls aperture length in a dosage-dependent manner.

PS3-5

Epigenetic changes and autophagic features accompany the developmental programmed cell death of tapetum cells

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The tapetum, nursing tissue inside anthers undergoes cellular degradation by programmed cell death (PCD) during late stages of microspore-early pollen development. Despite the key function of tapetum, little is known about the molecular mechanisms regulating this cell death process. Epigenetic marks, such as DNA methylation, have been revealed as hallmarks that establish the functional status of chromatin domains, but no evidences on the epigenetic regulation of PCD have been reported. Several pathways of PCD can be found in plants, some of them showing apoptotic-like and/or autophagic features. Increasing evidences indicate that autophagy plays critical roles in plant PCD processes.

In this work, we studied the changes in DNA methylation and expression of MET1 DNA methyltransferase, as well as the existence and dynamics of autophagy compartments and machinery during the PCD of tapetal cells of Brassica napus, by a multidisciplinary approach. Results showed that tapetum PCD progresses with the increase in global DNA methylation and MET1 expression, that accompany high chromatin condensation, activity of caspase 3-like proteases and cytochrome C release, as well as an increase of vesicles, vacuoles, autophagic-like structures and autophagy markers in the cytoplasms.

This data suggests the participation of autophagy in the tapetum PCD and a possible new role of the epigenetic marks in PCD processes, giving new insights in the knowledge of the epigenetic control of plant PCD.

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PS3-6

High-temperature-induced downregulation of MADS-box B-class gene expression leads to a loss of anther identity and reduced fertility in tomato

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Sexual reproduction of plants is strongly influenced by environmental conditions. Studies in both, monocot and dicot species, have identified the pollen as the component that is most sensitive to high ambient temperature. So far, it is thought that high temperature disturbs the cellular metabolism of the developing pollen or that of the surrounding tapetum. When growing tomato plants under continuous mild heat conditions (CMH) that reduced male fertility, we observed a concomitant increase in the frequency of anthers with pistil-like morphological characteristics. Gene expression analysis confirmed that pistil-specific genes were significantly up-regulated in anthers grown under CMH and that expression of B-class MADS-box genes, necessary for anther identity, was significantly reduced. Supporting a role for the latter in the heat-induced flower phenotype, a partial knockdown line of one of the tomato B-class genes, showing only a weak phenotype under control conditions, reacted hyper-sensitively to CMH. Interestingly, this knockdown line showed a strong reduction in pollen number and viability, resembling CMH treatment, already at control temperature. Together, our results support the hypothesis that the loss of anther identity, caused by reduced B-class gene expression, contributes to the often-observed decrease in pollen fertility at high temperature.

PS3-7

The genetic basis of pollen number variation revealed by a genome-wide association study in Arabidopsis thaliana

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Although self-fertilization (selfing) has independently evolved numerous times in flowering plants, selfing species tend to share numbers of floral traits collectively known as the 'selfing syndrome', which involves a reduced pollen number instead of increased ovule number, i.e., changes in so-called pollen/ovule ratio.

Despite a wealth of knowledge about the ecological significance of the changes, the molecular genetic mechanisms are still poorly understood. To reveal the genes that are involved in pollen number variation, we first performed a genome-wide association study (GWAS) using world-wide natural accessions of Arabidopsis thaliana. GWAS identified several loci that are significantly associated with pollen number variation, and some of them encompass genes that have already been reported to be important for pollen function, suggesting the validity of our GWAS scan. GWAS also identified several unknown genes, including one we named Reduced Pollen Number 1 (RDP1). Secondly, we confirmed that mutant lines of RDP1 show a significant reduction in pollen number as well as reduced expression of RDP1 compared to wild type lines.

PS3-8

Long-term stored pollen transcripts and their role in pollen and embryo development

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We have aimed to identify transcriptional regulators acting during male gametophyte development to better understand many important processes required for succesfull gametogenesis. Better understanding of gamete production in the male gametophyte represents crucial aspect how to improve plants vigour and fertility.
Candidate genes were chosen exploiting pollen developmental transcriptomic data (Honys and Twell, 2004). Based on a wide screen of T-DNA mutant lines (Reňák and Dupľáková et al. 2012) we have identified several promising candidates and subjected them to the functional characterization.
This selection includes bZIP family of trascription factors (TFs), counting 75 members in Arabidopsis thaliana. Out of these, few genes exhibit pollen enriched expression profile and sharing properties for possible interaction analyzed in amino acid sequences alignment. At present, we are focusing on several members, whereas one candidate is showing very similar characteristics as previously characterized AtbZIP34 (Gibalová et al. 2009), implying possible functional link. As a next step we aimed to extend our knowledge to uncover downstream targets of our candidate bZIP transcription factors and to outline mode of action within the network of functional homo- and hetero-dimers identified in Yeast two Hybrid analysis.

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PS3-9

Novel players in pollen germination and peroxisome biogenesis

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Pollen germination on stigma is a prerequisite for sexual reproduction in higher plant. This process requires intercellular interaction between the pollen and papilla cells. Exudes of wet stigma and pollen coat of dry stigma are suggested to be involved in this process. Lipid in stigma is known to be the essential factor for pollen penetration. However, pollen factor is still unclear. We isolate a mutant named dayu which is specifically defective in pollen germination on stigma. The dayu pollen germination in vitro is comparable to the wild type. Genetics and molecular complementation analysis shows that DAYU encodes APEM9 which has been shown to be involved in peroxisomal matrix protein import. By confocal and transmission electron microscopy analysis, we found that dayu pollen lack integral peroxisomes. This data suggest that DAYU/APEM9 is directly involved in peroxisome biogenesis. We also investigated the role of other peroxisomal membrane proteins involved in peroxisomal matrix protein import on pollen germination. PEROXIN13, another peroxisomal membrane protein reported to be essential for pollen germination on stigma, is also required for peroxisome biogenesis witnessed by the lack of intact peroxisome in pex13 pollen. In dayu pollen, the content of hormone JA is substantially reduced, and application of JA manually on the dayu and pex13 inflorescence can partially rescue the pollen defect. Biochemical experiments showed that DAYU interaction with PEX13 and PEX16 in planta. Together, this research revealed the function of peroxisome in pollen germination on stigma and sheds light on the assembly of membrane proteins during the peroxisome biogenesis. Currently, we are investigating the molecular function of DAU in the de novo peroxisome assembly through advanced fluorescence microscopy and conditional complementation of dayu mutant to further dissect its role in pollen germination.

PS3-10

Establishing cell lineages in the ovule primordium of *Arabidopsis thaliana*

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Within the ovule primordia of flowering plants, the megaspore mother cell (MMC) is the pre-meiotic precursor of the female gametophyte. Although the ovule primordium of Arabidopsis thaliana has a relatively simple architecture, the cell division patterns that prevail in the nucellus have not been determined, as cell lineages within the L2 and L3 layers are not established. We used a reporter transgenic line containing a transposon (Tag1) placed between the 35SCaMV promoter and the GUS gene (35S-Tag1-GUS) to monitor excision and define clonal sectors in the developing ovule. Tag1 in the 35S-Tag1-GUS line is active in somatic and reproductive tissues and shows high frequency of excisions during ovule and seed development (Liu and Crawford, 1998). We also identified mitotic configurations in hundreds of fixed ovules through propidium iodide staining and confocal microscopy. Using these two approaches a total of 155 multicellular sectors have been so far identified, analyzed, and interpreted. The highest frequency of excision-based sectors is in the proximal region of the primordium, and the lowest frequency occurs in the nucellar region containing the MMC. The overall interpretation of cell division patterns is progressively allowing the establishment of a 3-dimensional fate map of cell divisions in the pre-meiotic ovule, a contribution to the overall understanding of cell lineage prior to female gametogenesis.

Reference:
Liu, D. and N.M. Crawford (1998) Characterization of the germinal and somatic activity of the Arabidopsis transposable element Tag1. Genetics, 148: 445-456.

PS3-11

Three-dimensional visualization of the female gametophytes of Oryza sativa and Arabidopsis thaliana using multiphoton microscopy

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Imaging the female gametophyte (or embryo sac) of angiosperms is complicated by the protective sporophytic tissues of the ovule, which typically necessitate sectioning, dissection or other preparations that can disrupt the functional integrity of the embryo sac. Ideally, it should be possible to observe whole, intact tissue in such a way as to avoid structural disturbance without sacrificing resolution. The advent of two-photon and three-photon microscopy used in conjunction with optical clearing of tissues provides a technique for the analysis of thick specimens at high-resolution. Using this method, three-dimensional images can be routinely obtained via a series of optical sections, spaced apart such that each voxel (3D pixel) has equal, cubic dimensions in all axes. Such a system permits a spatially accurate reconstruction to be made of thick specimens while retaining their original intact structure. In this study, we imaged the embryo sacs of Oryza sativa ssp. japonica and Arabidopsis thaliana at various stages during early fertilization with a Leica TCS SP8 multiphoton-equipped microscope. Using a 63× or 20× objective, ovaries and ovules fixed in PFA and cleared/mounted in methyl salicylate (n = 1.53) were excited using a multiphoton laser set at 830 nm. Broad-spectrum autofluorescence emission was detected using two HyD detectors, set at 350 nm–550 nm and 550 nm–750 nm or using a two channel photo multiplier tube in the non-descanned position, with autofluorescence data being divided into distinct sub-spectra. A spectral scan analysis of emission data from multiple structures within the tissue reveals differential patterning of emitted autofluorescence. Merged channels provide contrasting and complementary images of the structurally complex, three-dimensional organization of these embryo sacs providing new insights of sexual reproduction and subsequent development in plants.

PS3-12

Distribution of 5-methylcytosine and total transcriptional activity of *Hyacinthus orientalis* L. female gametophyte cells before and after fertilization

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Our previously immunocytochemical studies were shown that egg cell and central cell of Hyacinthus orientalis have a low metabolic activity and they are transcriptionally silenced (Pięciński et al., 2008; Niedojadło et al., 2012). On the other hand ultrastructural analysis indicated different chromatin organization in these cells. Chromatin in the egg cell nucleus was highly decondensed. After fertilization the restart of RNA synthesis was observed in the zygote and endosperm. Also chromatin organization in the zygote was changed. In the zygote nucleus chromatin condensed and occurred in the form of larger and smaller aggregates. Based on this data we assumed that different genes activity and chromatin structure of embryo sac cells could be regulated by epigenetic modification, especially methylation of DNA. The aim of the present investigations was to determine in vivo the distribution of 5mC (5-methylcytosine) in female gametophyte cells of Hyacinthus orientalis. 5mC was localized by immunofluorescence techniques. The obtained results have shown that before fertilization in the egg cell the level of genome methylation was higher than in the central cell. Although chromatin in the egg cell nucleus was decondensed the level of 5mC was high. The fusion with sperm cells caused a change in the methylation in the nuclei of both cells. After the transcriptional activation of the zygote slightly lower signal of fluorescence, than observed before fertilization, was visible. Unlike chromatin of the central cell, the primary endosperm DNA was almost completely demethylated. We confirm that the level of 5mC in these cells both before and after fertilization was positively correlated with transcriptional activity. In summary, our results indicate that the process of DNA methylation play important role in the regulation of gene activity and chromatin organization in the cells participating in double fertilization.

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PS3-13

Immunolocalization Survey of Methylation Marks during the male gametophyte development of Lilium longiflorum and characterization of the GRSF repressor

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We have conducted an immunlocalization survey of ten histone methylation marks in the developing male gametophyte of Lilium longiflorum. The methylation status of K4me2, K9me1, K9me2, K27me1, K27me3, K36me1, K36me2, K36me3, H4 R3me2s and H3 R17me2as were assessed in seven different developmental stages that cover pre-meoisis cells up to the mature binucleate pollen including detection of marks in the meiosis InterphaseI to TelophaseI. Developmental patterns of dynamics methylation marks will be described. In parallel, we have identified a histone methyl transferase protein that interacts with the germ cell-specific gene repressor GRSF. This preliminary result could link GRSF to a repressive methylation marks present in every anther cells, but excluded from the generative nuclei as shown by our immunological survey of histone methylation marks.

PS3-14

Evaluation of the presence of arabinogalactan proteins and pectins during *Quercus suber* male gametogenesis

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Quercus suber (cork oak) is a dominant Fagacea tree in the forests of South-West Iberian Peninsula. It is a monoecious tree with a long progamic phase that provides a comprehensive system for comparative studies in development and sexual reproduction. In this study the distribution of arabinogalactan protein (AGPs) and pectin epitopes in anthers of Q. suber was assessed in order to map these hydroxyproline-rich glycoproteins and the galacturonate-rich acidic polysaccharides during pollen development.
Immunocalization in male flowers was performed with a set of monoclonal antibodies directed against the carbohydrate moiety which recognizes AGPs and pectins. Ubiquitous labeling was obtained with anti-homolgalacturanos antibodies for pectins methyl-esterified in all cell types. By contrast the antibody that labelled non methyl-esterified homogalacturans had a preferential presence in microsporocyte cells walls at the beginning of pollen development. Intense labeling was obtained with anti-AGP antibodies both in tapetum and in intine wall near the pollen apertures and later in the generative cell wall and vegetative cell.
In order to identify AGP genes involved in cork oak pollen development, we searched for annotated AGP genes in the available transcriptome data of the Cork Oak EST Consortium database (www.corkoakdb.org). The evaluation of the putative AGPs highly expressed in male gametophyte was achieved by quantitative RT-PCR analysis in male and female cork oak flowers. Four putative AGPs that are preferentially expressed in the male gametophyte were identified. These AGPs probably play a significant role in reproduction due to their enhanced expression in male gametophyte.

PS4-1

Back to the Rhizoids of Plant Sexual Development

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The mode of sexual plant reproduction has fundamentally changed in both development and mechanism throughout the course of land plant evolution. Whereas fertilization in more primitive plants requires spermatocytes that swim from the male to the female gametophyte, flowering plants deliver the immotile sperm cells to the embryo sac (female gametophyte) inside the growing pollen tube (male gametophyte). In the flowering plant *Arabidopsis thaliana*, many developmental aspects of fertilization, such as pollen tube growth and reception, are dependent on the joint functions of several members of a family of receptor-like kinases, the *Catharanthus roseus* RLK1-like (*Cr*RLK1L) receptor-like kinase subfamily. The *Cr*RLK1L family comprises 17 homologs in *A. thaliana*, and different members can sometimes have very distinct developmental functions, making it difficult to assess the core or original function of the *Cr*RLK1Ls. In probably the most ancestral of land plants and emerging model organism, the liverwort *Marchantia polymorpha,* the CrRLK1L family is represented by a single gene homolog, which was named *MpFERONIA* (*MpFER*) after the family's most prominent member. This indicates that the evolution of the new structures and mechanisms in angiosperm reproduction required the recruitment of ancestral genes to fulfill completely new developmental roles. Here, we show that *MpFER* is broadly expressed in vegetative and reproductive tissues by fusing the endogenous promoter to a triple-VENUS fluorescent protein. Functional analyses demonstrate that *MpFER* is involved in the vegetative development of *M. polymorpha* by controlling rhizoid formation and overall growth of the plant. These phenotypes indicate a conserved and basal function of the *Cr*RLK1Ls in cell elongation. Finally, phylogenetic analyses suggest that *Mp*FER is basal and orthologous to all other land plant homologs analyzed, indicating that parts of flowering plant reproductive development originate from vegetative processes, like rhizoid formation, in evolutionary older plants.

PS4-2

The dilemma of the ovules of the Ficus

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All Ficus species are characterized by a closed inflorescence (syconium) with a minute opening (ostiole). Each syconium hosts hundreds of flowers along its inner wall. All Ficus species also require mutualistic fig wasps for pollination. Besides the pollinator, most Ficus host also other wasp species. Those pollinators which penetrate the syconium through the ostiole deposit pollen on the stigmas, insert their ovipositor through the style and lay one egg in the ovules. Usually one wasp species is a pollinator, others are parasites.

Ficus sycomorus is a monoecious species; within the same syconium are scattered female flowers with variable style length and male flowers around the opening. All flowers have the same potential of becoming seeds or forming galls *F. sycomorus* In Israel, hosts Sycophaga sycomori only, which does not convey pollen and does not pollinate. Sycophaga inserts its ovipositor through the style and lays one egg in the embryo sac of the ovule.The larvae are fed on the proliferated nucellus, in this case pollination is not essential for galls formation and in Israel all syconiums are lack of seeds.

In Kenya, as in other East and South African countries, together with Sycophaga sycomori, co-exists a pollinator: Ceratosolen arabicus, which convey pollen in special pockets, smear pollen on stigmas and iserts its ovipositor between the inner integument and the nucellus of the embryo sac , its larvae develops on the plant's embryo.In this case pollination is essential.

In many places in Kenya, both Sycophaga and Ceratosolen wasps are followed by the parasite Ceratosolen galili and it is not clear what its larvae feeding on.

The interaction between different flowers and each of the wasp species will be described, and the dilemma of the flower to become a seed producer or larvae bearer will be discussed.

PS4-3

Omics-based improvement of doubled haploid line production efficiency for wheat breeding

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Wheat is the third most important crop worldwide and the most important one in Europe. Breeding of new wheat cultivars can be accelerated by 2-3 years when using doubled haploid (DH-) lines instead of conventional line breeding. This acceleration during the breeding process is combined with an acceleration of the breeding progress: adaption to changing climatic environments, safeguarding the global needs of food for a growing population, and exploitation of new and up to now unsuitable growing areas via better stress resistance of new cultivars will faster become possible.
The goal of our work is to unravel the developmental pathways underlying androgenic microspore development during anther culture of wheat. This knowledge shall be used to increase the efficiency of doubled haploid wheat production.
Therefore, microspores of three developmental stages will be manually selected from developing anther cultures and probed at the DNA-, RNA-, and metabolite-level. In combining results of all three regulatory mechanisms, we hope to gain hints for a knowledge-based improvement of wheat anther culture. Here, first results of our work will be presented.

PS4-4

Is the threatened endemic Dianthus morisianus Vals. (Caryophyllaceae) affected by inbreeding depression?

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Isolation and small population size resulting from habitat destruction and fragmentation may negatively affect plant fitness through pollinator limitation and increased levels of inbreeding; therefore these factors could have important consequences for the viability of threatened plant populations. In order to understand the mechanisms which could affect the persistence of threatened plant populations it is necessary to examine in detail the main phases of the species life cycle.
Dianthus morisianus Vals. (Caryophyllaceae), one of the 10 most endangered taxa of Sardinia (Italy) is a psammophilous chamaephyte which grows on stabilized dunes in a small area of Southwest Sardinia. In this study, the inbreeding depression was investigated in 92 ex situ cultivated plants in order to verify the outcomes of self and cross-pollination.
First of all, our results suggest that the self-pollination treatment affected the fruit set (81.81% and 97.14% for self and cross, respectively), the number of seeds per fruit (42.86±1.83 and 46.8±1.72, respectively), the seed weight (1.15×10-2 and 1.19×10-2 g, respectively), the germination rate (93.88% and 95%) and the T50 (5.82±0.875 days for self and 3.95±0.398 days for cross). Additionally, Cruden's categories indicate that the breeding system of D. morisianus could be classified from facultative to strictly xenogamaous (P/O = 287.42). Moreover, a significant inbreeding effect on germination time (T50) (δ=0.47) and seed set (δ=0.23) was found as a result of the pollination treatment.
Our study therefore demonstrates that D. morisianus is an outcrossing fertile and self-compatible species which is negatively affected by inbreeding depression at the germination and the seed production stages. Further aspects are now under study to investigate a larger set of attributes in this threatened plant and to evaluate responses to inbreeding in the natural population.

PS4-5

Study of the *INNER NO OUTER (INO)* gene in *Prunus* species. Implications for the evolution of ovule development in angiosperms.

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While gymnosperms ovules have a single integument, most angiosperms have bitegmic ovules although unitegmic ovules have arisen independently several times during the evolution of flowering plants. The genetic regulation behind integument development has been mainly studied in the model plant Arabidopsis where the INNER NO OUTER (INO) gene, expressed exclusively in the outer integument, has been found essential for ovule development, but also for ovule fertility. The Rosaceae, a family among the core eudicots and the third most economically important angiosperm family in temperate regions, shows great ovule morphological diversity. Thus, in the same genus (Prunus) species with bitegmic and unitegmic ovules can be found. In this work we perform a microscopic study on ovule development and follow the expression pattern of the INO gene in P. armeniaca (apricot), P. persica (peach) and P. incisa. The morphologic study of ovule development showed bitegmic ovules in P. armeniaca, intermediate bifid ovules in P. persica and unitegmic ovules in P. incisa. However, the characterization of INO expression by in situ hybridization revealed similar expression patterns in the outer integument of P. armeniaca and P. persica and in the single integument of P.incisa. These results show the conservation of INO gene expression in Prunus species and confirm that the identity of the outer integument is also present in the intermediate bifid species P. persica and the unitegmic species P. incisa supporting the hypothesis of fusion of the outer and inner integuments that could represent a widespread situation in other unitegmic species in flowering plants.

PS4-6

Differentiation in reproductive behaviour within a population of the relict plant species *Sonchus fragilis (Asteraceae)*

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The influential model of evolution for reproductive systems by Lande & Schemske (1985) advocates that two alternative stable situations are exclusively possible in plant populations: complete selfing and outcrossing, being the intermediate situations transitional. The relevant number of cases of mixed mating reported during last two decades has determined a changing view, and has led to significant efforts in creating new models devoted to explain the stability of such mixed systems in the wild. However, experimental analyses addressed to describe this variability and the relevance of ecological conditions involved are scarce.
We describe the variability in the reproductive system of a large population of Sonchus fragilis (Asteraceae), a cliff-dwelling endemic, relict species to Western-Rif Range (Morocco). Along the population studied, a bimodal distribution pattern of the incompatibility index (ISI) was found, being the strong self-incompatible (ISI = 0.8−1) and self-compatible (ISI ≤ 0.2: 0.2−[-0.4]) individuals much more common than those with intermediate condition (0.8>ISI>0.2). The comprehensive analysis of the selfing ability and the pollen-stigma interactions allow us to define five reproductive behaviour groups, although they are not clearly delimited: i) fully autog-amous plants, ii) self-compatible plants with deficient self-pollination, iii) changing self-incompatible plants, iv) self-incompatible plants at stigma level, and v) self-incompatible plants at style level. The spatial pattern of distribution of self-fecundation ability denotes a notable case of genetic polymorphism, probably related to ecological heterogeneity. We postulate that relatively small differences in pollination services may be the responsible of the reproductive variability, and that limited ability for gene flow in the species may contribute to the maintenance of the system.

References:
Lande, R. & D.W. Schemske (1985) Evolution, 39: 24-40.

PS4-7

Impedance Flow Cytometry – A novel method for pollen viability determination

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Analysis of pollen viability plays an important role at various aspects of plant breeding and plant production processes. Pollen viability is generally determined by various classical methods like staining techniques or in vitro germination assays. The disadvantage of the various staining techniques is that they have to be adapted per species and the resulting data do not always correlate with *in vitro* germination. Both, the current methods analysing pollen viability and germination are limited in the number of cells that can be analysed in a certain time frame and they are laborious in preparation and analysis. Here, we present a novel, label-free approach for the determination of pollen viability and maturation grade based on high-throughput single cell analysis by impedance flow cytometry (IFC). The technique is based on an improved Coulter counter analysing individual pollen grains via a microfluidic chip. It permits impedance measurements in the radiofrequency range (from 0.1 to 30 MHz), allowing more detailed cell characterisations than conventional cell counters. Various pollen from different plant species like e.g. *Saintpaulia*, *Campanula*, tomato, pepper, barley have been successfully analysed by IFC demonstrating the potential of this technique to simplify and standardize pollen viability analyses.

PS4-8

Evaluation of fitness in the progenies from a mixed mating plant population

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The influential model by Lande and Schemske (1985) for the evolution in reproductive systems of plant populations proposes that major evolutionary force lies in inbreeding depression. According to them, transition to selfing is favored when fitness in selfed progenies is at least 50% of those of outcrossed progenies. The empirical data available to test this theory are not common because of the difficulty of designing ecologically significant comparisons between strains of both types.
The description of a population of the relict species Sonchus fragilis (Asteraceae) showing a mixed mating system provides a valuable study material for testing the role of fitness in the evolution of sexual systems in plants. We carried out an experiment using three families of progenies raised from seeds of diverse origin from this population: i) wild seeds from self-incompatible mother plants, ii) wild seeds from self-compatible mother plants, and iii) seeds obtained by forced geitonogamous crosses of self-compatible mothers in the greenhouse. We quantified vegetative fitness components (germination rate and germination dynamics, juvenile vigor, and final aerial and root biomass) and reproductive fitness components (flowering phenology, fecundity and flower production). Fitness of the progenies obtained from seeds of self-incompatible plants is higher in general, mainly for biomass; however, differences among families are moderate, never reaching values of 50%. No notable significant differences were found between the progenies obtained from wild and forced geitonogamous seeds from self-compatible mothers, which indicates an effective purging of major deleterious alleles. In addition, the analysis of reproductive components shows slight differences among reproductive strategies. Our results support that shifts of reproductive systems are not merely governed by inbreeding depression level but also for cumulative effects of fitness through the life cycle with important incidence of competitive ability.
a notable case of genetic polymorphism, probably related to ecological heterogeneity. We postulate that relatively small differences in pollination services may be the responsible of the reproductive variability, and that limited ability for gene flow in the species may contribute to the maintenance of the system.
References:
ande, R. & D.W. Schemske (1985) Evolution, 39: 24-40.

PS4-9

Floral nectaries and elaiophores in *Cypella* Herb. (Iridaceae: Iridoideae): their distribution, diversity and anatomy.

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Iridaceae is one of the few angiosperm families in which both floral nectaries and elaiophores evolved to reward pollinators. Trichomal elaiophores constitute a major innovation among the Iridoideae of South and Central America, but the current knowledge about additional resources available for pollinators in oil flowers is still limited. Cypella is one of two largest genera of the tribe Tigridieae (Iridoideae) in South America and the occurrence of trichomal elaiophores is recorded for members of this genus, even though the only well-documented account concerns a single species. Indeed, flowers of Cypella herbertii produce simultaneously floral oil and nectar, a unique feature among the Iridoideae of the American continent. This work aims to characterize the secretory structures involved in the production of nectar and oils at the genus level and to study their evolution in a broader phylogenetic context. Morphological and structural observations, as well as histochemical tests, were conducted on fresh and fixed material of 19 species of Cypella and several species of the related genera Calydorea, Catila, Herbertia, Kelissa and Onira for generic comparisons. All species of Cypella and Onira examined produce nectar and oil, while Herbetia and Kelissa produce only oil and Calydorea and Catila are apparently devoid of nectaries and elaiophores. Based on the topology of the latest comprehensive phylogeny of the tribe, the character optimizations suggest that both elaiophores and nectaries evolved only once at the base of the most species rich clade of Cypella, with one or few subsequent reversions. Cypella is the only example among Iridaceae where both floral nectaries and elaiophores evolved simultaneously at this taxonomic level. This unusual combination of pollination rewards may testify to the existence of a bimodal pollination system, such strategy being probably important for the successful reproduction of these plants.

PS4-10

Potential utility of reproductive anatomical features in subgeneric classification of Passiflora (Passifloraceae)

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Passiflora L. is the largest genus of Passifloraceae, with more than 530 species. Their representatives inhabit primarily the neotropics and are composed by climbing herbs, trees, and woody lianas. The genus was formerly divided into 22 subgenera, but recent studies, based primarily on floral morphology, proposed its rearrangement into four subgenera. Subsequent studies, mostly based on molecular data, confirmed this subdivision. Some defining characters of subgeneric classification are the presence of tendrils, leaves shapes, habit and morphology of hypanthium and operculum. However some of these states are shared between groups. In the present study we demonstrate the potential taxonomic utility of anatomical features of reproductive structures (anthers and pollen grains) on the subgeneric delimitation in Passiflora. External and internal morphology of anthers and pollen grains from thirteen species (belonging to Astrophea, Decaloba and Passiflora subgenera) from southern Brazil were investigated under light and scanning electron microscopy. Differences found in thirteen anatomical features were accessed. A character-state matrix was used for phylogenetic analysis under parsimony method. The resulting consensus tree placed P. haematostigma (the only sampled species from Astrophea subgenus) as the most divergent one, while grouped all sampled species from Passiflora subgenus into a well-supported clade. The species belonging to Decaloba subgenus did not form a consistent group. Some features found in this study deserve attention for their utility, as the anthers venation pattern, which were not branched in Decaloba, sparsely branched in Astrophea and intense branching in Passiflora subgenus. Other relevant characters are the size of the anthers, the presence of elongated stomial cells, endothecium thickeness pattern, pattern of exine reticulum (amplitude and presence of baculli) and sporoderm structure. These findings indicate that such embryological features may be useful in studies on species with problematic taxonomic delimitation at this taxonomical level. Support: CNPq, CAPES.

PS4-11

Common control of fruit dehiscence and seed abscission in Arabidopsis thaliana.

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Seed dispersal is an essential trait for plants, allowing the seed detachment from the mother plant and their spread. Seed dispersal in Arabidopsis depends on two main factors, the separation of the fruit valves that protect the seeds –fruit dehiscence- and the separation of the seeds from the funiculus that connect them to the mother plant seed abscission

The fruit dehiscence has been studied during the last decades providing a detailed molecular mechanism of this process. In the accepted model the redundant MADS-box transcription factors SHATTERPROOF1 (SHP1) and SHP2 play an essential role on the differentiation of the valve margin tissue, as in the shp1shp2 mutant the valve margin disappears and the fruits become indehiscent. During valve margin establishment the SHP expression is restricted to the narrow zone that will form the valve margin by factors expressed in the replum and valves. Downstream SHPs several bHLH transcription factors also contribute to the specification of the lignified and separation layers that conform the differentiated valve margin. The lignification pattern established will provide the mechanical force to open the fruit

The control of seed abscission is less understood. It has been described that mutations in the MADS-box gene SEEDSTICK (STK) produce the lack of seed abscission. Interestingly, STK and SHP genes are phylogenetically related, and play a redundant role in ovule identity establishment, indicating a similar activity. In contrast, STK and SHP have been involved independently in the two separation processes presented above. For these reasons we decided to study the mechanistic control of STK on seed abscission comparing it with the role of SHP on fruit dehiscence. Our results suggest a similar molecular mechanism controlling both seed abscission and fruit dehiscence in Arabidopsis. Thus, a common mechanism for tissue separation could have evolved to control both kind of processes.

PS4-12

Controlled pollination in cork oak (*Quercus suber* L.) to support its genome sequencing project

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The high economic, environmental and biodiversity importance of Cork oak (Quercus suber L.) for the Portuguese forest is presented.
The advantages of controlled crosses to obtain full-sib progenies to use in genetic mapping are discussed considering the species high heterozygotic profile. The reasons for the choice of the mating design based on four female parents and ten male parents are presented.
Cork oak is a species of complex and rather unpredictable reproductive behavior throughout the years and among trees and female flowering is more unpredictable than male flowering. On controlled pollination it is crucial to choose trees offering high probabilities on female flowering. Hence the selection of female parents was performed only at the controlled and permanent plot at Quinta da Serra, to benefit from the long run population genetics studies performed since 1992 where 24 trees are being studied the reproductive behavior at the level of flowering/fruiting ability and flowering phenology.
The pre-selection was focused on 10 trees known to have good to high flowering capacity and intermediate or late flowering phenology, in order to guarantee the use of pollen from male parents with unknown phenology. From the initial sub-set of 10 trees, four were selected as female parents (trees QS1, QS4, QS13, and QS20).
To minimize the chances of having a close genetic relationship between the 10 male parent trees, we have chosen trees geographically distant, including the tree selected for genome sequencing (HL8).
The key aspects of flowering receptivity, pollen processing, artificial pollination procedures and pollen germination tests as well as the pollination and fecundation processes leading to annual and biennial acorn formation are discussed.
After appropriated traceability of putative full-sib plants the controlled progenies will be used to construct a genetic map that will support the national cork oak genome sequencing project.

PS5-1

Oakleaf: an S locus-linked mutation of Primula vulgaris that affects leaf and flower development

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Floral heteromorphy in *Primula* species is a specialised breeding system that promotes out crossing through reciprocal herkogamy, with insect-mediated cross-pollination occurring between pin and thrum form flowers. Development of the different forms of flower is coordinated by genes located at the di-allelic *S* locus. The *S* locus genes that orchestrate floral heteromorphy have not yet been described, but previous studies have identified *S* locus linked genes that have helped define the *S* locus region as a step towards identifying the key genes. *Oakleaf* is an additional *S* locus-linked mutant of *Primula vulgaris* characterised by changes in leaf-morphology and petal shape. *Oakleaf* is dominant to wild type, and this characteristic, together with development of lobed leaves and ectopic meristem development is reminiscent of the phenotype observed through over-expression of *KNOX*-homeodomain transcription factor genes in other species. The present study has identified the full complement of *KNOX*-like genes within the *P. vulgaris* genome and enabled analysis of their differential expression in wild type and *Oakleaf* plants. These studies reveal two potential candidate genes for *Oakleaf*. Further comparative analysis of leaf and flower transcriptomes has identified cohorts of up-regulated and down-regulated genes in *Oakleaf* which will facilitate future analyses of the regulatory networks perturbed by the dominant *Oakleaf* mutation.

PS5-2

SFBB-containing canonical and noncanonical SCF complexes in pollen of apple (Malus × domestica)

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Gametophytic self-incompatibility of Rosaceae, Solanaceae and Plantaginaceae is genetically controlled by a single polymorphic S locus. The S locus contains at least two genes, S-RNase and F-box protein encoding gene SLF/SFB/SFBB that control pistil and pollen specificity, respectively. Generally, the F-box protein forms an E3 ligase complex, SCF complex with Skp1, Cullin1 (CUL1) and Rbx1, however, in Petunia inflata, SBP1 (S-RNase binding protein1) was reported to play the role of Skp1 and Rbx1, and form an SCFSLF-like complex for ubiquitination of non-self S-RNases. On the other hand, in Petunia hybrida and Petunia inflata of Solanaceae, Prunus avium and Pyrus bretschneideri of Rosaceae, SSK1 (SLF-interacting Skp1-like protein1) is considered to form the SCFSLF/SFB complex. In this study, we isolated pollen-expressed apple (Malus × domestica) homologs of SSK1 and CUL1, and named MdSSK1, MdCUL1A and MdCUL1B. MdSSK1 transcript abundance was significantly (>100 times) higher than that of MdSBP1. In vitro binding assays showed that MdSSK1 and MdSBP1 interacted with MdSFBB1-S9 and MdCUL1, and MdSFBB1-S9 interacted more strongly with MdSSK1 than with MdSBP1. The results suggest that both MdSSK1-containing SCFSFBB1 and MdSBP1-containing SCFSFBB1-like complexes function in pollen of apple, and the former plays a major role.

PS5-3

Identification of proteins modified by ROS/NO during self-incompatibility response in *Papaver rhoeas*

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Many higher plants use self-incompatibility (SI) mechanism to prevent inbreeding and thus encouraging out-crossing. Upon a self-challenge in Papaver rhoeas, a Ca2+-dependent signalling cascade is initiated resulting in the destruction of the self-pollen through a Programmed Cell Death (PCD) network. Upstream of PCD, several SI-specific events are triggered in incompatible pollen, including phosphorylation of soluble inorganic pyrophosphatases (sPPases) and a MAPK (p56); alterations to the cytoskeleton; increases in Reactive Oxygen Species (ROS) and Nitric Oxide (NO) and activation of several caspase-like activities. This results in pollen tube growth inhibition and programmed cell death (PCD) in incompatible pollen, which effectively prevents self-fertilization.
ROS and NO are well established signalling molecules involved in various biological reactions in both mammalian and plant systems including PCD. ROS and NO participate in signal transduction and also modify cellular compo-nents, causing macromolecular damage and cell death. NO is known to modify proteins by S-nitrosylation in animal systems but very little is known about how they regulate plant responses, especially in the context of a defined physiologically relevant stimulus. We recently showed that increases in ROS and NO are involved in SI-mediated PCD in incompatible pollen. We recently initiated a project to identify proteins modified by oxidation and S-nitrosylation as a result of SI signalling, as this might provide insights into SI-mediated events and how ROS and NO might mediate SI-induced PCD. We have used a mass spectrometry approach to identify proteins that are modified by oxidation and S-nitrosylation. Recent data obtained regarding proteins modified in this manner will be presented.

PS5-4

The establishment of regeneration and transformation systems in *Primula* and *Silene*

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The development of transformation systems for Primula vulgaris and Silene dioica are important for the identification of genes that are involved in the control of floral heteromorphy and sex determination. Primulaceae species almost certainly provide the best known examples of heteromorphic flower development. Molecular approaches give the best opportunity to define and understand the role of genes involved in floral heteromorphy in the common primrose, Primula vulgaris, along with other Primula species. Primula plants either produce pin form flowers or thrum form flowers and crosses only occur between these two different forms. The underlying genes responsible for these different flower forms control anther position, style length and pollen size, and are clustered within the S locus. Primula transformation is key to identifying and assessing the genes within the S locus. To enable analysis of sex determination in dioecious Silene dioica (red campion) we have taken similar molecular approaches. Silene produces separate male and female plants the gender of which is determined by sex chromosomes. Our aims are to identifying male specific genes located on the male determining Y chromosome, then test gene function through plant transformation.
For Primula vulgaris and Silene dioica we have established new efficient in vitro regeneration systems, which have proven invaluable in the maintenance and propagation of sterile genotypes. We have developed a transient transformation system which provides a quick and efficient method of testing gene function. Significant progress has been made towards the development of stable transformation systems, through the optimisation of gene delivery and plant regeneration.

PS5-5

Comparative 2D-proteome analyses of non-pollinated, incompatibly and compatibly pollinated *thrum* and *pin* pistils of common buckwheat

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Common buckwheat (*Fagopyrum esculentum* Moench) is a self-incompatible dicot species with two morphologically different flower types: thrum (shorter style, longer anthers, larger pollen grains) and pin (longer style, shorter anthers, smaller pollen grains). In this species fertilization is allowed only between flowers of different morphology (compatible pollination), while it is prevented between flowers of the same morphology (incompatible pollination) through self-incompatibility (SI). Two flower morphs have different expression site of the SI response: in thrum pistil at the junction of stigma and style, and in pin pistil at 2/3 of style's length. It is well documented that SI response includes various protein-protein interactions in other studied plant SI species, but so far there are no such reports for buckwheat. The aim of our study was to track and compare changes in proteome profiles of thrum and pin pistils' upon their incompatible and compatible pollination. Total proteins extracted from non-pollinated, incompatibly and compatibly pollinated thrum and pin pistils were separated by 2D-PAGE and analyzed using Image Master 2D Platinum software v6.0. In each sample, proteome profiles revealed distribution over a wide mass range (10-130 kDa), with the prevalence of acidic to neutral proteins (pI 3.5-7) and only a few basic proteins (pI 7.5-9). The most abundant proteins were those shared by two morphs, which were present independently of pollination type (incompatible/compatible) (c. 90-95% of detected spots per sample). Proteins specific for morph and pollination type were also discovered (c. 5-10% of detected spots per sample) and will be identified by MS analysis.

PS5-6

Cross incompatibility between wheat and rye

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Triticeae cereals (wheat, barley, and rye) are major crops in Europe and efficient breeding of improved Triticeae varieties is essential in the context of environmental changes. Cultivated cereal gene pools contain only a fraction of the available diversity in the species. To improve agronomical important traits (yield, quality, tolerance to abiotic and biotic stresses), breeders make use of interspecific hybrids. However, this process is generally very inefficient in wheat due to the presence of dominant genes (Kr) inhibiting interspecific crossability in most wheat species. A few wheat varieties, which are of no agronomic interest (like the international wheat reference variety Chinese Spring), are able to produce hybrids after pollination with rye or barley. Interestingly, they all originate from the Asian (China, Japan) wheat pool raising the question of the origin and evolution of the genes controlling interspecific crossability. Our team has discovered a novel QTL (SKr locus) controlling the inhibition of crossability between wheat and rye, located on the short arm of chromosome 5B. Genetic and physical mapping allowed locating the SKr locus in an interval of 0.6 cM. In parallel to the physical mapping and sequencing of the SKr locus in the non-crossable wheat, and to the genetic diversity of the SKr locus analysis, we are investigating molecular basis of cross incompatibility between wheat and rye through proteomics of pollinated (sampled 30 and 60 min after pollination) and non-pollinated ovaries from both compatible and incompatible lines to identify the protein(s) involved in incompatibility. Understanding the molecular basis of incompatibility is of major interest scientifically to overcome hybridization barriers, thus allowing enhanced exploitation of genetic diversity in the future by transferring alien chromatin into wheat from its wild relatives carrying new favorable alleles for genes of agronomic importance.

PS5-7

Pollen-stigma interactions in Brassicaceae

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As for all organisms, reproductive success is critical for survival in plants. Success of Flowering plants is assigned to several unique evolutionary adaptations that aid in reproduction. Several of these adaptations relate specifically to the female part of the flower also named pistil. Once a compatible pollen grain lands on the stigma, the apical surface of the pistil, adhesion and hydration occur followed by pollen germination. Species of Brassicaceae, including Arabidopsis and Brassica, have dry stigmas and contact with a stigmatic cell is a prerequisite for the activation of pollen and subsequent pollen tube development. In addition, plants that exhibit genetic self-incompatibility reject self-pollen by blocking pollen adhesion and hydration at the earliest stages of contact with stigmatic cell. Therefore, stigma receptivity is strictly connected with pollination dynamics, reproductive success and plant productivity.
The stigmatic cells expand anisotropically and can be view as a model for polarized cell growth. We know, from others cell systems, that cytoskeleton, microtubules and actin filaments, has a fundamental role in directional cell expansion. We have generated Arabidopsis lines that specifically express, in the stigma, fluorescent proteins targeting individual cytoskeleton components to characterize the cytoskeleton architecture in course of stigma development. Our preliminary results shown that microtubules and actin filaments have perpendicular orientation during stigmatic cell growth. Moreover, it has been suggested that cytoskeleton is re-organized during compatible and incompatible pollinations. We have developed a live imaging approach to dissect the role of cytoskeleton in pollen acceptance or rejection, adding a dynamic view to the current published data. We showed that, about 15 minutes after compatible pollen deposition at the stigma surface, actin microfilaments seem to reinforce, surrounding the pollen tube contact site. We are currently monitoring the cytoskeleton behavior after incompatible pollination.

PS5-8

Molecular characterization of self-incompatibility factors in the grass, *Hordeum bulbosum*

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Self-incompatibility (SI) in the grasses (Poaceae) is gametophytically controlled by a distinct two-locus genetic system. It is governed by the multiallelic loci S and Z, and the incompatible reaction occurs when both the S and Z alleles in the pollen grain match those in the pistil. This suggests that the specificity of SI is determined by complementary interaction of S and Z genes. We have studied the SI of diploid Hordeum bulbosum, a wild relative of barley (H. vulgare), to elucidate the molecular mechanisms of the grass SI system. To date, we have identified and analyzed a good candidate of the femele S gene, named HPS10 (Hordeum pistil S-specific 10). This gene fulfills the requirements for the female S, that is complete linkage to the S locus, stigma-specific expression and a high degree of allelic sequence polymorphisms. The gene product was predicted to be a small hydrophilic protein of unknown function. To identify HPS10 as the female S determinant, we newly developed an in vitro pollen bioassay system in H. bulbosum. We will present our data on functional characterization of HPS10 protein using the bioassay system. We will also report our comparative transcriptome analysis of pollen and pistil from different haplotypes to find new candidate genes coding for the remaining SI determinants.

PS5-9

Loss of self-incompatibility in the allopolyploid *Arabidopsis kamchatica* by degradation of the male component

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The genetic basis of the evolutionary transition from outcrossing to selfing has been a major focus in evolutionary biology. Selfing, most commonly, evolved through the breakdown of the self-incompatibility (SI) system. Sporophytic SI system consists of the male specificity component, S-locus cysteine-rich protein (SCR), the female specificity component, S-locus receptor kinase (SRK) and genes that are involved in the downstream signaling pathway. SCR is expressed at the pollen coat and acts as the ligand of SRK, a transmembrane serine/threonine receptor kinase that expresses on the stigma. Interaction between SCR and SRK from the same S-haplogroup triggers downstream mechanism to inhibit pollen tube germination on the stigma.

Polyploidization is common in plant genomes and it has been suggested that polyploids self more frequently than their diploid relatives. However, the underlying mechanisms associating self-compatibility and polyploidization are still largely unknown. We are interested in elucidating the molecular mechanisms involve in the loss of SI in Arabidopsis kamchatica. It is a self-compatible allotetraploid species, originated through allopolyploidization of multiple individuals from two diploid species, Arabidopsis halleri and Arabidopsis lyrata that are predominantly outcrossing. Previous study shows that SRK and genes involved in the downstream signaling pathway are still functional in some A. kamchatica accessions. On the other hand, SCR is not functional in all A. kamchatica accessions. In order to isolate the short and polymorphic SCR genes, we performed next-generation sequencing of the anther cDNA of A. halleri. Potential SCR gene of S-haplogroup A, B and D, respectively were isolated. Mutations disrupting the function or expression of the potential SCR genes were identified in A. kamchatica. These indicate that the degradation of the male component, SCR, is responsible for the loss of SI in A. kamchatica.

PS5-10

Pollen modifier genes in Prunus Gametophytic Self-Incompatibility. Study of the self-compatible apricot mutants cv. 'Canino' and 'Katy'

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RNases and F-Box proteins belonging to *S*-locus are essential for the gametophytic self-incompatibility (GSI) specific recognition in *Prunus* species. However, *S*-locus unlinked factors have also been suggested as essential for the correct functioning of the mechanism. Particularly, Pollen Part Mutations (PPMs) unlinked to *S*-locus have been described to confer self-compatibility in apricot cvs. 'Canino' and 'Katy' and, following a similar mapping strategy based on segregation distortion loci, both were mapped in the so-called *M* and *M'*-locus, respectively. Interestingly, these two loci are located within an overlapping region of 273 Kb in the distal part of the chr. 3, according to the peach (*Prunus persica*) genome sequence. No evidence is yet available to discern whether they affect the same gene or not, but molecular markers seem to indicate that both cultivars are genetically unrelated suggesting that every PPM may have arisen independently. Genomic and transcriptomic strategies are being currently implemented to pave the way for positional cloning of the underlying gene(s), which identification might be crucial to shed some light on *Prunus* GSI system

PS6-1

Arabidopsis Galacturonosyltransferase (GAUT) 13 and GAUT14 Have Redundant Functions in Pollen Tube Growth

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Cell wall biosynthesis is indispensable for pollen tube growth. Despite its importance to sexual reproduction, the molecular mechanisms of pollen tube wall biosynthesis remain poorly understood. Here, we report functional characterization of two putative Arabidopsis galacturonosyltransferase genes, GAUT13 and GAUT14, which are essential for pollen tube growth. GAUT13 and GAUT14 encode the proteins that share a high amino acid sequence identity and are located in the Golgi apparatus. The T-DNA insertion mutants, gaut13 and gaut14, did not exhibit any observable defects, but the gaut13 gaut14 double mutants were defective in pollen tube growth; 35.2–37.3% pollen tubes in the heterozygous double mutants were swollen and defective in elongation. The outer layer of the cell wall did not appear distinctly fibrillar in the double mutant pollen tubes. Furthermore, distribution of homogalacturonan labeled with JIM5 and JIM7 in the double mutant pollen tube wall was significantly altered compared to wild-type. Our results suggest that GAUT13 and GAUT14 function redundantly in pollen tube growth, possibly through participation in pectin biosynthesis of the pollen tube wall.

PS6-2

The pollen-expressed AtTFIIB1 and TFIIB-Related Transcription Factor 2 are required for pollen tube growth and seed development in *Arabidopsis*

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Pollen tube growth and seed development require a large number of genes to be expressed. Transcription of eukaryotic nuclear genes is accomplished by three conserved RNA polymerases acting in association with a set of auxiliary general transcription factors (GTFs), including B-type GTFs. The roles of B-type GTFs in plant reproduction remain poorly understood. Our group is currently characterizing a group of pollen-expressed B-type GTFs that play roles in pollen tube growth and embryogenesis. The pollen-expressed AtTFIIB1 shares 86% and 44% similarities with AtTFIIB2 and AtTFIIB3/AtpBRP2 respectively (Zhou et al., 2013, J Exp Bot. 64:2205-2218). Mutations in AtTFIIB1 caused a drastic retardation of pollen tube growth and endosperm development, as well as impaired pollen tube guidance and reception, leading to disruption of fertilization and seed development. Pollen-Expressed Transcription Factor 2 (PTF2) shares a lower amino acid sequence similarity with other known TFIIB and TFIIB-related proteins in Arabidopsis (Niu et al., 2013, Mol Plant 6:1091-1108). It can interact with TATA-box binding protein 2 (TBP2) and bind to the double-stranded DNA (dsDNA). In addition, PTF2 can form a homodimer and also interact with the subunits of RNA polymerases (RNAPs), implying that it may be involved in the RNAPs transcription. Mutation in PTF2 caused failure of pollen germination. Pollen-rescue revealed that the ptf2 mutation also disrupted embryogenesis and resulted in seed abortion. These results suggest that the B-type GTFs plays crucial roles in pollen tube growth and seed development in Arabidopsis, possibly through interaction with TBP and the subunits of RNAPs. (1These authors contributed equally to this work. #To whom correspondence should be addressed: De Ye, fax +86-10-62734839, e-mail: yede@cau.edu.cn.)

PS6-3

Storage lipid mobilization pathways during pollen germination in *Olea europaea*

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Organelles called oil bodies (OBs), which are gradually mobilized during pollen germination. Our current knowledge regarding the molecular mechanisms that regulate OB mobilization in the pollen tube is scarce. In this work, we characterized several OB-associated enzymes involved in storage lipid breakdown. First, we showed that phospholipase A (PLA) and peroxygenase (PX) proteins were early incorporated during OB biogenesis, whereas acyl lipase (LIP) and lipoxygenase (LOX) enzymes were recruited after pollen germination. Drug inhibition of LIP and LOX enzymes hampered both pollen germination and pollen tube growth, leading to a characteristic accumulation pattern of OBs at the germinative aperture. We propose that two independent pathways involving LIP and LOX/PX enzymes, respectively, are responsible for early mobilization of neutral lipids in the pollen tube, while PLAs may promote the access of these enzymes to the OB matrix. Moreover, pollen performance in vitro was not affected under sugar-deprivation conditions but OB-associated enzyme activities were enhanced and OB mobilization was sped up. These data suggest that OBs are sufficient to promote the autonomous growth of the olive pollen tube at the onset of germination, while sugar availability may modulate the activity of these degrading enzymes.

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PS6-4

Involvement of pectin methylesterase during imbibition and germination of *Arabidopsis* pollen grain

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Sexual plant reproduction involves the growth of tip-polarized pollen tubes through the female tissues in order to deliver the two sperm cells to the ovule. The pollen grain is composed of two cell wall layers: the intine, composed of complex polysaccharides including homogalacturonan (HG), and the exine. It is assumed that the degradation of the intine wall is of main importance to insure the proper pollen germination. The modulation of the degree of HG methylester is regulated in the cell wall by the action of Pectin methylesterases (PMEs). Variations of the cell wall stiffness, which are likely to be related to the level of HG methylesterification has been proposed to participate in the tube growth. The Arabidopsis genome contains 66 genes coding for putative PMEs and 14 PME transcripts are specifically expressed in the pollen grain and/or the pollen tube. Disruption in VANGUARD1 and PPME1 showed a reduction of the pollen tube growth [1,2]. In order to understand how other PMEs act during pollen germination and/or pollen tube growth, we have selected one mutant line (knock-out mutant for PME48). PME48 is expressed in dry pollen grains and pollen tubes. pme48 pollen displayed a strong delay in the germination process compared to wild-type pollen and showed remarkable phenotypes with multiple pollen tube tips emerging from the pollen grain apertures. The data suggest that in wild-type pollen grains, (1) PME48 acts before the dehydration step of the pollen grains during pollen maturation and (2) a low level of HG methylesterification in the intine is a prerequisite to ensure fast and proper pollen grain imbibition and germination as low methylesterified HGs, more hydrophilic, can also be the target of polygalacturonases and/or pectate lyases.

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PS6-5

The cell wall pectic polymer rhamnogalacturonan-II is required for proper pollen tube elongation: implication of a putative sialyltransferase-like protein

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Rhamnogalacturonan type-II (RG-II) is known to be the most complex pectic polysaccharide present in the primary cell wall of all land plants [1]. Although present as a minor component, its role in cell wall strengthening is undoubtedly necessary. RG-II exists in the cell wall predominantly as a dimer that is cross-linked by a borate di-ester between two apiosyl residues, as such, cell wall, and most probably RG-II is a sink for boron. Pollen of most plant species requires boron to germinate [2] and boron is essential to control the mechanical properties of the cell wall [3]. RG-II has been detected in pollen tubes using immunocytochemistry [4, 5] and GC-EIMS [5]. Even though the RG-II structure and in muro dimerization have not been investigated in pollen tubes, we postulate that boron-induced RG-II cross-linking is crucial for pollen tube germination and/or elongation. Through a bioinformatic study, Voxeur and co-workers [6] identified 24 new putative glycosyltransferases possibly involved in the Arabidopsis RG-II biosynthesis. Using pollen tubes as a model, we studied the effect of At3g48820 down expression, predicted to encode a sialyltransferase-like protein, possibly involved in the transfer of Kdo and/or Dha on the HG backbone of RG-II. Analyses of two heterozygous lines revealed a strong reduction in pollen germination and pollen tube growth in vitro and in vivo suggesting that sialyltransferase-like proteins, and so the RG-II integrity, are required for the proper pollen tube growth.

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PS6-6

An AGP gene, BcMF8, involved in pollen development in Brassica campestris

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Background and Aims: The arabinogalactan protein (AGP) gene family is involved in plant reproduction. However, little is known about the function of individual AGP gene in pollen development and pollen tube growth. In this study, Brassica campestris male fertility 8 (BcMF8), a putative pollen-specific AGP-encoding gene was isolated from Brassica. campestris ssp. chinensis and the function was investigated.
Methods: RT-PCR and in situ hybridization were used to analyze the expression of BcMF8. Prokaryotic expression and western blots were used to ensure that BcMF8 could encode a protein. Antisense RNA technology was applied to silence gene expression, while morphological and cytological approaches (e.g. scanning electron microscopy and transmission electron microscopy) were used to reveal abnormal phenotypes caused by gene silencing. Homology cloning was used to isolate the homolog of BcMF8 in species from genus Brassica, genus Raphanus and genus Orychophrogmus of family Cruciferae.
Key Results: BcMF8 encoded a putative AGP protein, which was located in the cell wall, and started to express at the uninucleate stage and maintained the expression till the pollen tubes at pollination stage. The functional interruption of BcMF8 resulted in slipper-shaped and bilaterally sunken pollen with abnormal intine development and aperture formation, led to a significant decrease in pollen germination in vitro, while in those who did germinate, the pollen tubes were abnormally shaped and burst frequently, which corresponded to the in vivo arrest of pollen germination at the stigma surface and retarded pollen tube growth in the stylar transmitting tissues. Sequence analysis showed BcMF8 was highly conserved in family Cruciferae.
Conclusions: BcMF8 has a crucial function in modulating the physical nature of the pollen wall and in maintaining the integrity of the pollen tube wall matrix.
Key words: AGP, arabinogalactan proteins, Brassica campestris, Chinese cabbage, aperture, intine, pollen tube, pollen wall development, aperture formation.

PS6-7

Evolution and expression analysis of polygalacturonase gene family in *Brassica campestris ssp. Chinensis*

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The present research analyzed the constituents, locations, structures, evolution stories and expression patterns of PG genes in *Brassica campestris* L. *ssp. chinensis*. The evidence from multiple sequences anlignment, domain and evolution analysis indicated that the PG gene families can be classified into 7 groups, namely group A to group G. Orthological and syntenic analysis of PG genes from B. campestris and Arabidopsis, together with comparative analysis of gene retention rates demonstrated that PGs in group B and C as well as endo-PGs show higher retention rate than the others. The expression analysis of PG genes in *B. campestris* incicated that different groups of PG gene family show different expression patterns, as most of the genes from group D have a specific expression in inflorescence or stamen; many genes from group A are expressed in the development of siliques and fruits; and most of the genes from groups B, C, F, E seem to be expressed in all the tissues.

Key words Brassica campestris, polygalacturonase, evolution, expression pattern

PS6-8

The effects of hydrogen peroxide on *Nicotiana tabacum* L. pollen tube protoplasts

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The role of H2O2 in sexual plant reproduction is poorly understood. Endogenous ROS production is crucial for pollen germination and tube growth; H2O2 is also accumulated in pistil tissues awaiting pollination. These facts point towards the possible signaling role of hydrogen peroxide during pollen germination in vivo. We have made a first step to test this hypothesis by performing experiments with tobacco pollen protoplasts in vitro.

We have observed a rapid increase in [Ca2+]cyt after H2O2 addition (10 μM), measured by Fluo-3 staining. Ca2+-channel inhibitor nifedipine (100 μM) abolished this effect, indicating that Ca2+ influx is carried by plasmalemmal Ca2+-channels. Since these channels can be voltage-gated, we assumed that H2O2-induced Ca2+ influx can be associated with membrane potential shifts.

Therefore, we have used voltage-sensitive dye Di-4-ANEPPS to record membrane potential dynamics during H2O2 treatment. We have observed membrane hyperpolarization occurring at the same time as [Ca2+]cyt increase. These data was confirmed by measurement of absolute membrane potential values using the DiBAC4(3) staining: it has also revealed H2O2-induced hyperpolarization in a large protoplast population.

The two observed membrane effects could cause various delayed physiological changes. One of the key processes determining pollen tube growth rate is cell wall assembly. We have found that H2O2–treated protoplasts were able to regenerate cell wall significantly faster than the control ones. Nifedipine removed this effect, indicating that elevation of [Ca2+]cyt is crucial for H2O2–mediated acceleration of pollen wall synthesis.

Thus, rapid and delayed effects of H2O2 revealed in this study point towards the possible role of ROS in exogenous signal transduction in growing pollen tubes. The key targets for H2O2 are plasmalemmal ion-transport systems, which in turn activate the cell wall assembly.

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PS6-9

Factors affecting *in vitro* pollen germination and pollen tube growth of elderberry (*Sambucus nigra* L.)

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The development of biotechnological methodologies based on in vitro elderberry Sambucus nigra L. pollen treatments would be of particular interest for plant breeding approaches, in order to influence its pharmacological properties and/or culinary value. The main objective of our research was therefore to develop techniques for in vitro germination of S. nigra mature pollen. Optimization of germination procedure was well established by modification of Brewbary and Kwak medium (BK). Effect of stirring on a rotary shaker during incubation was found to be highly efficient. Using this method, a similar germination rate as obtained in 20 hours without stirring was achieved in just one hour. In these conditions, germination rate and pollen tube length was assessed to evaluate the choice of carbohydrates (different combination of sucrose and maltose). Studies related to the optimization of the mature pollen in vitro germination procedure were found to be very useful for identifying an appropriate maturation medium.

Keywords: elderberry (Sambucus nigra), in vitro, germination, pollen

PS6-10

Production of reactive oxygen species by NADPH oxidase in olive during pollen

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Among the large number of signaling mechanisms involved in plant reproductive processes, ROS and redox metabolism are emerging as a key elements controlling numerous cell to cell interactions. NADPH oxidase (NOX) enzymes are a family of transmembrane proteins able to transport electrons across the membrane from a cytosolic electron donor to oxygen, generating superoxide radical. Their activity seems to be involved in the ROS production in plant reproductive tissues and it has been particularly demonstrated to be essential for pollen tube growth [1].
In this study we determine the occurrence of NOX activity in the reproductive tissues of the olive (Olea europaea L.), an agronomically important tree in the Mediterranean basin. Focusing on the pollen tube growth, we have identified a Rboh-type gene (OeNOX1), mainly expressed in the mature pollen grain and during anther development. Transfection of olive pollen tubes with OeNOX1-specific antisense ODNs showed that the oxidative burst in olive pollen tip mainly depends on NADPH oxidase and that this activity is essential for proper pollen tube growth. As regard to the regulation of pollen NOX activity in vitro, calcium ions and the signalling phospholipid Phosphatidic Acid (PA) seem to positively stimulate such activity, possibly in a synergistically way. This activation is also affected by small GTPases. These data suggest a conserved mechanism for pollen NOX regulation among different species [2].

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PS6-11

Cellular localization and quantitation of glutathione during olive (*Olea europaea* L.) pollen development and germination

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Glutathione performs a crucial role as an antioxidant against biotic and abiotic stresses, although it also contributes to redox signaling, modulation of gene expression and regulation of the activities of various enzymes. Further, it is considered a low molecular weight metabolite essential for plant growth and development.
Glutathione involvement in plant reproduction recently began to be characterized by the study of mutants deficient in several key enzymes (Zechmann B, Koffler BE, Russell SD. 2011. BMC Plant Biology). Cellular localization of glutathione has been successfully analyzed by TEM immunolocalization or by derivatization to fluorescent components and fluorescence microscopy localization in various plant sources (Zechmann B, Stumpe M, Mauch F. 2011. Planta). In this work, we utilized a commercial antibody to glutathione to define tissue and subcellular localization of glutathione during microsporocite development and pollen germination in olive (Olea europaea L.). These studies revealed the extensive presence of glutathione in numerous and different subcellular locations like plastids, mitochondria, the cytosol, the nucleus, the apertural regions of the pollen grain and the pollen tube. Also, glutathione was present in other tissues of the anther (including the tapetum).
In order to further assess the relevance of glutathione metabolism in reproductive tissues, we used a recently developed LC-ES/MS analysis (Morad Airaki, Lourdes Sánchez-Moreno, Marina Leterrier, Juan B. Barroso, José M. Palma and Francisco J. Corpas. 2011. Plant Cell Physiol.) to detect and quantify GSH, GSSG, GSNO and ascorbate in olive pollen. The results show that this metabolite in its different forms was patent throughout the whole developmental course, and in the pollen grain and the elongating pollen tube with an extensive distribution, thus suggesting a key role for this component in pollen metabolism and physiology, as it has been determined already for somatic tissues.

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PS6-12

The effect of temperature on papaya *in vitro* pollen germination

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Papaya (Carica papaya L.) is a very sensitive species concerning to temperature, especially during the reproductive phase, since that it is common to observe sexual reversion of its flowers (hermaphrodite to male) during summer season. Considering that, this research was set up in order to determine the effects of temperature on papaya pollen germination and to estimate the cardinal temperatures for two genotypes, the elite line JS12 and UENF/CALIMAN01, a hybrid from the elite line. In order to reach the objectives, first, it was set up an experiment to determine the better medium for in vitro pollen germination. So, pollen grains from the genotypes, JS12 and UENF/CALIMAN01, were dust on artificial growth medium consisted of sucrose (0, 5, 10, and 15%) and boric acid (0; 7.5, and 15ppm), at room temperature for six hours. Significant differences were observed at 1% of probability for all sources of variation, except the interaction genotype x boric acid. The medium composed by sucrose 5% and boric acid 15ppm gave the best pollen germination percentage, 75.4% and 82.3% for JS12 and UENF/CALIMAN01, respectively. The second experiment aimed to study the effect of temperature on in vitro pollen germination. Pollen grains from plants growth on the field of UENF/CALIMAN01 and JS12 were sprinkled on germination medium (5% sucrose/15ppm boric acid) and incubated, for four hours, in six temperatures ranging from 15 to 40ºC at 5ºC intervals. Significant differences on pollen germination percentage were observed at 1% probability for genotype, temperature and genotype x temperature. At low temperature (15ºC) few pollen grains germinated, and the highest pollen germination was reached at 30°C, with mean germination of 81.2% and 78.0% for JS12 and UENF/CALIMAN01, respectively; above this temperature, the germination rates decreased. The mean cardinal temperatures (Tmin, Topt, Tmax) for pollen germination were 15.9ºC, 27.9ºC and 39.9ºC for JS12 and 16.5ºC, 28.5ºC and 40.3ºC for UENF/CALIMAN01.

PS6-13

Polyamine effects on apical growth of the pollen tube

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Pollen tube growth is a rapid process restricted to the only tip region. Many factors cooperate to allow this apical growth, creating an intricate signalling network. The continuous rebuilding of the cell wall and apical migration of the cytoplasm sustained by cytoskeleton re-organisation are the most important driving forces needed for growth1, but many other factors are involved in this process, among which polyamines (PAs), that are essential during pollen tube emergence2 and ROS, that support the apical growth, at physiological concentration3. We investigated the effect of natural and synthetic PAs on the apical growth of Pyrus communis pollen tube and observed that among the natural PAs, spermine (Spm) inhibited the growth from 10 mM onwards. Among the synthetic ones, BD23, an aromatic derivative of Spm also showed similar effects. Thanks to a FITC-labelled Spm we were able to observe, that PAs enter through the pollen tube tip, then diffuse in the sub-apical region. The same region underwent drastic morphological changes, showing loss of polarity and enlarged tip when Spm and BD23 were supplied at 100 mM or higher. The effects of PAs were related, at least in part to their ability to act as ROS scavengers of both O_2^- and H_2O_2 in the apical zone, probably disrupting the balance between ROS species that affect cell-wall relaxation or stiffening. Consequence of this redox state perturbation was a decrease in pollen viability, stimulation of DNA-laddering after 30 minutes incubation with 500 mM of both PAs and, as final result, the complete degradation of both vegetative and generative nuclei as shown by DAPI labelling of pollen tube. The degradation of nuclear DNA was inhibited when pollen had been pretreated with the caspase-3 inhibitor I peptide, Ac-DEVD-CHO (DEVD).

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PS6-14

Studies of biogenesis of small noncoding RNAs in *Hyacinthus orientalis* L. male gametophyte

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Small noncoding RNA (sRNAs) have emerged as important factors in regulation of gene expression involved in many biological processes in plants such genome stability, development and adaptive responses to biotic and abiotic stresses. They belong to two classes micro RNA (miRNAs) and short interfering RNA (siRNAs) and act by silencing gene expression on post-transcriptional (PTGS) or transcriptional level (TGS). Additionally, sRNA plays an important role in some cases such regulations were followed by DNA methylation and chromatin remodeling. Our knowledge of small RNA biogenesis and mechanisms of action has dynamically expanded in the past decade but their functions in regulation of sexual plant reproduction is still not fully understood. In the present study we examined the spatial and temporal distribution of the molecules involved in the biogenesis of the small noncoding RNAs: AGO1 (Argonaute 1), HYL1 (Hyponastic Leaves 1) and AGO4 (Argonaute 4) in mature pollen grain and in vitro growing pollen tubes of Hyacinthus orientalis. AGO1 and HYL1 are specifically associated with miRNA pathway. AGO4 is involved in siRNA gene silencing and in RNA-directed DNA methylation. Using immunofluorescence techniques we have shown changes in the distribution of examined antigens in male gametophyte cells. Different localization of AGO1, HYL1 and AGO4 were observed in mature pollen grain. Also the changes in the pattern of the labelling were visible during pollen tube growth in the vegetative and generative nuclei and after division the generative cell in sperm cells in the pollen tube after 8-12 h of growth in medium. In summary, our observations in H. orientalis indicate that the processes including sRNAs take place and play important role in regulation of gene expression in these cells during male gametophyte development and probably fertilization.
The research was supported by the National Science Centre (NCN) grant 2011/03/D/NZ3/00603

PS6-15

Characterization of two *Pollen Specific Kinase* coding genes from *Arabidopsis thaliana*

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Pollen grains are the male gametophyte of plants and thus are essential for plant reproduction and productivity. However, despite their biological and agronomical importance, little is known about the molecular mechanisms that regulate its development and function. In Arabidopsis, three cells compose mature pollen grains: a large vegetative cell and two small sperm cells engulfed in the cytoplasm of the vegetative cell. During fertilization, the vegetative cell must germinate and produce a pollen tube, a growing tip structure that directionally transports the sperm cells to the ovule to produce the double fertilization event. Currently, little is known about signal transduction pathways and molecular components involved in these processes. Using microarray data we have previously identified 2 genes encoding kinases proteins (PSK2 and PSK4, for POLLEN SPECIFIC KINASE) that are expressed exclusively during the last stages of pollen development, germination and tube elongation. We have analyzed the promoter activity of each PSK gene using GUS as reporter gene. To analyze the physiological relevance of PSK2 and PSK4, we have generated transgenic plants expressing specific amiRNAs for these genes under the control of a pollen-specific promoter (LAT52) and we have analyzed pollen development and tube elongation in insertional mutants and also in transgenic plants expressing amiRNAs. Tube guidance was affected in PSK2 mutant plants, suggesting a role for this protein in male-female interaction. On the other hand, pollen tubes from PSK4 mutant and amiRNA silenced plants lack of a callose plugs. Also, we have determined the sub-cellular localization of each kinase protein using 35S:PSK:GFP constructions in agroinfiltration experiments in tobacco leaves. Our results suggest that both proteins have an important role for pollen tube elongation and guidance in Arabidopsis thaliana. Funded by Fondecyt 1120766 and UNAB DI-74-12/R.

PS6-16

The calcineurin B-like Ca^{2+} sensors CBL1 and CBL9 function in pollen germination and tube growth in *Arabidopsis*

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Ca2+ has been established as an important second messenger regulating pollen germination and tube
growth. Ca2+ forms a concentration gradient across the length of the pollen tube, with its concentration being high-est at the tip and gradually decreasing towards the shank. The presence of a Ca2+ gradient in growing pollen tubes represents information to the cell which demands the existence of Ca2+ decoding mechanisms by Ca2+ binding proteins. However, to date, only a few signaling components have been identified to decode and relay Ca2+ signals in growing pollen tubes.
Here, we report a function for the calcineurin B-like (CBL) Ca2+ sensor proteins CBL1 and CBL9 from Arabidopsis in pollen germination and tube growth. CBL1 and CBL9 are expressed in mature pollen and pollen tubes and impair pollen tube growth and morphology if transiently expressed in tobacco. The induction of these phenotypes requires efficient plasma membrane targeting of CBL1 and is independent of Ca2+ binding to the fourth EF-hand of CBL1. Overexpression of CBL1 or its closest homolog CBL9 in Arabidopsis renders pollen germination and tube growth hypersensitive towards high external K+ concentrations while disruption of CBL1 and CBL9 reduces pollen tube growth under low K+ conditions.
These findings indicate that a faithfully regulated accumulation level of CBL1 and CBL9 at the plasma membrane is crucial for efficient implementation of pollen germination and polar tube growth. Moreover, these data suggest a function of CBL1 and CBL9 in the regulation of ion homeostasis in pollen and pollen tubes.

PS6-17

Glutamate receptors in the pollen of Arabidopsis thaliana - On the calcium branch

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The genome of Arabidopis thaliana contains 20 genes homologous to mammalian ionotropic Glutamate Receptors (iGluRs), denominated glutamate receptor-like genes (GLRs). Some of the GLRs occur in different splicing variants and - as their mammalian counterparts - are likely to oligomerize, therefore increasing the number of possible functional channel units. Plant GLRs group into three clades and show a broad, overlapping expression pattern with no obvious preferential tissue expression.
Functional redundancy and genetic compensation is therefore likely to occur. Indeed, inactivation of single GLR genes often only causes mild macroscopic phenotypes.
We therefore generated plants lacking up to four GLR genes and studied their functional and phenotypic characteristics, along with their sub-cellular localization. Since it allows for studying their implication in reproduction, we focused on pollen-abundant GLRs.
In vitro germination of pollen from all generated knock-out lines yielded in an increased number of branching pollen tubes with significantly lower growth rates when compared to wild type control pollen, especially upon Ca2+ deprivation.
In accordance with the Ca2+ conductance of GLRs, vibrating probe measurements in pollen tubes of GLR knock-out plants revealed decreased fluxes of Ca2+ at the tip of the growing tube.
We are currently analyzing sub-cellular expression patterns of members from all three clades in order to deduce possible clade-specific functions, potential patterns in oligomerization and localization.
For the latter, we recently identified a new class of proteins that interact with GLR's and are necessary for their proper trafficking.

PS6-18

Small molecules interfere with the tip-polarized growth of Arabidopsis and tomato pollen tubes

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Sexual plant reproduction is of economic importance because it allows the production of seeds that are used directly or after processing for human or animal consumption, biofuel production and many other applications. During this process, pollen grains land on the stigma, rehydrate and produce pollen tubes that grow through the female transmitting tract tissue. Pollen tubes perceive different signals promoting their adhesion and guidance that allow a correct delivery of the male gametes to the ovule and ensures the proper fertilization. Using a chemical screen approach of 300 small bioactive molecules, 8 compounds were selected for their abilities to perturb *Arabidopsis thaliana* and *Solanum lycopersicum* pollen tube growth and shapes in a dose-dependent manner (from 0.1 to 30 µM) and for their inhibiting effects on the adhesion of pollen tubes onto an *in vitro* adhesion matrix. Here, we present preliminary data on the phenotype observed after treatment with these compounds with a focus on pollen germination, pollen tube growth and shape as well as immuno-localization of cell wall polysaccharides on treated pollen tubes

PS7-1

Does cell specific hormone action determine female attractiveness?

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In flowering plants, sperm cells are transported by pollen tubes. While initially a large group of pollen tubes sets out to target ovules, typically only a single pollen tube arrives in the female gametophyte. The restriction of pollen tube number requires fertilization-dependent degeneration of two pollen tube attracting synergids. We have previously shown that ein3 eil1 mu-tants, which are hyposensitive for the gaseous hormone ethylene, attract supernumerary pollen tubes. The defect traces back to a failure of one of the synergids to undergo programmed cell death. This results points towards a key role of ethylene in the establishment of a pollen tube block.
To further investigate the molecular mechanisms underlying ethylene dependent synergid degeneration, we follow two differ-ent approaches. First, we identify the sites of ethylene production by determining the transcriptional dynamics of ethylene biosynthesis genes in the process of fertilization. Second, we aim at modulating ethylene content in a tissue and cell-type specific manner. Here, we discuss our results and potential implications on ethylene dependent pollen tube block regulation

PS7-2

Two-photon imaging of pollen tube growth and guidance in the pistil

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The plant fertilization process including pistil–pollen interactions provides an attractive model for fundamental study of female–male communication in plants. Especially, guidance of the pollen tube (male) to the embryo sac (female) is an important for successful fertilization in flowering plants. However, the actual molecular mechanisms of pollen tube guidance remain largely unknown because the process of pollen tube guidance occurs in the deep inside the female tissues, pistil.

Two-photon microscopy provides non-invasive tool for deep imaging in living organisms. To observe the process of pollen tube guidance in the pistil, we established live-cell imaging methods using two-photon microscopy. We visualized pollen tubes, synergid cells and other female tissues by fluorescent proteins in Arabidopsis thaliana.

Two-photon microscopy reveals pollen tube growth inside the transmitting tract of the pistil. We also observed that pollen tubes emerged on the surface of septum, and one and more pollen tubes were guided to each ovule. Subsequently, we observed that pollen tube enters into the embryo sac, and pollen tube discharges its contents in the embryo sac. Finally, we succeeded in continuously observing the pollen tube growth, guidance and discharge from 1 to 24 hours after pollination.

We will discuss the differences among pollen tubes in the pistil comparing with pollen tubes under the conditions in vitro. We will also discuss the pollen tube competition and the mechanism of block to polyspermy.

PS7-3

Dynamic redistribution of MLO proteins during pollen tube reception

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Plants have evolved elaborate signaling mechanisms to insure that sperm cells are delivered to the female gametophyte during sexual reproduction. During pollination, a pollen grain is recognized at the stigma and germinates to produce a pollen tube that grows through the style and transmitting tract and finally into the micropyle of the ovule, attracted by a signal produced by the female gametophyte. The final step of pollination is the reception of the pollen tube at one of the synergid cells that flank the egg cell followed by the cessation of pollen tube growth and rupture to release the sperm so that double fertilization can occur and viable seeds can be produced. In Arabidopsis thaliana feronia (fer) and nortia (nta) mutants, cell-to-cell communication at early stages of pollination is normal, but upon reaching the synergid the pollen tubes continue to grow instead of bursting to release the sperm, leading to infertility. Both FER, a CrRLK1L family receptor-like kinase, and MULTIPLE RESISTANCE LOCUS-O (MLO) proteins related to NTA have been shown to be involved in fungal invasion of plant epidermal cells, indicating that mechanisms for penetration of plant cells by tip-growing pollen tubes and fungal hyphae may have been conserved over evolution. In both cases, the MLO protein becomes redistributed to the site of interaction with a tip-growing cell (pollen tube or fungal hyphae). FER activity is necessary for the redistribution of NTA protein to the site of pollen tube entry into the synergid, but little else is known about the molecular mechanisms involved in this pollen tube-synergid communication system. Progress toward understanding the mechanism behind pollen tube-triggered redistribution of NTA to the filiform apparatus of synergid cells will be presented.

PS7-4

Relationship between karyogamy progression and onset of *de novo* gene expression in rice zygotes produced by *in vitro*

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The conversion of an egg cell into a zygote involves two sequential gametic processes: plasmogamy, the fusion of the plasma membranes of male and female gametes, and karyogamy, the fusion of the gametic nuclei. Karyogamy involves the approach and fusion of the male and female nuclei in a zygote. Nascent synthesis of mRNAs and proteins from the zygotic genomes has been reported to be initiated either during karyogamy or within hours after fertilization in some plants, including maize, Arabidopsis and tobacco. However, a relationship between a precise karyogamic stage and the onset of de novo gene expression in the zygote was not clearly presented.
For identification of de novo synthesized genes, cell-type-specific transcriptomes were successfully obtained by microarray analyses for egg cells, sperm cells and zygotes isolated from rice flowers, and up- or down-regulated genes in zygotes after fertilization were identified as well as genes enriched in male and female gametes. Then, in order to observe karyogamy progression in the zygotes, rice gametes expressing histone H2B-GFP/RFP or SUN2-GFP were fused in vitro and the resultant zygotes were monitored. The results indicated that the sperm nucleus migrates adjacent to the egg nucleus via an actin cytoskelton, and the egg chromatin then spreads unidirectionally into the sperm nucleus via a possible nuclear bridge. Thereafter, sperm chromatin begins to decondense to complete karyogamy. The development of early rice zygotes from plasmogamy to karyogamy was divided into eight stages, and RT-PCR analyses for several up-regulated genes in zygotes during the early zygote development suggested that possible paternal and de novo synthesized transcripts were separately detected in zygotes at early and late karyogamy stages, respectively.

PS7-5

Dynamics of male and female chromatin during karyogamy in rice zygotes

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In angiosperms, the conversion of an egg cell into a zygote involves two sequential gametic processes: plasmogamy, the fusion of the plasma membranes of male and female gametes, and karyogamy, the fusion of the gametic nuclei. To investigate the mechanisms of gametic and/or early zygotic development, we previously established a procedure to isolate rice gametes and an in vitro fertilization (IVF) system to produce zygotes that can develop into fertile plants. In the present study, zygotes were prepared by IVF of rice gametes heterologously expressing histone H2B-GFP/RFP and SUN2-GFP, which labeled the nuclei and nuclear membranes, respectively, and the dynamics of karyogamy in the rice zygotes were monitored. The results indicated that the sperm nucleus migrates adjacent to the egg nucleus 5 to 10 min after plasmogamy via an actin cytoskelton. Then the egg chromatin spreads unidirectionally into the sperm nucleus via a possible nuclear bridge, possibly resulting in enlargement of the sperm nucleus, and 30 to 70 min after fusion the sperm chromatin begins to decondense with the complementation of karyogamy. Based on the present observations, the development of early rice zygotes from plasmogamy to karyogamy was divided into eight stages. Moreover, by RT-PCR analyses for several genes which have been identified as up-regulated genes in zygote after fertilization, paternal and de novo synthesized transcripts were separately detected in zygotes at early and late karyogamy stages, respectively.

PS7-6

Role of Arabidopsis pollen coat CRPs in reproductive signalling

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The process of sexual reproduction in plants is strictly regulated by molecular communication between cells of male and female reproductive structures and gametes. A diverse class of small cysteine-rich proteins (CRPs) has been identified as having multiple roles in pollination, including the early stages of the pollen-stigma interaction. The secreted CRPs found in the pollen coat of members of the Brassicaceae, the pollen coat proteins (PCPs), are emerging as important regulators of this process. Previous studies have shown that members of the PCP-B class in Arabidopsis thaliana play a central role in pollen hydration, a crucial regulatory checkpoint during the early events of pollination. The PCP-Bs, encoded by only four genes in Arabidopsis, are likely to be ligands for as yet unidentified receptors, which is the case for most CRPs characterised to date. In this project all four Arabidopsis PCP-Bs have been heterologously expressed in E.coli for use in both biochemical and cell biological experiments aimed at understanding their precise roles in pollination. Ongoing work focuses on identifying stigmatic targets for the PCP-Bs along with functional analyses utilizing T-DNA knockout / RNAi lines. Confocal microscopy will also be utilised to uncover early stigmatic cellular responses to PCP-Bs. This study will shed new light on the roles of PCP-B class CRPs in Arabidopsis reproductive signalling.

PS7-7

A genetic approach to identify novel components of gamete fusion mechanisms in Arabidopsis

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Fertilization occurs when the plasma membranes of two distinct gamete cells, a sperm cell and an egg cell, merge to produce a novel diploid zygote. Despite its fundamental importance, the mechanism of sperm-egg fusion is not well understood in any species. Since proteins involved in reproduction are expected to evolve rapidly and the process of membrane fusion is transient, it has proved difficult to identify gamete fusion proteins using traditional biochemical or homology-based approaches. Genetic analysis in Arabidopsis has revealed a single sperm-specific gene, HAP2(GCS1), that is essential for gamete fusion, conserved across many eukaryotic species, and represents an opportunity to define a mechanism for gamete fusion. To identify new genes that may participate in gamete fusion, we are characterizing putative mutations that restore gamete fusion to mutant sperm expressing only a HAP2(GCS1) hypomorph. he initial EMS screen provided 20 candidate suppressors of the HAP2(GCS1) hypomorph phenotype. Self-fertilization events as well as male and female-specific crosses were analyzed to determine whether the suppressor mutation is specific to male gametes, female gametes, or expected to affect both gametes. These studies will allow us to prioritize extragenic suppressors for gene identification using next-generation sequencing. Genes identified from this screen will help elucidate the mechanism of HAP2(GCS1)-mediated gamete fusion in A. thaliana and will provide insights into HAP2/GCS1 function in other eukaryotic species.

PS7-8

Molecular mechanism controlling pollen tube attraction toward AtLURE1 peptide

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Pollen tube guidance is an essential mechanism for successful fertilization, which is achieved by a series of male-female interactions. Despite the importance of the guidance mechanism in plant reproduction, little is known about molecular mechanisms underlying pollen tube guidance. Previously, we have identified species-specific LURE peptides as key attractant molecules in the final step of pollen tube guidance in Torenia fournieri (Okuda et al., 2009, Nature) and Arabidopsis thaliana (Takeuchi and Higashiyama, 2012, PLoS Biology). LURE peptides induce reorientation of pollen tube tip growth toward the peptides. However, little is known about molecular mechanism for LURE reception and signal transduction. Here, we show our progress on searching for candidate receptor molecules involved in AtLURE1 signaling in A. thaliana pollen tube. Candidate receptor molecules were assumed to be transmembrane proteins expressed in the pollen tube because the LURE peptide was bound to the surface of the tip of growing pollen tubes (Okuda et al., 2013, Molecular Plant). We searched for the candidates by using expression data and analyzed them by genetic approaches. We also analyzed dynamics of intracellular molecules which could act downstream of AtLURE1 reception. We will propose a model for molecular mechanism of pollen tube reorientation induced by the LURE attractant peptide.

PS7-9

Live-cell analysis of gamete communication during double fertilization in *Arabidopsis thaliana* by laser disruption technique

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During double fertilization in angiosperms, two sperm cells delivered by a pollen tube are released into an embryo sac and fertilize an egg cell and a central cell, followed by embryo and endosperm development. Double fertilization is not only biologically but also agriculturally an important phenomenon because it serves our staple foods. Live-cell observation during double fertilization process is essential to study double fertilization mechanism. However, it had been challenging for a long time because double fertilization occurs in the deep of flowers. Recently, live-cell imaging of double fertilization process in Arabidopsis thaliana was achieved by semi-in vivo fertilization system (Hamamura et al., Curr. Biol., 2011). It was shown that released sperm cells had stationary phase in the region between the egg and central cell before sperm-egg and sperm-central cell fusions occur. But, it still remains unknown how two sperm cells precisely decide the fertilization targets there.
In this study, we established egg or central cell-specific disruption technique with femtosecond pulse laser of the multi-photon microscopy and performed live-cell imaging of the behavior of two sperm cells in ovules with one female gamete disrupted. In some cases, single fertilization of the central cell was observed in the egg cell-disrupted ovules, indicating that a central cell might have the mechanism to fertilize with only one sperm cell (i.e. polyspermy block) independently of an egg cell. We used a plasma membrane marker line to check the degree of the cell-disruption and found that the level of laser damage on the egg or central cell membrane might be related to the fertilization patterns of released sperm cells. In addition, we analyzed the position of released sperm cell using fertilization-defective mutants. We discuss the importance of the sperm cell position in the stationary phase for successful double fertilization.

PS7-10

Role of defensins/DEFLs during defence and reproduction in *Zea mays*

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Defensins as well as defensin-like proteins (DEFLs) are small cysteine-rich proteins (CRPs), which were first found in animal granulocytes with a critical function in the host defence response. They also occur as toxins of snakes, scorpions and honeybee. Recent studies demonstrated that gene families encoding CRPs are expanded in flowering plants and possess additional functions to pathogen defence. Especially plant DEFLs were shown to be involved in plant growth, development and defence against bacteria, fungi and insects, but more importantly they also play key roles in plant reproduction processes. While DEFLs LURE1 and LURE2, for example, are secreted from synergid cells for pollen tube attraction in Torenia (Okuda et al., 2009; Nature.), ZmES1-4 are secreted from the egg apparatus to induce pollen tube burst (Amien et al., 2010; PLoS Biol.). Until now there is no report for the function of male-specific DEFLs in plants or a report about the role of DEFLs during pollen-stigma interaction in the economically important grasses.
Among other CRPs we are foucussing in our lab on the functional role of two pollen-specific DEFLs in maize, named as Pollen Defensin1 (PDEF1) and Pollen Defensin2 (PDEF2). Based on our RNAseq data and expression profiles of Sekhon et al. (2011; Plant J.), these two DEFLs are highly and exclusively expressed in mature maize pollen and germinated pollen tube, indicating that they may have critical functions during pollen development, pollen tube growth and/or double fertilization. PDEF1/2 belong to different DEFL subgroups, but share similar features consisting of an N-terminal signal peptide and a Gamma-thionin/Knottins motif. We generated stable transgenic maize lines using RNAi technology to study their biological functions and PDEF1/2-GFP lines to investigate their subcellular localization and secretion. We also aim to investigate potential antimicrobial function(s) in vitro. We aim to elucidate the function(s) of these male-expressed DEFLs in maize and to identify their targets in future studies.

PS7-11

Analysis of vacuolar CBL-CIPK complexes and their contribution to pollen tube growth and plant fertility

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Calcium is an important second messenger in plants. It can be released from internal stores and imported from the apoplast in response to various stimuli like abiotic and biotic stresses as well as in developmental processes. The perception and signal transduction of such calcium signals happens through several decoding machineries, like for example the CBL-CIPK network. This network consists of calcium sensors (CBLs) and their interacting kinases (CIPKs) which can regulate adequate responses. Of the 26 Arabidopsis CIPKs, CIPK12 is the most strongly expressed in pollen which suggests a functional role of this kinase in regulation of pollen germination and/or pollen tube growth. CIPK12 and its closest homologue, CIPK19, both interact with the tonoplast-localized calcium sensors CBL2 and CBL3, which are also expressed in pollen. Analyses of T-DNA insertional mutant lines and transient overexpression studies in germinating tobacco pollen tubes strongly suggest that these vacuolar CBL-CIPK pairs contribute to proper pollen tube growth and fertilization in Arabidopsis.

PS7-12

Pollen Tube-in-a-Chip: A microfluidic devise for quantitative analysis of pollen tube guidance

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Plant sexual reproduction relies on successive interactions between female tissues and growing pollen tubes inside the pistil. Many factors have been reported to influence on pollen tube growth both in vivo and in vitro, including pollen tube attractant LURE proteins (Okuda et al., Nature, 2009). Because pollen tubes grow inside the pistil, it is difficult to evaluate the concentration and distribution of these attractants in vivo. To understand the function of pollen tube attractants quantitatively, we have developed a new microfluidic devise (Horade and Kanaoka et al., RSC Advances, 2013). This devise is made of biosafety PDMS resin. Pollen tube growth medium was filled in the flow channel in the devise 100μm in height. The main flow channel splits into a T-shaped channel and following reservoirs. When a pollinated Torenia fournieri pistil was placed at an entrance of a narrow flow channel and ovules were placed in one reservoir, pollen tubes grew into the channel connected to the ovule reservoir. When embryo sacs were killed with UV laser and these ovules were placed in one reservoir, pollen tubes evenly grew toward both channels. This result suggests that pollen tube attraction by ovules is embryo sac-dependent. Moreover, a fluorescent material injected in a reservoir made a gradient of fluorescence in the reservoir, indicating that this device is a good platform for analyzing pollen tube attractants in a concentration gradient manner.

PS7-13

Sperm activation during double fertilization: Identification of EC1 interactors on the sperm cell surface and downstream signaling events

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The delivery of two functional sperm cells into the female gametophyte and their fusion with two female reproductive cells (egg cell and central cell) is a characteristic feature of flowering plants, called double fertilization. Recently it has been shown that the rapid fusion of the two sperm cells with the egg and the central cell in Arabidopsis thaliana depends on a family of small cysteine-rich EC1 (EGG CELL1) proteins, which are secreted by the egg cell upon sperm cell arrival. Sperm cells released from pollen tubes respond to synthetic EC1 peptides and recombinant EC1 proteins by increased exocytosis, indicated by the shift of the potential gamete fusogen GCS1/HAP2 (GENERATIVE CELL SPECIFIC1/HAPLESS 2) from the endomembrane system to the cell surface. This suggests that Arabidopsis sperm cells are rapidly activated by egg cell-secreted EC1 when they arrive at the fusion site. However, nothing is known about the molecular mechanism of EC1-mediated sperm activation, or the nature of the downstream signaling components.
In order to identify EC1-interacting proteins on the sperm cell surface, and to investigate putative downstream signaling components involved in sperm cell activation, we established a protocol to isolate sperm cells from Zea mays. Here we will present our current data on the protein composition of sperm cell membranes as analyzed by high-throughput proteomics, our strategy to identify EC1-interacting proteins on the sperm cell membrane, and our in vitro sperm cell activation assays in which we are testing different second messengers as well as recombinant maize EC1.

PS7-14

Differential expression patterns of Arabinogalactan Proteins in *Arabidopsis thaliana* reproductive tissues

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Arabinogalactan proteins (AGPs) are heavily glycosylated proteins existing in all plant kingdom and differentially distributed through distinctive developmental stages. Here we show the individual distribution of specific *Arabidopsis* AGPs: AGP1, AGP9, AGP12, AGP15 and AGP23, throughout reproductive tissues and point out their possible roles in several reproductive processes. AGP genes specifically expressed in the female tissues were identified using available microarray data. This selection was confirmed by promoter analysis using multiple GFP fusions to a nuclear localization signal (NLS), GUS fusions, and *in situ* hybridization as an approach to confirm the AGPs expression patterns. Promoter analysis allowed the detection of a specific and differential presence of these proteins along the pathway followed by the pollen tube during its journey to reach the egg and the central cell inside the embryo sac. *AGP1* is expressed in the stigma, the style, the transmitting tract, the funiculus, and in the chalazal and funiculus tissues of the ovules. *AGP9* is present along the vasculature of the reproductive tissues and *AGP12* is expressed in the stigmatic cells, the chalazal and funiculus cells of the ovules, and the septum. *AGP15* is expressed in all pistil tissues, except in the transmitting tract, while *AGP23* is pollen grain and pollen tube specific. The expression pattern of these AGPs brings new and significant evidences for the detection of a subset of specific AGPs involved in plant reproductive processes, being of great significance for this field of study. AGPs are prominent candidates for male-female communication during reproduction.

PS7-15

Nanovesicles are secreted during pollen germination and pollen tube growth: a possible role in fertilization

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Earlier reports support the presence of exosome-like vesicles in plants. However, no data are available on exosome-like vesicle secretion during germination and pollen tube growth, despite the importance of vesicular trafficking in such events. In this study, we showed that fresh olive (Olea europaea) pollen grains release nanovesicles during in vitro pollen germination and pollen tube growth. We proposed the term pollensomes to designate these nanovesicles, which can be isolated from germination medium by ultracentrifugation. Pollensomes display typical exosome-like size and morphology, with a density on sucrose gradient of 1.24-1.29 g/ml. Proteomic analysis of pollensomes revealed proteins characteristic of exosomes, such as actin, heat shock protein 70 and glyceraldehyde 3-phosphate dehydrogenase. In addition, proteins associated with cell wall expansion (PME or Ole e 11), pollen hydration and pollen tube growth (e.g., Ole e 1), and defense/stress (e.g., PCBER or Ole e 12) were also detected. The presence of esterified pectin was also demonstrated. These findings suggest that pollensomes comprise a heterogeneous population of secretory vesicles, based on their molecular cargo. Interestingly, ultrastructural studies showed that Ole e 12 localized into vesicles of 200-600 nm highly enriched in the pollen aperture region and the pollen tube in germinating pollen grains. Our findings suggest that pollensomes may play an important role in pollen tube growth, with potential implications in fertilization. However, in the absence of specific markers, we cannot from our data assign pollensomes to canonical exosome-like vesicles.

PS8-1

Two Key-elements in BABY BOOM-mediated Somatic Embryogenesis

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BABY BOOM (BBM), is a member of the AINTEGUMENTA-LIKE (AIL) sub-clade of AP2/ERF transcription factors. AIL transcription factors are expressed in dividing tissues where they regulate stem cell niche specification and meristematic growth. BBM overexpression induces adventitious growth, including somatic embryogenesis (SE) and ectopic organ formation. Using a steroid-inducible (DEX, GR) system, we show that BBM overexpression phenotypes are both dosage- and context-dependent. CHIP-seq combined with expression analysis showed that BBM activates genes in the seed maturation (LEC1 and LEC2) and auxin biosynthesis pathway (TAA1, YUCCA). BBM-mediated SE is suppressed in the lec2 mutant, as well as by biochemical inhibition of the TAA1 and YUCCA auxin biosynthesis enzymes. Our results suggest that BBM induces SE by activation of seed maturation and auxin biosynthesis pathways.

PS8-2

Chromosome elimination in Triticeae or oat x pearl millet hybrid: centromere specific histone H3 (CENH3) dynamics in embryogenesis

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In crosses between wheat (Triticum aestivum L.) and subfamilies distant related species such as maize (Zea mays L.), sorghum (Sorghum bicolor L.) and pearl millet (Pennisetum glaucum L.), paternal non-wheat chromosomes are elimination during embryogenesis. We have used pearl millet to pollinate a range of the tribe Triticeae, as well as oat (Avena sativa L.). Seven days after pollination, the dynamics of pearl millet chromosomes in the embryos were analyzed by FISH using a pearl millet centromere-specific probe and GISH using labelled genomic DNA of pearl millet. In addition, we analyzed the centromere function using centromere histone H3 (CENH3)-specific anti-bodies which cross reacts with the active centromere of both parental species. In the Triticeae species x pearl millet cross, paternal pearl millet chromosomes were gradually eliminated from the hybrid embryo. Pearl millet chromo-somes showed chromosome rearrangements, non-disjunction and formation of micronuclei. On the other hand, in oat x pearl millet cross, paternal pearl millet chromosomes were not eliminated in the hybrid embryo. Embryo cul-ture confirmed the true hybrid nature of oat-pearl millet, but true hybrids showed light sensitive necrosis. It was revealed that the CENH3 encoded by the maternal genome of oat was incorporated in the centromeres of pearl millet during early embryogenesis. CENH3 gene activity of pearl millet was revealed in oat x pearl millet hybrid embryos. However, pearl millet CENH3 protein was not found in active centromeres of hybrid cells. Phylogenetic analysis of grass CENH3s revealed that the sequence of CENH3 histone-hold domain of the genes were more simi-lar between oat and peal millet than between wheat and millet. In oat x pearl millet cross, because of CENH3 his-tone hold domain similarity, pearl millet centromeres could localize oat CENH3 and form functional kinetochores, and stabilized pearl millet chromosomes in the embryogenesis despite subfamily distant related species hybrid.

PS8-3

NtCYS, a multifunctional player in plant PCD during embryogenesis

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Zygote first division gives rise to a larger basal cell and a smaller apical cell with a distinct developmental fate. The small apical cell will develop into the main body of the embryo proper, whereas the larger basal cell will mainly grow into a suspensor that goes through predetermined degeneration at late stages of embryo development. Critical roles of suspensor degeneration at certain time point and molecular mechanism underlying it are two core questions in the field of plant embryogenesis.We have previously proved that the suspensor degeneration is a typical process of programmed cell death (PCD) and discovered a basal cell exclusively located cysteine protease inhibitor, NtCYS, which exert its anti-cell death effect by directly inhibiting cathepsin H-like protease NtCP14 to protect the basal cell lineage from precocious activation of PCD in early embryogenesis. Thus, NtCYS-NtCP14 works as a molecular switch to control suspensor cell fate. Further study revealed that NtCYS has multifunctional role in embryogenesis and even in whole process of sexual plant reproduction. These works suggest that NtCYS is a key regulator for PCD during both developmental and stress-induced cell deaths.

PS8-4

DNA hypomethylation bypasses the interploidy hybridization barrier in Arabidopsis

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Plants of different ploidy levels are separated by a strong postzygotic hybridization barrier (triploid block) that is established in the endosperm and often results in non-viable offspring. Deregulated parent-of-origin specific genes are causal for the response to interploidy hybridizations, revealing an epigenetic basis of this phenomenon. We found evidence that in paternal excess hybridisations hypomethylation of the paternal genome can bypass the interploidy hybridization barrier by alleviating the requirement of the epigenetic Polycomb Repressive Complex 2 (PRC2) in the endosperm. This bypass of the barrier is mediated by suppressed expression of imprinted genes. Using whole genome bisulfite sequencing of endosperm tissue we show that the hypomethylated pollen genome causes *de novo* CHG methylation directed to FIS-PRC2 target genes, revealing that different epigenetic modifications can functionally substitute for each other. Our work presents a method and the underlying mechanism for the generation of viable triploids, providing an impressive example for the potential of epigenome manipulations for plant breeding.

PS8-5

Epigenome profiles of the Arabidopsis endosperm

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In most angiosperms, the endosperm is a polyploid tissue containing two maternal genomes and one paternal genome. This particular genomic configuration makes the endosperm prone to epigenetic regulatory processes. One common example of epigenetic regulation occurring in the endosperm is the parent-of-origin specific expression of genes (genomic imprinting). In order to understand how chromatin marks trigger imprinting regulation and endosperm development, we aimed at identifying allele-specific epigenetic modifications in the endosperm. To isolate endosperm-specific nuclei we employed the INTACT (Isolation of Nuclei TAgged in specific Cell Types) system. Endosperm nuclei were tagged with a biotinylated nuclear envelope transgenic protein, permitting us to purify these nuclei using streptavidin-coated magnetic beads. After nuclei purification we were able to perform chromatin immunoprecipitation (ChIP) followed by deep sequencing. Using the sequence polymorphisms between Ler and Col Arabidopsis accessions we were able to differentiate the parental origin of the epigenetic marks in the endosperm. This methodology provides us with a powerful tool for deciphering epigenetic processes in the endosperm, allowing us to link epigenetic modifications with imprinted gene regulation.

PS8-6

Maternal Control of *Arabidopsis* Early Embryo and Endosperm Development by a Mitochondrial PPR Protein NUWA

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Pentatricopeptide repeat (PPR) proteins are well known to be involved in embryo and endosperm development of plants, but the mechanism and regulation of PPR proteins during these early developmental stages remain poorly understood. We identified a mitochondrial localized PPR protein NUWA, which regulated Arabidopsis early embryo and endosperm development by affecting mitochondria genesis and function. We demonstrated by genetic and molecular biology approaches that NUWA is a maternally expressed imprinted gene, and not all the PPR motifs are essential for its activity in plant early development, suggesting that the expression and function of PPR protein are precisely regulated. Our study suggests that maternal inheritance of mitochondria and maternal effect of nuclear gene NUWA are synergistically involved in regulation of early embryo and endosperm development shortly after double fertilization.

PS8-7

Mitochondria- and plastid-localized protein DEE plays an essential role in the communication of embryo and endosperm in *Arabidopsis*

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Double fertilization is a characteristic and important process in angiosperms reproduction. During double fertilization, the egg cell and the central cell are fused with a sperm cell separately, which initiate the development of embryo and endosperm respectively. There are hypotheses that communication exists between embryo and endosperm, but the nature and the mechanism of the communication is not clear.

We isolated a recessive early embryo lethal mutant, designated defective early embryogenesis (dee). Both embryo and endosperm development were defective in dee, i.e., embryo development was arrested at 2-cell stage, whereas the division of endosperm nuclei was blocked and the subsequent cellularization events did not occur.

DEE encodes a protein that is conserved in plant kingdom, with a signal peptide at the N-terminus. We found that DEE protein was localized both in mitochondria and plastids and that deletion of this signal peptide disrupted the localizations, suggesting that this dual-target signal peptide is responsible for the localization of DEE in these two organelles.

To investigate the function of DEE in embryo and endosperm development, we generated constructs of DEE driven either by an embryo specific promoter ABI3 or an endosperm specific promoter FWA, and transformed into homozygous dee mutant. We found that both constructs partially rescued the development defects in embryo and endosperm development of dee. This result suggests that DEE protein has played an essential role in the communication between embryo and endosperm.

PS8-8

Enhancing the Heat Stress Response in tomato anther to improve pollen thermo-tolerance

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The ever increasing environmental temperature threatens plant development, especially that of reproductive organs. When plants are exposed to high temperatures, a variety of responses are triggered to protect them. One of the most important response is the HSR (Heat Stress Response), which is regulated by heat stress transcription factors (Hsfs). These serve as the components of signal transduction, mediating the expression of heat shock proteins (Hsps) and other HS-induced transcripts.
Plant reproductive development is very sensitive to heat stress, and such sensitivity is often reflected in decreased crop yields. Our study addresses the hypothesis that heat shock factors are engaged in the protection of tomato flower development, especially in anthers under heat stress. According to past studies, the microspore formation stage is extremely vulnerable to heat stress during flower development. Our research focuses on uncovering which Hsfs are active and if their expression can be modulated to increase the HSR especially in anther/pollen at this stage.
To test this hypothesis, the expression level of candidate Hsfs is investigated in anther/pollen under heat stress, and overexpressed or downregulated under control of tissue-specific promoters. To test the activity of the anther/pollen specific promoters before and after HS, we transformed tomato plants with promoters::GUS fusions.

PS8-9

Live-cell analysis to reveal the cell fate determination during early embryogenesis in *Arabidopsis thaliana*

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Multicellular animals and plants develop from the single-celled zygote to form the mature embryo. In Arabidopsis thaliana, the zygote divides asymmetrically to form cytoplasmic apical cell, which is the precursor of the embryo proper, and a large vacuolated basal cell, which develops into the suspensor, along the apical-basal axis after fertilization. Although the cell fate is thought to be determined by positional information, not cell lineage, it is remains unknown how the cell fate is determined during early embryogenesis.
Here, we established the in vitro embryogenesis system and optical manipulation for disruption of a single cell in Arabidopsis embryo to analyze the cell fate in real time. First, we succeeded in time-lapse recordings of cell division from zygote to globular-stage embryos using the in vitro embryogenesis system. These movies supported that the cell division of apical lineage in early embryogenesis (until 16-cell stage) is not absolutely synchronized as shown by 3D volume analysis of early Arabidopsis embryogenesis (Yoshida et al., 2014). Moreover, the durations of cell division between apical lineages and basal lineages are significantly different just after first asymmetric division from zygote. These results also suggested that the apical and basal cell are functionally different after first asymmetric division. Moreover we are currently performing optical manipulation for disruption of a single cell with early embryo in this in vitro system. We will discuss the determination of cell fate during early embryogenesis in Arabidopsis.

PS8-10

Origin and survival of extranumerary embryos in Neotropical Inga species (Leguminosae, Mimosoideae)

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This study elucidated the origin and survival of the extranumerary embryos recorded in *Inga laurina* and *I. vera* legume trees. Ovules and seeds were processed for light and transmission electron microscopy. Moreover, we evaluated the average numbers of seeds/fruit, of embryos/seed, the average area of seeds and embryos, the dry seed mass, the percentage of polyembryonic seeds, of seeds with multiple emergences, and the speed index and percentage of emergence in a germination chamber. After a month of evaluation, the experiment was transferred to a greenhouse to evaluate the number of seedlings, the number of emergences and the number of embryos that only germinated. The presence of one embryo sac per ovule and the nucellar origin of embryos characterize the polyembryony as simple, with adventitious apomixis. The cells of the embryo sac and nucellus exhibited characteristics related to embryo nutrition: labyrinth walls and amyloplasts in the central cell; plasmodesmata connecting nucellar, central and embryonic precursor cells. Species are similar in seedling establishment, with high average numbers of embryos and seedlings per seed. The number of embryos in a seed was similar to the average number of emergences per seed, as well as the high percentage of seedling emergence. Thus, both species, although presenting low genetic variability, display a great competence for adaptation and survival (Fapesp).

PS8-11

Involvement of auxin biosynthesis, action and transport in stress-induced microspore embryogenesis initiation and development

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Immature pollen grains (microspores) can be reprogrammed in vitro towards an embryogenesis pathway. In isolated microspore cultures of Brassica napus, the embryogenic switch and embryo formation are induced by heat treatment in a culture media free of plant growth regulators. Despite the abundant data on the auxin involvement in plant growth and development, no information on the role and dynamics of endogenous indol-acetic acid (IAA) on microspore embryogenesis is available. In this work IAA levels and distribution, expression of BnNIT2, responsible of conversion of indol-3-acetonitrile (IAN) to IAA, and the effects of inhibition of IAA transport and action by N-1-naphthylphthalamic acid (NPA) and α-(p-Chlorophenoxy) isobutyric acid (PCIB) treatments were analyzed during B. napus microspore embryogenesis. Results indicate de novo synthesis of IAA at early stages of microspore embryogenesis and a progressive IAA increase during embryo development. IAA is abundant and localized in every cell of early embryos, whereas it is concentrated in apical and basal regions in polarized torpedo embryos. Both, the inhibition of the IAA transport by NPA and the inhibition of IAA action by PCIB diminish the embryogenesis efficiency. NPA also alters IAA distribution, being concentrated in cytoplasmic compartments in early embryo cells and showing a non-polarized pattern in advanced embryos. Taken together, results indicated that endogenous auxin biosynthesis, action and transport are involved in microspore embryogenesis initiation and development.
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PS8-12

Long-term stored pollen transcripts and their role in pollen and embryo development

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Long-term stored pollen tube mRNAs are suitable candidates for the delivery of paternal transcripts into female gametophyte, since they could migrate during the fertilization process and contribute to the already existing gene expression. The concept of mRNA storage in specialized EPP particles has been previously described, suggesting the precise translational repression and activation in a time-related manner during pollen tube growth and post-fertilization. Transcriptional profiling of subcellular fractions from in vitro-grown tobacco pollen tubes led to the identification of several genes exhibiting increasing or stable expression levels in EPP particles even after 24h of cultivation. Three tobacco homologs (EB433007, DW005251 and EB447794) in Arabidopsis (ED1, ED2, ED3) display pollen- as well as lethal embryo-defective phenotype resulting in a developmental arrest at various stages of embryogenesis. Screening of respective T-DNA insertion lines revealed a number of phenotypic aberrations that correlated with the segregation of the transgenes in two subsequent generations. However, reciprocal crosses did not reveal any significant shift in transmission of the traits through male or female, respectively. Our data suggests that the embryo-defective phenotype is induced only when two mutant gametes fuse. Assays focused on pollen tube growth in vitro and semi-in vivo address the fitness of mutant pollen tubes compared to wild type, whereas ovule fitness is described via the blue dot experiment. Mutant lines were crossed with specific markers (egg cell, sperm cell, vegetative cell and endosperm) to study cell fate of selected tissues in the mutant background. Subcellular localization of mRNAs and proteins, complementation of the mutant plants and a detailed study of the gene functions are planned as future experiments. We would like to thank the Czech Grant Agency (P501/11/1462 and 14-32292S) for the support of our research.

PS8-13

Early endosperm expansion dependents on endosperm composition

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Reproduction in flowering plants critically relies on a complex fertilization process involving synergid-mediated pollen tube attraction, degeneration of the first synergid, and gamete fusion. Successful double fertilization triggers programmed cell death of the second synergid and results in an early seed stage comprising of a zygote and a triploid endosperm. We have previously shown that the second synergid fails to undergo PCD in ethylene hyposensitive ein3 mutant. Intriguingly, the persistent synergid expresses two endosperm markers and divides synchronously with the nuclei of the endosperm after fertilization, indicating that this otherwise terminally differentiating cell can be reprogrammed into endosperm. Here we investigate the influence of the asexual endosperm fraction on early seed development. We discuss evidence according to which the synergid-derived maternal endosperm fraction affects endosperm expansion rates.

PS8-14

Microspore-derived embryo recovery trough isolated microspore culture in *Corylus avellana* L.

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Hazelnut (Corylus avellana L.) is the fourth nut tree worldwide, followed by cashew, Anacardium occidentale L., almond, Prunus dulcis and Persian walnut, Juglans regia L. (FAOSTAT 2013). All cultivated forms of hazelnut are diploid with a monoploid number of chromosomes n=x=11. It is a monoecious, dichogamous, self-incompatible and wind pollinated plant (Rovira et al. 1993). The presence of incompatibility is a main factor that does not allow reaching homozigozity by conventional methods, restricting its genetic improvement.

Haploid (H) and doubled haploid (DH) technology can facilitate the traditional breeding of this high value crop, allowing the production of homozygosity in one step and shortening the breeding times. Actually, in woody species, such as hazelnut, generally characterized by a long juvenile periods, a high degree of heterozygosity, large size and, often, self-incompatibility, the only way to obtain homozygous breeding lines is the gametic embryogenesis (Germanà 2011a; 2011b).

In this study, isolated microspore culture of six hazelnut cultivars (Carrello, Gentile romana, Imperatrice Eugenia, Meraviglia de Bollwiller, Minnulara and Tonda romana) was carried out, evaluating two different culture media (P and N6) and four different thermal stresses (only two for Meraviglia de Bollwiller): 30 min at 35°C, 60 min at 40°C and 30 min and 60 min at -20°C.

During the culture, observations were carried out by fluorescence microscope, after 4',6-diamidino-2-phenylindole staining. It was possible to notice both the gametophytic pathway with the presence of two asymmetrical nuclei, and the sporophytic pathway with the presence of bicellular (with symmetrical nucleus division), tricellular and multicellular structures.

After one year of culture, it was possible to observe the presence of embryos and calli in the most of the tested cultivars.

To our knowledge, this is the first time that the production of microspore-derived embryos was obtained in Corylus avellana.

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PS8-15

Distribution of arabinogalactan proteins (AGPs) and pectin epitopes in *Quercus suber* (cork oak) zygotic embryo

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Arabinogalactan proteins (AGPs) are a class of hydroxyprolin rich glycoproteins, ubiquitous in the plant kingdom that have been implicated in different processes of plant growth and development. Pectins are important cell wall polysaccharides, together with AGPs have been reported to play important roles in growth and development, plant defense, cell–cell adhesion, signaling, cell expansion and in somatic and zygotic embryogenesis. Pectins are mainly composed of homogalacturonan (HG), rhamnogalacturonan I (RG-I) and RG-II. The Fagacea tree cork oak (*Quercus suber*) is a dominant tree from the Southern Iberian Peninsula economically very important which was chosen to study the involvement of AGPs and pectins during zygotic embryogenesis due to the socio-economic interest for the production of acorns destined either for nursery production or for animal food.

Immunofluorescent localization of AGP and pectin epitopes were performed with a set of antibodies against AGPs JIM13 and JIM8 and antibodies binding to pectin HG epitopes with different degrees of methyl-esterification, JIM 5 and LM19 for de-esterified homogalacturonans and JIM7and LM20 for highly methyl-esterified homogalacturonans. The anti-pectin de-esterified antibodies do not label cell walls of the shoot and of the root apical meristems of the embryo, and the methyl -esterified epitopes recognized by JIM7 and LM20 are present in all cell walls throughout the embryo. AGP epitopes recognized by Jim13 were distributed with high intensity all over the cell walls of the mature embryo and with less intensity at the apical meristems and in the inner layers of the root cap. Interestingly, in the mature embryo cells AGPs labeling is present in several cell membrane vesicles in the protoderm and in the procambium, and these vesicles are not visible in the ground meristem cells.

PS9-1

A new class of non-coding RNAs that are 29-37 nts in length which are expressed in seeds of angiosperms and gymnosperms

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Prior research in small non-coding RNAs (sncRNAs) describes an abundance of 21 and 24 nucleotide (nts) classes of miRNA and siRNAs that are either processed by DICER-LIKE1 (DCL1) or by a series of other DCL members found in the different plant genomes. These particular classes of miRNAs and sncRNAs, are well defined and function together with RNA-induced silencing complexes (RISCs) to target transcripts or various RNA molecules for cleavage, translational inhibition, silencing and PTGS. Many of these molecules function in a variety of plant processes, and point towards a central role for miRNAs in the control of gene regulatory networks, such as developmental regulation, reproductive development and hormonal response for example. We have now identified new groups of short non-coding RNAs (sncRNAs) that have lengths around 29-37 nts, with a peak at 35 nts, and these are found in mature seeds from angiosperms and in seeds from nine diverse gymnosperms. Small RNA libraries were sequenced on the Ion Torrent platform and analyzed with CLC Genomics, and their presence was also confirmed by Illumina platform data. Their absence in vegetative tissues, and the fact that we could identify the 29-37 nt class in female megagametophytes, mature seeds and other female reproductive tissues across different seed plants suggests an important reproductive function. Filtering non-redundant sequences shows that this class of sncRNAs are produced from discrete loci in angiosperm and gymnosperm seeds, and that these occupy relative amounts of up to 35.5% and 46.9% of the total sRNAs produced from the sum of the 21-24 nt and 29-37 nt sRNA classes in angiosperms and gymnosperms respectively. We are in the process of defining these loci further and characterizing the biosynthesis of this class of sncRNAs using mutants in Arabidopsis.

PS9-2

Seed-larva interaction in two unrelated seed-feeder Chalcidoidea species

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The association between ontological processes of plant structures and strategies of resource utilization by phytophagous insects is not traditionally considered in studies of insect-plant interactions. Morphological, chemical and physiological properties of plant tissues vary along ontological stages, exerting selective pressure on insect traits related to strategies of resource utilization. We assessed the seed and larval development of two pairs of interacting chalcid wasps and Angiosperm species, to unveil processes involved on seed exploitation by larvae of these chalcid species. We studied the wasp Megastigmus transvaalensis (Torymidae) associated with Schinus terebinthifolia (Anacardiaceae), and Bephratelloides pomorum (Eurytomidae) associated with Anonna spp. (Annonaceae). Our results showed that M. transvaalensis and B. pomorum, although belonging to distinct Chalcidoidea families, adopt the strategy of oviposit in young fruits, when seed resources are still developing. The egg in both cases is deposited inside the endosperm from which first larval instar nourish. The first larval instar has little impact on seed development, allowing that both seed and plant embryo keep growing. When the infested seed reaches the maximum size of a normal seed, the larva grows rapidly, and consumes almost all the seed content. Although the gross larva-host plant relationship was similar for M. transvaalensis and B. pomorum, we observed that M. transvaalensis modify the endosperm cells while B. pomorum apparently does not induce any tissue change. Future studies should focus on other chalcid families, as well as other Hymenoptera superfamilies and insect orders in order to investigate convergent patterns among seed-feeder insects.

PS9-3

Correlation between sexual reproduction in *Phragmites australis* and die-back syndrome

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The common reed Phragmites australis (Cav.) Trin. ex Steud. have a widespread distribution in both hemispheres, in different habitat types from river/lake shores, wetlands, to ruderal, disturbed and even urban areas, hence it is considered a subcosmopolite species. In the last decades, reeds are dying back at a fast rate in sizeable areas of Europe, with significant impacts on important wetland functions (biodiversity, stability of river and lake margins, water quality) and local economy. Similar symptoms have been detected even in central Italy. Besides ecological, morphological or anatomical parameters studies concerning some reproductive aspects might also be interesting in order to detect the health condition of reed-dominated ecosystems. In all the plant species the flower biology can be a good indicator of the health condition as the seed production and the seed viability. In plants exhibiting both vegetative and sexual reproduction, the energy allocation can be shifted from one to the other strategy in response to environmental stress; it is well known that sexual reproduction decreases the vulnerability of a population to disturbances and biotic stresses by increasing the genetic variability. On this ground, we took into account the reproductive features of a declining reed stand in central Italy, where the die-back syndrome was recently detected, with the aim both to extend knowledge on sexual reproduction in P. australis and to highlight links between sexual reproduction and die-back symptoms. In this frame, cyto-histological analyses of inflorescences at different developmental stages were carried out in permanent plots where morphological investigations were also performed. Histochemical analyses were also carried out to verify pollen and seed viability. An interesting link between some decline symptoms and the rate of spikelets containing at least one viable seed. In detail, it appears that higher rates of viable seeds are recorded in the declining reed stands.

PS9-4

Regulation of Polycomb group protein activity in *Arabidopsis thaliana* seed development

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Seed formation in Arabidopsis thaliana requires a coordinated development between embryo, endosperm and seed coat. While the first two structures are originated by a simultaneous fertilization event of the egg and the central cell, the seed coat is solely of sporophytic origin. Still, initiation of its development is triggered by the sexual endosperm.
In the absence of fertilization, both seed coat and endosperm development are repressed by the activity of Polycomb group proteins (PcG). Thus, in order for successful seed development to take place, PcG protein activity needs to be tightly regulated. PcG proteins are chromatin-associated factors that repress transcription of several specific target loci. This transcriptional repression is achieved through trimethylation of lysine 27 of histone 3 (H3K27me3) at the target loci. PcG proteins are organized in multimeric complexes, called Polycomb Repressor Complexes2 (PRC2), which control several developmental processes during the plant life cycle. In seed development specifically, the PRC2 complexes VRN and EMF repress the autonomous development of the seed coat, while the FIS complex represses the autonomous development of the endosperm. Therefore, it can be hypothesized that upon fertilization PRC2 activity both in the developing endosperm and seed coat is repressed. The main goal of this study is to understand the post fertilization regulatory mechanisms that control PRC2 activity in the early seed. Furthermore, we are particularly interested in knowing how the regulation of the sporophytic PRC2 complexes is processed, and which signaling mechanisms need to take place between the gametophytic and sporophytic tissues, in order to achieve a synchronized modulation of PRC2 activity in the different structures of the early seed.

PS9-5

BEL1-type homeobox gene SH5induces seed shattering by enhancingabscission-zone development and inhibiting lignin biosynthesis.

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Seed shattering is an important trait that influences grain yield. A major controlling QTL in rice is qSH1. Although the degree of shattering is correlated with the level of qSH1 expression,some qSH1-defective cultivars display moderate shattering while others show a non-shattering phenotype.SH5on Chromosome 5 is highly homologous to qSH1. Although we detected SH5transcripts in various organs, this gene was highly expressed at the abscission zone (AZ) in the pedicels. When expression of this gene was suppressed in easy-shattering ‘Kasalath’, AZ development was reduced and seed loss was thereby reduced. By contrast, the extent of shattering, as well as AZ development, was greatly enhanced in moderate-shattering ‘Dongjin’ ricewhen SH5 was overexpressed. Likewise, over-expression of SH5 in the non-shattering ‘Ilpum’led to an increase in seed shattering because lignin levels were decreased in the basal region of spikeletsin the absence of AZ development. We also determined that two shattering-related genes, SHAT1 and Sh4,which are necessary forproper AZ formation, were regulated by SH5. Based on these observations, we propose that SH5 modulates seed shatteringby enhancing AZ development and inhibiting lignin biosynthesis.

PS9-6

Effect of X-rays on fruit anatomical and nutritional traits in tomato plants irradiated at different phenological phases

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Understanding the effect of ionising radiation on plant growth and reproduction is a many-sided goal which arouses interest within different frameworks. On one hand, the response of plants to low doses of ionising radiation is analysed for Space-related issues; on the other hand, higher doses are applied to generate information useful for terrestrial applications in the field of radio-ecology. Moreover, irradiation can be used in breeding programs to induce mutations. Differently from animals, plants are very resistant to ionising radiation which can also induce positive reactions.
The aim of this study was to evaluate the effect of different X-rays doses (from 0.3 Gy up to 100 Gy) on fruit traits of Solanum lycopersicum L. 'Microtom' plants irradiated at four phases of the life cycle: 1) dry seeds, b) young plants with two true leaves; c) adult plants showing at least one flower in blossom, and d) during fruit ripening. Anatomical and nutritional analyses were performed on ripe fruits to detect possible radioprotective strategies based on mechanical or biochemical defences. Light and epi-fluorescence microscopy observations, coupled with digital image analysis, allowed to quantify anatomical traits such as the thickness of cuticle and of sub-epidermal layers of cells accumulating phenolic compounds. Nutritional analyses were focused on anti-oxidant compounds and included the quantification of various carotenoids and phenolic compounds.
The overall results showed that irradiation at specific phases with high doses can constrain the completion of the reproductive cycle, thus preventing fruit formation. When the exposure of plants to specific doses of X-rays allowed fruit development, ripe fruits were characterised by an increased amount of some carotenoids and phenolic compounds, especially in sub-epidermal cell layers which were also more numerous than in non-irradiated control fruits. Such compounds, having antioxidant properties, can act as natural screens against radiation.

PS9-7

Embryo development and aril ontogeny in two species of *Passiflora*

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Passiflora is the largest genus in Passifloraceae and most of the commercially used species develop an aril surrounding the seed, which is commercially important for fresh fruit consumption, and concentrate juice. Reproductive developmental studies associating morpho-anatomical and molecular characteristics are essential for a better understanding of particularities of this genus. The present project aimed to characterize embryo and aril development in Passiflora edulis and Passiflora alata. Fruits were collected at regular intervals and ovaries processed for scanning and light microscopic. The endosperm is nuclear and starts developing soon after fertilization through successive divisions forming a syncytium. Later, cell walls start developing and the endosperm begins cellularization approximately 20 days after pollination. Embryogenesis initiates with the first division of the zygote, approximately 7 days after pollination. This division is transversal and asymmetrical; the apical cell undergoes successive divisions leading to the subsequent stages of embryo development such as 4- and 8-celled, globular, heart-shaped, torpedo. Approximately 30 days after pollination, the embryo reaches the cotyledonary stage. Aril primordium is observed in pre-anthesis when the embryo sac is organized. Epidermic cells at the base of the funiculus undergo periclinal divisions forming a rim surrounding the raphe. Differentiation stops until after fertilization when cell divisions are reactivated and the aril starts to develop into a multicellular structure surrounding the developing seed from the funicle towards the chalazal end. At approximately 14 days after pollination the aril already covers one third of the seed, when mostly cell expansion is observed until the whole seed is covered. These observations allowed the definition of two specific stages of aril development for laser-capture-microdissection and further molecular analysis aiming to evaluate the molecular basis of the control of aril differentiation in Passiflora. (Acknowledgements FAPESP, CNPq)
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PS9-8

Fruitlet abscission in apple (*Malus × domestica Borkh.*), the role of hormones

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Apple is one of the most appreciated fleshy fruits around the world. Apple fruit qualities are improved by reducing the orchard fruit set, usually applying thinning chemicals during fruitlet development. Such treatment, increases the physiological fruitlet abscission to allow only the so-called dominant fruitlets to continue their development until ripening. Most of the studies on the molecular mechanisms involved in apple fruit development have been focused on the last steps of development or ripening. Nevertheless, it is well characterized that ethylene and auxin have a main role in the establishment of fruitlet dominance inside the apple fruit cluster, although little is known about the molecular mechanisms controlling fruitlet abscission.
Nutritional stress is responsible for starting the signalling cascade (hormonal crosstalk) between the fruitlet cortex and in the seeds. This stress leads to the disruption of the polar auxin transport (PAT) in the smaller fruits. The PAT disruption determine the increase of sensitivity to ethylene in the abscission zone, the peduncle, and its activation leads to the fruitlet drop.
To determine the role of the seeds in the signal translation and the molecular mechanisms involved in the process of fruitlet abscission, an RNA-seq was performed in order to find differentially expressed genes between lateral and central fruits in an orchard of Golden Delicious apple trees. Gene expression dynamics during fruitlet development was characterised, in particular focusing in the hormonal pathways.

PS9-9

Cyto-histological analysis of *Theobroma cacao* l. Seed

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Cacao, an economically important tropical-fruit tree crop that is the source of chocolate, in recent years, has acquired a considerable importance not only for its economic value but also from the nutritional point of view. Proanthocyanidins, flavonoid polymers that are present in large amounts in Theobroma cacao seeds, may be beneficial to human health by improving cardiovascular health, providing cancer chemo preventative effects and also through neuroprotective activities. The morphological and anatomical characteristics of cacao seeds are closely related to the nutritional characteristics of this crop and to the cacao aroma; the large poliphenol and storage cells of seeds as well as the permeability of the seed coat of the fermenting seeds may affect its final flavour quality. Therefore it can be very useful to collect more information about the accumulation of cocoa seed compounds in the various components of this seed. Macronutrients, such as lipids, proteins and carbohydrates were localized in the different tissues/organs of the cocoa seed, by hystochemical analysis and microscopic observations. The presence and localization of flavonoids were also investigated. Seeds were collected from a fresh fruit of cocoa and portions of the seed coat, endosperm, cotyledons and embryo were embedded in epoxy resin. In cyto-histological studies, semithin sections were stained with toluidine blue, while to localize macronutrients PAS staining and the osmium treatment were carried out. In the teguments soluble polysaccharides were detected, in the endosperm the polysaccharides and lipids; polyphenols and polysaccharides were detected in the embryo axis and lastly polyphenols and polysaccharides in the cotyledons. Our studies confirmed that all portions of the mature seeds contribute to store macronutrients.

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