Wednesday September 6

Session 4: Sexual Plant Reproduction

Chair: Colombo Lucia

PLENARY SPEAKER

9:00-9:30 **HIGASHIYAMA TETSUYA** (Nagoya, japan) "Pollen tube guidance inside flowers"

9:30-10:00 **TUCKER MATTHEW** (Adelaide, Australia): "Intercellular communication during female germline development in flowering plants"

SELECTED TALKS

10:00-10:15 **MENDES MARTA** (Milan, Italy): "Live and Let Die: a REM complex promotes fertilization through synergid cell death in Arabidopsis"

10:15-11:00 Coffee break

PLENARY SPEAKER

11:00-11:30 **PALANIVELU RAVISHANKAR** (Tucson, USA): "Closing the deal for a new beginning: Arabidopsis LORELEI is required for pollen tube reception by the female gametophyte"

SELECTED TALKS

11:30-11:45 **PEREIRA ANA MARTA** (Porto, Portugal): "Who's got the moves like JAGGER? An AGP Essential for Persistent Synergid Degeneration and Polytubey Block in Arabidopsis"

11:45-12:00 **WILKINSON LAURA** (Adelaide, Australia) "Identification of molecular cues influencing ovule development in barley"

17:30-20:00 Posters

Marie Skłodowska Curie Actions
Research and Innovation Staff Exchange - RISE

SexSeed Mid-Term Meeting Agenda
Thursday September 7 (Consortium only)

9:30h Introduction by Thierry Jacquin (Research Executive Agency Representative) and Sílvia Coimbra (Coordinator)

9:40 Coordinator's Report Presentation focused on the following topics:

. Scientific achievements

. Training, Knowledge Transfer & Networking Development

. Project Management Strategy

. SexSeed Impact History

10:45 Coffee break

11:15 Partner Organizations Presentation

. Sílvia Coimbra – University of Porto

. Lucia Colombo – University of Milan

. Emidio Albertini – University of Perugia

. Charles Spillane – Galway University Ireland

11:45 Seconded staff members presentations

. Ana Marta Pereira - University of Porto

. Silvia Manrique – University of Milan

. Mónica Costa – University of Porto

. Rossana Petrella - University of Milan

. Sara Pinto – University of Porto

. Mário Costa – University of Porto

. Giovanni Beccari – University of Perugia

12:45 Lunch

14:15 Meeting between seconded staff members and the Research Executive Agency Representative

15:15 Open discussion & Debate

SESSION 4 | SEXUAL PLANT REPRODUCTION

O35

Pollen Tube Guidance inside Flowers.

Higashiyama T.

Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Furo-cho, Chikusa, Nagoya 464-8601, Japan

Pollen tube guidance for successful reproduction of flowering plants involves complex cell-to-cell communication. Defensin-like peptide LUREs are pollen tube attractants of *Torenia* and *Arabidopsis* secreted by two egg-accompanying "synergid cells" (Science 2001; Nature 2009; PLoS Biol. 2012). To understand the molecular mechanism of pollen tube guidance, we have been taking two approaches of live-cell study (for reviews, Cell Growth Differ. 2013; Annu. Rev. Plant Biol. 2015). The first approach is to use precisely defined semi-in vitro system (semi-in vivo system), including development of various microfluidics devices (e.g., RSC Adv. 2013). Semi-in vitro studies combined with synthetic chemistry lead to discovery of novel intercellular signaling molecules involved in pollen tube guidance. Arabinogalactan sugar chain AMOR derived from *Torenia* ovular sporophytic tissues is critical to make pollen tubes competent before attraction by TfLUREs (Curr. Biol. 2016; Plant Physiol. 2017). PRK6 is a receptor kinase of *Arabidopsis*, which is critical in sensing of AtLURE1 peptides (Nature 2016). Development of fluorescent small molecules is also in progress for single molecule or super-resolution imaging of cellular signaling (e.g., J. Am. Chem. Soc. 2017). The second approach is based on in vivo imaging. We have shown that pollen tube guidance is intimately related with double fertilization (e.g., Dev. Cell 2013; Cell 2015). By using two-photon microscopy, we have succeeded in visualizing pollen tube guidance in the pistil tissue (e.g., Development 2015, Protoplasma 2015). In this talk, I will talk about recent progresses of our pollen tube guidance study to discuss how pollen tubes are navigated to ovules, focusing on precise directional control, species-specific attraction, and one-to-one relationship between multiple ovules and pollen tubes.

E-mail of the presenting Author: higashi@bio.nagoya-u.ac.jp

SESSION 4 | SEXUAL PLANT REPRODUCTION

O36

Intercellular communication during female germline development in flowering plants.

Leong WH[1,2], Juranic M[3], Wilkinson L[1,2], Tan H[1,2], McKee L[4], Houston K[5], Shirley NJ[1,2], Bulone VB[1,2], Koltunow AM[4] and Tucker MR[1,2].

[1]*School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, SA 5064, Australia.*

[2]*Australian Research Council Centre of Excellence in Plant Cell Walls, University of Adelaide, Urrbrae, SA 5064, Australia*

[3]*Division of Glycoscience, School of Biotechnology, Royal Institute of Technology, AlbaNova University Center, 106 91 Stockholm, Sweden.*

[4]*CSIRO Agriculture, Hartley Grove, Waite Campus, Urrbrae, SA 5064, Australia.*

[5]*Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK.*

Female germline development in plants initiates when a single somatic ovule cell differentiates and enters a reproductive pathway that includes meiosis, mitosis and cell specification. Correct development of the germline results in the formation a female gamete, which is required for the successful and stable production of seed. Studies of mutants showing altered ovule development indicate that cross-talk between reproductive and somatic cells is critical for germline development. Using *Arabidopsis*, barley and *Hieracium* as model systems, we have been studying the role of different cell wall polysaccharides in this process. Cell wall-related genes implicated in 1,3-β-glucan (callose), pectin and arabinogalactan biosynthesis and modification are abundant in specific ovule cell types. Callose is particularly abundant in the expanding germline cells, and its accumulation coincides with a loss of intercellular transport with surrounding cells, possibly through constriction of intercellular channels (plasmodesmata). Using cell-type specific promoters, we show that expression of an atypical 1,3-β-glucanase (GLUC) in the Arabidopsis female germline has a pronounced effect on germline development. Heterologous assays in *E.coli* and *Nicotiana benthamiana* confirm that GLUC expression hydrolyses PD-associated callose, and alters 1,3-β-glucan deposition in the Arabidopsis germline cell wall. In ovules, GLUC expression coincides with aberrant accumulation of mobile tracer dyes and proteins that are typically excluded from the germline precursor in wild-type. Using this system, we have been exploring the molecular basis for germline isolation and its role in sustaining the downstream events of female germline development. The information gained may provide avenues for the targeted modification of cell fate in ovule and seeds with diverse agricultural and industrial benefits.

E-mail address of the presenting Author: matthew.tucker@adelaide.edu.au

SESSION 4 | SEXUAL PLANT REPRODUCTION

O37

Live and Let Die: a REM complex promotes fertilization through synergid cell death in *Arabidopsis.*

Marta Adelina Mendes[1], Beatrice Castelnovo[1], Rosalinda Guerra[1,2], Yuriria Velazquez[3], Piero Morandini[1], Hugh Dickinson[3] and Lucia Colombo[1]

[1]*Dipartimento di Bioscienze, Università degli Studi di Milano, 20133 Milan, Italy*

[2]*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.*

[3]*Center for Biomolecular Interactions Bremen, University of Bremen, Leobener Straße NW2, 28359 Bremen, Germany.*

Fertilization in flowering plants is complex and requires a series of coordinated events involving the male and female gametophytes to ensure successful seed production. In the female gametophyte the two synergid cells are responsible both for the attraction and reception of the pollen tube - the male gametophyte. Once the pollen tube penetrates the receptive synergid it ceases growth and enters a programmed leading to cell death; this allows the release of the two sperm cells into the receptive synergid – which also undergoes a similar process of cell death. Two REM transcription factors, VAL and VDD, both targets of the ovule identity MADS-box complex SEEDSTICK-SEPALLATA3, form a complex that can control the expression of the cytokines oxidase/dehydrogenase CKX7 and CKX6. The correct maintenance of cytokinin levels is required for the receptive synergid to die, allowing sexual fusion to take place.

E-mail address of the presenting Author: marta.miranda@unimi.it

SESSION 4 | SEXUAL PLANT REPRODUCTION

O38

Closing the deal for a new beginning: Arabidopsis LORELEI is required for pollen tube reception by the female gametophyte.

Yanbing Wang, Xunliang Liu, Jennifer Noble, Rebecca A Mosher, and Ravi Palanivelu

School of Plant Sciences, University of Arizona, Tucson, AZ 85721

Double fertilization requires successful sperm cell delivery by the male gametophyte (pollen tube) to the female gametophyte (embryo sac). We isolated a null allele of LORELEI (LRE) and implicated it in inducing pollen tube reception by the synergid cells of the female gametophyte. LRE fused to citrine yellow fluorescent protein (LRE-cYFP) remains functional and localizes to the synergid plasma membrane-rich filiform apparatus, the first point of contact between the pollen tube and the female gametophyte. LRE contains domains that are critical for adding a glycosylphosphatidylinositol (GPI) anchor to the pre-protein in ER. Consistent with these observations, deletion of GPI anchor addition domains led to decreased localization of LRE-cYFP in the filiform apparatus. Biochemical analysis of ectopically-expressed LRE-cYFP expressed in leaves demonstrated that LRE is indeed a GPI-anchored membrane protein. Ectopically expressed and delivered LRE-cYFP from pollen tubes could non-cell-autonomously complement the pollen tube reception defect in *lre* female gametophytes, only if they expressed FERONIA, a receptor-like kinase, indicating that LRE and FERONIA jointly function in pollen tube reception at the interface of the synergid cell and pollen tube. Expression analysis showed that LRE is expressed in the female gametophyte before fertilization and the developing seed up to 24 hours after pollination. Interestingly, *LRE* expression is imprinted: the *LORELEI* matrigenic allele contributes nearly all the *LORELEI* expression after fertilization, making it a rare imprinted gene that functions immediately after double fertilization. Our results show that a strict maternal and matrigenic expression of *LORELEI* in the female gametophyte and fertilized ovules, respectively, allows the maternal parent to control two critical events – pollen tube reception and initiation of seed development – just before and soon after double fertilization using the same gene.

E-mail address of the presenting Author: rpalaniv@email.arizona.edu

SESSION 4 | SEXUAL PLANT REPRODUCTION

O39

Who's got the moves like JAGGER? An AGP essential for persistent synergid degeneration and polytubey block in Arabidopsis.

Pereira, A.M.[1], Nobre, M.S.[1,3], Pinto, S.C.[1], Lopes, A.L.[1], Costa, M.L.[1], Masiero, S.[2], Coimbra, S.[1]

[1] *Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal;*

[2] *Università Degli Studi di Milano, Dipartimento di Bioscienze. Via Celoria 26, 20133, Milan, Italy.*

[3] *Present address: Institute of Plant Biology and Zürich-Basel Plant Science Center, University of Zürich, Zolikerstrasse 107, 8008 Zürich, Switzerland*

In flowering plants, sperm cells delivery by the pollen tube into the female gametophyte is tightly controlled by the two synergids at its micropylar entrance. By a series of complex signalling pathways, not yet fully understood, these two cells control pollen tube attraction, arrest and burst. Together with the central and the egg cells, they also control their own death, and consequently the polyspermy block, by cessation of pollen tube attraction. This tightly controlled process will end with the development of seeds and the establishment of a new plant generation. We have recently identified JAGGER, Arabinogalactan Protein 4 (AGP4), as an important player to avoid the growth of multiple pollen tubes into one embryo sac in *Arabidopsis thaliana*. AGPs are glycoproteins bearing an interesting high proportion of sugars, and have long been implied in several steps of the reproductive process. In *jagger*, a knock-out mutant for AGP4, the pistils show impaired pollen tube blockage as a consequence of the survival of the persistent synergid. JAGGER appears to be involved in the signalling pathway that leads to a blockage of pollen tube attraction. Our results present relevant data that will help to shed light into the mechanism responsible for preventing polyspermy in Arabidopsis and for safeguarding a successful fertilization of all ovules in one pistil.

E-mail address of the presenting Author: ambacpereira@gmail.com

SESSION 4 | SEXUAL PLANT REPRODUCTION

O40

Identification of molecular cues influencing ovule development in barley.

Wilkinson LG[1], Houston K[2], Byrt CS[1], Burton RA[1] and Tucker MR[1]

[1]*ARC Centre of Excellence in Plant Cell Walls and School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, South Australia, Australia*

[2]*Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK*

The number of fertile florets per spike of cereal crops is regulated by successful development of male and female reproductive tissues. A key component of reproductive development is the establishment of a germline that leads to the production of gametes. In the ovule, this takes place in the nucellus, a mass of somatic cells located at the distal tip of the ovule that is surrounded by a ring of integuments.

The nucellus generally fulfils a similar role between species; it gives rise to the female gametophyte, provides a protective physical barrier, releases signals that regulate female gametophyte progression, and after fertilization it acts as a nutrient transfer tissue to support downstream stages of seed development. However, unlike model dicots, the cereal nucellus persists as a multilayered somatic tissue until after fertilisation, accounting for 70% of the mature ovule tissue mass. Genetic and molecular understanding of female reproductive development in cereals is far from complete, and even in the case of dicots such as Arabidopsis, the molecular identity of the nucellus remains unclear. In this project we have been characterising genetic and molecular regulatory pathways that underlie development of the barley ovule, with a particular focus on nucellus and gametophyte development.

To identify genes regulating barley ovule development we have employed various molecular, microscopic and genetic techniques. Transcriptional profiling of developing barley ovaries has revealed genes exhibiting dynamic changes in abundance across female gametophyte development. In parallel, a genome wide association study (GWAS) on a panel of 165 two-row spring barley cultivars, revealed four loci that influence morphological variance. Alignment of genetic, physical and transcriptional datasets enabled identification of specific candidate genes for further analysis. These genes are now being characterized to assess their expression and function during ovule development in different barley cultivars. The results will provide a foundation for future studies of ovule size and grain yield, in addition to molecular tools that can be used to investigate the role of the nucellus in reproductive stress tolerance.

E-mail address of the presenting Author: laura.g.wilkinson@adelaide.edu.au

POSTER SESSION

SEXUAL PLANT REPRODUCTION

POSTER SESSION | SEXUAL PLANT REPRODUCTION

SR 1

The Fasciclin-like arabinogalactan proteins (FLAs) multigene family differentially expressed in male and female flowers of monoecious *Quercus suber.*

Amorim, M.I.,[a, b] Sobral, R.,[c] Costa, M.L,[b] and Coimbra, S. [a, b]

[a] *Universidade do Porto, Faculdade de Ciências, Departamento de Biologia Rua do Campo Alegre, 4169-007 Porto, Portugal*
[b] *Universidade do Porto Biosystems and Integrative Sciences Institute (BioISI), Plant Functional Biology Center, Portugal*
[c] *Universidade do Minho Biosystems and Integrative Sciences Institute (BioISI), Plant Functional Biology Center, Campus de Gualtar, 4710-057 Braga, Portugal.*

Coark oak (*Quercus suber*) is one of the most important Fagacea tree species in the Western Mediterranean region due to its ecological and socio-economic value. *Q. suber* male flowers develop much sooner than the female flowers providing an interesting system for comparative studies of development and sexual reproduction in a monoecious non-model forest plant. Furthermore, the development of the female gametophyte occurs only after a delayed process of pollen tube growth, thus interactions between the pollen tube and the pistil tissues may involve different players, such as the Arabinogalactan proteins (AGP)s.

AGPs belong to family of highly glycosylated hydroxyproline-rich glycoproteins cell-wall components found in the entire plant kingdom, in almost all plant organs and cell types from root to flowers. Fasciclin-like AGPs (FLAs) are a sub-classe of AGPs that not only contain one or two AGP-like glycomodules, but also have one or two fasciclin domains, putative cell adhesion domains. Fasciclin domains were detected in several species, such as algae, lichens, plants and animals, which mediate cell-cell and cell-extracellular matrix adhesion. *Arabidopsis thaliana* FLAs, such as AtFLA4 has been involved in multidimensional cell growth, mucilage biosynthetic process involved in seed coat formation, abscisic acid-activated signaling pathway and in response to stress. AtFLA12 has been involved in plant-type secondary cell wall biogenesis, and AtFLA3 was implicated in microspore development. Furthermore, Arabidopis FLAs are expressed during the flowering stage, pollination, embryogenesis, vascular formation, and stem and root development. In eucalypt, EgrFLA2 and EgrFLA3 are involved in cellulose microfibril angle and stem flexural strength, respectively.

A bioinformatics search of FLAs in the *Q. suber* transcriptome using the BLAST tool in the cork oak Consortium database was performed. In these BLASTp analysis, Arabidopsis FLAs amino acids sequences were used as query. In an attempt to find FLA genes differentially expressed in male and female *Q. suber* flowers, we mine databases generated from sequencing non-normalised *cDNA* libraries of female and male inflorescences at different development stages. With this approach we identified 17 QsFLAs-like genes, all these genes have a coding sequence which contain a fasciclin domain, an AGP-like glycosylated region, a N-terminal signal and most of them show a C-terminal containing a glycosylphosphatidyl inositol (GPI) anchor signal sequence. The QsFLAs have several orthologs in Arabidopsis, and phylogenetically clustered into four major groups. At least five QsFLAs which clustered in the same group are differentially expressed in the male and female gametophyte.

The comparative transcriptomic analysis of *Q. suber* male and female flowers suggest that QsFLAs play a relevant role in the reproduction mechanism of Cork oak.

E-mail address of the presenting Author: mpamorim@fcup.pt

POSTER SESSION | SEXUAL PLANT REPRODUCTION

SR 2

VPS13 is involved in the miRNA-dependent translational repression pathway required for ovule development in *Arabidopsis thaliana*.

Balanzà, V.[2], Cucinotta, M.[1], Gatti, S.[1], Rattaggi, M. M.[1], Rigola, D.[3], Van Dijk, P. J.[3], Prins, M.[3], Colombo, L.[1].

[1] *Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy.*
[2] *Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas – Universidad Politécnica de Valencia, Avenida de los Naranjos s/n, 46022 Valencia, Spain.*
[3] *Keygene N.V., Agro Business Park 90, 6708 PW Wageningen, The Netherlands.*

Post-transcriptional repression mediated by miRNAs is a key regulator of developmental processes in all Eukaryotes. In plants, miRNAs repression can act in two different ways: by RNA cleavage (canonical) or through translational repression (non-canonical). While the canonical pathway has been extensively studied, non-canonical mechanisms are still largely unclear. In plants, the RNA-induced silencing complex (RISC), formed by AGO1 and the miRNA, is either able to bind mRNA, physically blocking ribosome movements by steric hindrance, or it can bind the endoplasmic reticulum (ER), sequestering target mRNAs to the polysome. Recently, the membrane protein associated with the ER named AMP1 (ALTERED MERISTEM PROGRAM 1) has been linked to the regulation of translational initiation. However, it remains unclear how AGO1 and AMP1 might interact. To address this question, we investigated the role of VACUOLAR PROTEIN SORTING-ASSOCIATED PROTEIN 13 (VPS13), a membrane-associated protein known to be involved in cytosolic vesicles trafficking in human and yeast.
We will show the genetics and molecular evidences suggesting that VPS13 protein is involved with AGO1 and AMP1 in the miRNA-dependent translational repression pathway essential for proper ovule development.

E-mail address of the presenting Author: stefano.gatti1@unimi.it

POSTER SESSION | SEXUAL PLANT REPRODUCTION

SR 3

The *S* locus Supergene in *Primula vulgaris* - Genetic Architecture and Control for Floral Heteromorphy.

Li, J., Cocker, J.M., Gilmartin, P.M.

University of East Anglia, School of Biological Sciences, Norwich Reserach Park, Norwich NR4 7TJ, UK; Earlham Institute, Norwich Research Park, Norwich NR4 7UZ, UK

In the *Primula* genus there are approximately 430 species and about 90% of them produce heteromorphic flowers - pin morph and thrum morph. Each individual plant produces only one floral morph, either pin or thrum. Pin flowers have a long style with low anthers and thrum flowers have a short style with high anthers. Both pin and thrum flowers produce fertile male and female gametes, however, successful sexual reproduction happens only if cross-pollination occurs between pin and thrum flowers, not by self-pollination or intra-morph pollination. This is believed to be controlled by a sporophytic self-incompatibility (SI) mechanism. Genetic analysis demonstrated both floral heteromorphy and self-incompatibility responses are controlled by the *Primula S* locus supergene, which has attracted the attention of many biologists and geneticists since the time of Darwin. A widely accepted genetic model of the *Primula S* locus supergene established over 60 years ago proposed a di-allelic multigene structure with three genes controlling the style length (*G/g*), anther height (*A/a*) and pollen size (*P/p*), respectively. Pin plants were believed to be homozygous for the recessive allele (*s/s* or *gpa/gpa*) and thrum plants were heterozygous for the *S* allele (*S/s* or *GPA/gpa*). This model proposed that self-fertile homostyles arose from *S* gene recombination between pin and thrum alleles. It also predicted a recessive lethal gene associated with the dominant *S* allele to prevent producing homozygous thrum plants. However, the identity of the *Primula S* locus supergene and the mechanism for floral heteromorphy control remained unknown until recently. We revealed the entire *S* supergene of *Primula vulgaris* using a combination of approaches including classical genetics, molecular genetics, molecular biology, Next Generation genome sequencing, genomics, transcriptomics and bioinformatics. We demonstrated that the pin genome of *Primula vulgaris* lacks a 278 kb sequence that contains five thrum-specific genes present in the thrum genome. Furthermore, this 278 kb region is the only thrum-specific genomic region transcribed in flowers. Therefore, thrums are hemizygous not heterozygous for the *S* locus. We characterized the five thrum-specific *S* locus genes and demonstrated that homostyles were resulted from gene mutation rather than recombination. We showed the long-homostyle phenotype was caused by mutations in CYPT and the short-homostyle was caused by a mutation in GLOT. The roles of other three thrum-specific *S* locus genes (PUMT, KFBT and CCMT) in floral heteromorphy and self-incompatibility control are under investigation. We also carried out transcriptome comparison analysis using flowers from pin, thrum, long-homostyle and short-homostyle plants, with the aim to understand the relationship of the thrum-specific *S* locus genes and their coordination in action.

E-mail address of the presenting Author: jinhong.li@uea.ac.uk

POSTER SESSION | SEXUAL PLANT REPRODUCTION

SR 4

Rice No Pollen 1 (*NP1*) is required for anther cuticle formation and pollen exine patterning.

Wanqi Liang[1], Ze Liu[1], Sen Lin[1], Jianxin Shi[1], Jing Yu[1], Lu Zhu[1], Xiujuan Yang[2], Dabing Zhang[1,2]

[1]*Joint International Research Laboratory of Metabolic & Developmental Sciences, State Key Laboratory of Hybrid Rice, Shanghai Jiao Tong University-University of Adelaide Joint Centre for Agriculture and Health, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China*
[2]*School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, South Australia 5064, Australia.*

Angiosperm male reproductive organs (anthers and pollen grains) have complex and interesting morphological features, but mechanisms that underlie their patterning are poorly understood. Here we report the isolation and characterization of a male sterile mutant of *No Pollen 1* (*NP1*) in rice (*Oryza sativa*). The *np1-4* mutant exhibited smaller anthers with a smooth cuticle surface, abnormal Ubisch bodies, and aborted pollen grains covered with irregular exine. Wild-type exine has two continuous layers; but *np1-4* exine showed a discontinuous structure with large granules of varying size. Chemical analysis revealed reduction in most of the cutin monomers in *np1-4*anthers, and less cuticular wax. Map-based cloning suggested that *NP1* encodes a putative glucose-methanol-choline (GMC) oxidoreductase; and expression analyses found *NP1*preferentially expressed in the tapetal layer from stage 8 to stage 10 of anther development. Additionally, the expression of several genes involved in biosynthesis and in the transport of lipid monomers of sporopollenin and cutin was decreased in *np1-4* mutant anthers. Taken together, these observations suggest that *NP1* is required for anther cuticle formation, and for patterning of Ubisch bodies and the exine. We propose that products of *NP1* are likely important metabolites in the development of Ubisch bodies and pollen exine, necessary for polymerization, assembly, or both.

E-mail address of the presenting Author: wqliang@sjtu.edu.cn

POSTER SESSION | SEXUAL PLANT REPRODUCTION

SR 5

Baby steps of AGP's crosstalk with CKX, from ovule to seed development, in *Arabidopsis thaliana.*

Lopes, A.[1,2,3], Mendes, M. [3], Pereira, A. [3], Amorim, M. [1,2], Colombo, L. [3], and Coimbra, S.[1,2]

[1] *Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.*
[2] *Biosystems and Integrative Sciences Institute – BioISI, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.*
[3] *Università Degli Studi di Milano, Dipartimento di Bioscienze. Via Celoria 26, 20133, Milan, Italy*

Agriculture depends on seeds and seeds on sexual reproduction. Understanding the factors regulating sexual plant reproduction is a complex biological process and currently an essential need to improve agricultural productivity. Seeds are essential for consumption and basal sources to generate high-value-added products. Understanding the factors regulating sexual plant reproduction is a complex biological process and currently an essential need to improve agricultural productivity. Combining transcriptomic and genetic approaches, we aim to increase our knowledge on transcriptional, signaling and hormonal functions of the network controlling seed formation in *Arabidopsis thaliana*, a model plant sharing a conserved developmental program with major crop plants. A key factor controlling seed development is SEEDSTICK (STK), a MADSbox factor that determines ovule identity, seed development and abscission. Furthermore, STK is indicated to play a huge role during seed development, as its mutant seeds are smaller. TFs, such as MADS-box, act as process integrators and connect other developmental processes as hubs in the network. The TF target gene analyses have revealed unexpected connections between processes previously considered to be unrelated, which could not have been predicted by classical genetic approaches. For example, the ChIP-seq data published during the last years demonstrated that MADS-domain TFs are able to bind to thousands of different genomic regions, several of which belong to genes involved in different hormonal pathways.
A recent ChIP-sequencing (ChIP-seq) analysis identified AGPs (AGP7, AGP9, AGP31) as STK targets, together with cytokinin (CK) oxidase-dehydrogenases (CKX) 6 and 7. Arabinogalactan Proteins (AGPs) are hydroxyproline rich glycoproteins that have been implicated in many important processes for plant development and growth, such as cell expansion, proliferation and differentiation, cell–cell recognition, somatic embryogenesis, pollen tube growth, programmed cell death, seed germination, and resistance to infection.CKX catalyze the irreversible degradation of CK. In the last decade, several studies have clearly proven that CK has also a significant role during ovule development and it has already been demonstrated that in plants that are defective in the production or perception of this hormone, correct ovule formation is compromised and/or the number of ovule is drastically reduced.
We aim to prove that STK regulates all these players and to understand their role during seed development and in particular in seed size. Some CK functions are executed through the control of cell cycle, so by regulating the expression levels of CKX6/7 proteins, these AGP genes may be the missing signals to modulate cell division in the seed coat and thus, seed size. This would demonstrate for the first time crosstalk between STK (TF), signaling and CK.

E-mail address of the presenting Author: ana.lopes@fc.up.pt