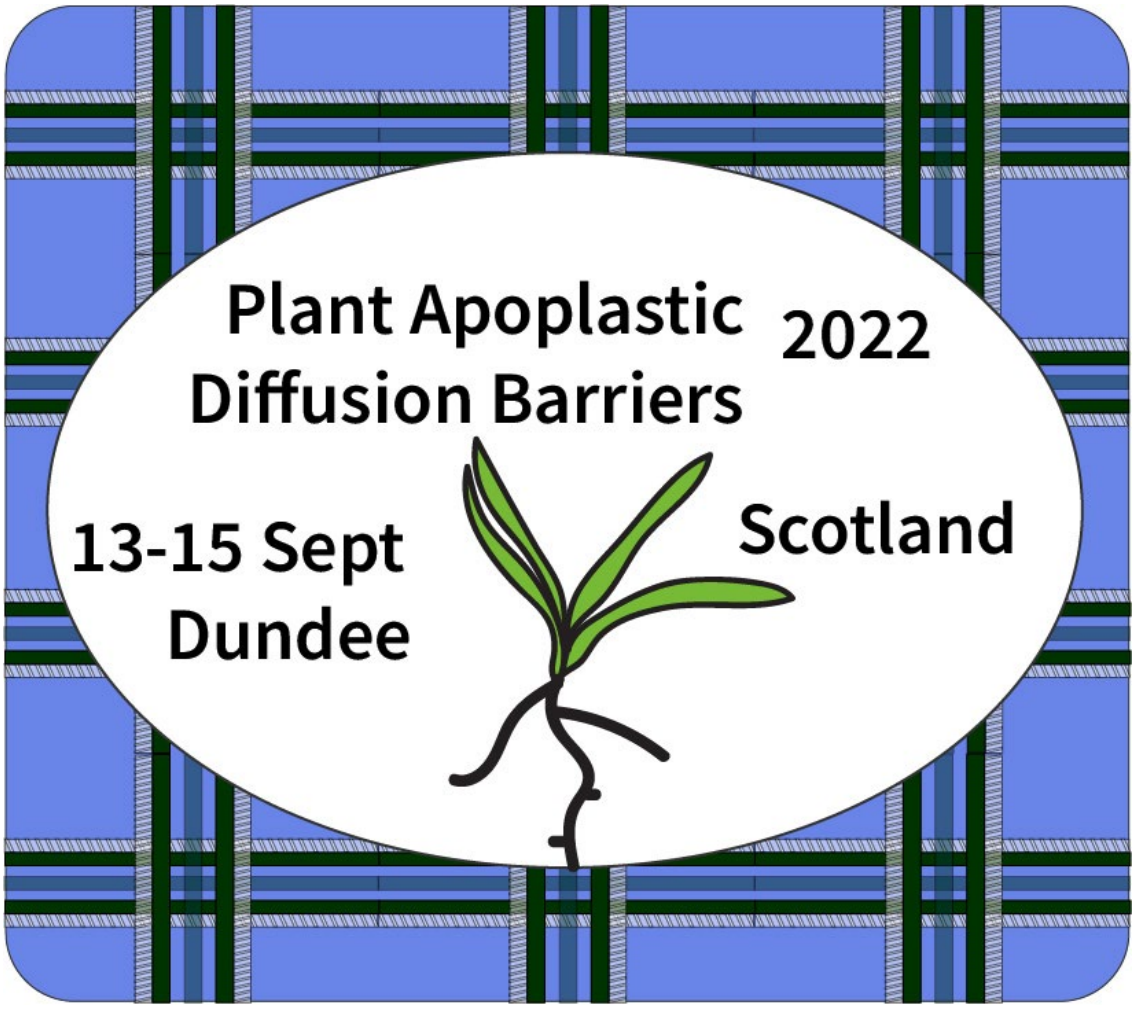
PADiBa 2022



Symposium programme and abstract booklet

ACKNOWLEDGEMENTS

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INVITED SPEAKERS

LUIS LOPEZ MOLINA

I am a group leader at the University of Geneva (UNIGE, Switzerland) since 2004. I studied theoretical physics at the UNIGE for my Master degree and then the molecular biology of mammalian circadian rhythms for my PhD degree, under the supervision of Prof. Ueli Schibler (UNIGE). I am currently interested in identifying the major processes withstanding the control of germination in Arabidopsis thaliana seeds. I initiated my research on this topic during my postdoctoral research in the laboratory of Prof. Nam-Hai Chua (Rockefeller University, New York, USA) where I studied ABA signaling and its role to control germination. Since then, my group has characterized the signaling pathways governing the control of seed germination in response to abiotic and biotic cues and studied the role of epigenetic modifications for the regulation of seed dormancy. A major focus of our work is to study the function of the endosperm since it plays an essential to control germination. More recently, we have been interested in the role of seed apoplastic barriers to regulate seed dormancy.

OHKMAE K. PARK

Ohkmae K. Park graduated from Seoul National University and obtained PhD in biochemistry from University of Virgina. She worked as a postdoc on the EGFR-JAK-STAT pathway in animals at Jones Hopkin University School of Medicine. She joined Kumho Life & Environmental Science Laboratory as a Principal Investigator in 1997, where she began her study of proteomics and defense signaling in plants. In 2005, she was appointed as a professor in the Department of Life Sciences at the Korea University. Her research interests include cell wall defense and lignin barrier, autophagy and proteostasis, and ethylene-jasmonic acid signaling and crosstalk in plant immunity.

INVITED SPEAKERS

INÊS BARBOSA

I am Portuguese. I did my undergraduate studies in Biology in Lisbon with a Masters in Ecology, on alpine meadows water use efficiency response to last century climate change. Shifting gears, I did a year of research training in Plant Molecular Biology Lab of Paula Duque (Instituto Gulbenkian de Ciência, Portugal) and I discovered my interest in plant molecular biology. For my PhD, I joined the lab of Prof Schwechheimer (TUM, Germany), to work on polarity control of the auxin transport regulators D6PK kinases. Here I got passionate about microscopy, cell biology and development.

For my postdoc I joined Niko Geldner's lab (University of Lausanne, Switzerland) to dissect the molecular function of CASP proteins, in the making of unique plasma membrane-wall polar domain - the Casparian Strip. I became fascinated about endodermal polarity and development and I hope I can combine this passion with my previous experience in auxin dependent development for future line of research.

TEAGEN QUILICHINI

Dr. Teagen Quilichini is a Research Officer at the National Research Council (NRC) in Saskatoon Canada and fascinated by how plants adapt to the stresses of sessile, terrestrial life. Her doctorate at the University of British Columbia in Vancouver Canada examined the toughened wall encasing spores and pollen that is critical for land plant reproduction. By merging leading-edge imaging with molecular genetics, her work contributed insights into the composition and transport of evasive 'sporopollenin', believed to be the strongest biopolymer on earth. After completing her doctorate, Dr. Quilichini transitioned into an industry-based postdoctoral position with Anandia Laboratories Inc., and revealed specialization among the multicellular trichome factories that produce the complex essential oils of *Cannabis sativa*. In her current position as a Research Officer at the NRC, Dr. Quilichini has focused on characterizing the genetic regulation of embryogenesis and seed development in agriculture-relevant species, including wheat, Brassicas (canola), legumes and their ancestral relatives. Dr. Quilichini is an active advocate for women in STEM and enjoys speaking with early career scientists about alternate research career paths.

INVITED SPEAKERS

MIRANDA SINNOTT-ARMSTRONG

Dr. Miranda Sinnott-Armstrong is a National Science Foundation Postdoctoral Fellow in Biology, currently based at the University of Cambridge. She completed her PhD at Yale University in 2019, studying global scale patterns in fruit colors as well as the evolution of biophotonic structures in the plant clade Viburnum. Her current research focuses on characterizing novel structural colors in fruits and on understanding their evolution and ecology. She is particularly interested in understanding the morphological origins of novel structural colors, such as whether some structural colors are derived from a modification of cuticle synthesis. Her research uses a variety of techniques, especially electron microscopy, optical simulations, phylogenetics, and comparative methods. Miranda currently serves on the Early Career Professional Development Committee for the Botanical Society of America.

MIKIO NAKAZONO

Mikio Nakazono is a professor of Graduate School of Bioagricultural Sciences at Nagoya University in Japan. He earned a PhD degree from University of Tokyo in 1995. After completion of his PhD, he worked at University of Tokyo as an Assistant Professor (1995 to 2003) and an Associate Professor (2003 to 2010) and then he moved to Nagoya University as Professor in 2010. He has studied molecular mechanisms of plant response and adaptation to abiotic stresses (especially flooding stress) for more than 20 years. In particular, he is interested in the mechanisms for the radial oxygen loss barrier formation and the aerenchyma formation, both of which are induced in some plant roots under flooded soil conditions.

INVITED SPEAKERS

YOSELIN BENITEZ-ALFONSO

Dr. Yoselin Benitez-Alfonso is Associated Professor in Plant Sciences at the University of Leeds (UK). She was born in Cuba and graduated in the Faculty of Chemistry in University of Cordoba, Spain. Yoselin did her PhD in plant biochemistry and molecular biology followed by postdoctoral research at Cold Spring Harbor Laboratory in New York (USA) and at the John Innes Centre (Norwich, UK). In 2017, she was appointed Lecturer at the University of Leeds, where she secured multiple funding including a recent UKRI Future Leaders Fellowship. Yoselin research group focuses on studying cell walls properties surrounding plant intercellular channels (named plasmodesmata) with the goal to unlock knowledge for the development of new strategies for crop improvement and biomaterial development. Yoselin is an advocate for equality and inclusion and outreach. She engages with social media via twitter @benitez_lab and @YoselinBenAlf. For more info visit benitezalfonso.wordpress.com.

TESSA BURCH-SMITH

Dr. Tessa Burch-Smith is an Associate Member and Principal Investigator at the Donald Danforth Plant Science Center in St. Louis, Missouri. Prior to this, she was an Assistant then Associate Professor in the Department of Biochemistry & Cellular and Molecular Biology at the University of Tennessee, Knoxville. She competed her graduate and post-doctoral work at Yale University and the University of California at Berkeley, respectively.

Her research focuses on intercellular communication in plants, particularly on structures called plasmodesmata that allow trafficking between cells. Her research uses a variety of molecular and cell biological approaches including advanced light and electron microscopy and plant viruses. Her lab also investigates chloroplast gene expression and how signals from chloroplasts can control expression of nuclear genes via retrograde signaling.

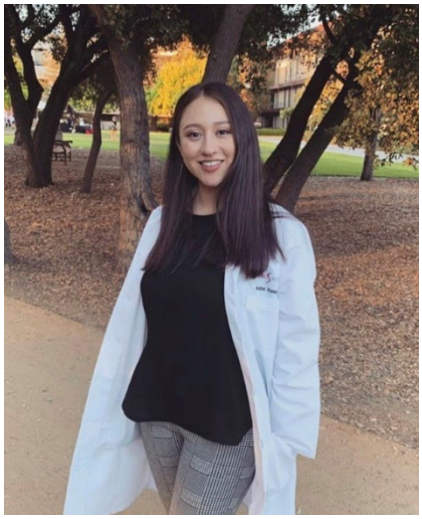
She is the author of numerous scientific articles and has received funding for her research from the National Science Foundation and the Defense Advanced Research Projects Agency. She is also a Senior Editor and Associate Editor-in-Chief of Molecular Plant-Microbe Interactions. She currently serves as Chair of the Science Policy Committee of the American Society of Plant Biologists and is a member of the ASPB Board of Directors.

INVITED SPEAKERS

HUGUES RENAULT

Hugues Renault obtained a PhD at the University of Rennes (France) during which he studied the functions of the GABA metabolism in plants. After a first postdoctoral experience at the University of Arizona (Tucson, USA) where he studied the plant MEDIATOR complex, Hugues Renault joined Danièle Werck's team at the Institute of Plant Molecular Biology in Strasbourg (France) to establish a research line centered on the evolution of cytochrome P450 enzymes. He then moved to the lab of Ralf Reski at the University of Freiburg (Germany) to expand his research to the origins of the plant phenylpropanoid pathway, using bryophyte models. In 2017, he got appointed tenured CNRS senior researcher at the Institute of Plant Molecular Biology in Strasbourg. The current focus of Hugues Renault's research lies in the understanding the determinants of plant metabolic diversity, and the associated adaptive functions. More particularly, his research investigates the origin and evolution of biopolymers that shape plant apoplastic diffusion barriers.

ANDREA RAMIREZ



Andrea grew up in Southern California in the town of Inglewood. She moved north to earn her undergraduate degree at UC Santa Cruz(majored in Molecular, Cellular, and Developmental Biology) and further north to do her Ph.D. at Stanford.

Andrea is now building a root anatomical atlas of diverse species in the Brassicaceae family to understand how innovation in tissue functions helps plants survive under stressful conditions.

INVITED SPEAKERS

BÉNÉDICTE BAKAN

Dr. Bénédicte Bakan, PI scientist at INRAE (Biopolymers Interactions & Assembly lab., Nantes, France), develops multidisciplinary research on the structure and biosynthesis of the cutin biopolymer in relation to its functional and technological properties. The different biochemical and biophysical strategies developed for the characterization of the polymer enabled to decipher the function of CUS1 (cutin synthase), in collaboration with INRAE Bordeaux, and to improve the understanding of the composite structure of the cutin-polysaccharide continuum.

DAY 1: TUESDAY 13TH SEPTEMBER

8:30 *Registration opens. Poster presenters to hang posters.*

9:15 Welcome: **Sarah McKim (University of Dundee)**

SESSION 1 – DEVELOPMENTAL EVENTS I
Chairs: Charles Hachez and Alice Berhin

09:20 Invited speaker: **Luis Lopez Molina (University of Geneva)**
Identification of a polarly localized oi1 barrier regulated by temperature and promoting dormancy in Arabidopsis seeds

09:50 **Joan Renard (ENS de Lyon)**
Seed apoplastic barriers ensure seed viability and seedling establishment

10:10 **Yiqun Gao (University of Nottingham)**
Dirigent protein complex regulates monolignol polymerization and deposition at Casparian strip

10:30 **David Molina (ZMBP - University of Tübingen)**
From Phellogen to Phellem, a tale of MYBs Transcription Factors

10:50 TEA

SESSION 2 – ABIOTIC/BIOTIC INTERACTIONS I
Chairs: Isabel Molina and Kiran Suresh

11:20 Invited speaker: **Ohkmae Kim Park (Korea University)**
Apoplastic lignin-based barrier spatially restricts invading pathogens and cell death in plant immunity

11:50 **Pedro M Barros (ITQB NOVA)**
The impact of drought on phellem development: assessing morpho-physiological adaptations and gene expression dynamics in cork oak stems

12:10 **Alvaro Luis Jimenez Jimenez (Center for Reasearch in Agricultural Genomics)**
Engineering structural defense responses in tomato for resistance against the bacterial wilt

12:30 LUNCH

DAY 1: TUESDAY 13TH SEPTEMBER (CONTINUED)

SESSION 3 – STRUCTURE/METABOLISM I
Chairs: Ljerka Kunst and Chiara Campoli

14:00 Invited speaker: **Inês Barbosa (University of Lausanne)**
CASPs: MARVELous proteins shaping and sealing the Casparian Strip

14:30 **Concepcion Manzano (UC Davis)**
The role of SlEXO1 and SlEXO2 genes in controlling the exodermis lignification

14:50 **Glenn Philippe (Cornell University)**
Cutin polymerization and remodeling in tomato fruit through the coordinated action of enzymatically diverse GDSL-hydrolases

15:10 Invited speaker: **Teagen Quilichini (National Research Council Canada)** – virtual presentation
Between a cell and a hard shell: how the toughest PADiBa, the pollen wall, is made

15:40 TEA

SESSION 4 – EVOLUTION/ DIVERSITY I
Chairs: Siobhan Brady and Alex Canto-Pastor

16:10 Invited speaker: **Miranda Sinnott-Armstrong (Cambridge University – University of Colorado-Boulder)**
How plants modify their fruit epicarps to generate structural color

16:40 **Jian-Pu Han (University of Geneva)** – virtual presentation
Understanding the significance of adaptive suberin plasticity

Early Evening Reception at

Discovery Point

17:30

DAY 2: WEDNESDAY 14TH SEPTEMBER

SESSION 5– ABIOTIC/BIOTIC INTERACTIONS II
Chairs: Jocelyn Rose and Glenn Philippe

9:00 Invited speaker: **Mikio Nakazono (Nagoya University)** – virtual presentation
Genetic and physiological analyses of a barrier that restricts radial oxygen loss and prevents the entry of phytotoxins into the root

9:30 **Juan de la Cruz Jimenez (Nagoya University)**
The rice wax synthesis-related gene Leaf Gas Film-1 (LGF1) is involved in the formation of the radial oxygen loss barrier

9:50 **Carlos J. S. Moreira (ITQB-NOVA)**
Cutin depolymerisation generates oligomeric structures able to trigger plant immunity in Arabidopsis thaliana

10:10 TEA

SESSION 6 – SPECIAL SESSION
Chairs: Jens Tilsner and Zoe Barr

10:40 Invited speaker: **Yoselin Benitez-Alfonso (Leeds University)**
Cell walls at plasmodesmata and the regulation of intercellular transport

11:10 Invited speaker: **Tessa Burch-Smith (Donald Danforth Plant Science Center)**
Novel insights into the mechanism of secondary plasmodesmata formation for intercellular communication

11:40 **Oona Lessware (University of Bristol)**
How does the Nepenthes trap rim get its ridges? Common processes in a new combination create a complex hierarchical pattern

12:00 **Ruth Stark (CUNY - City College of New York)**
Potato/potahto, tomato/tomahto: biological inspiration for the design of protective barrier materials

12:20 LUNCH

DAY 2: WEDNESDAY 14TH SEPTEMBER (CONTINUED)

SESSION 7– EVOLUTION/DIVERSITY II
Chairs: Marie Barberon and Maria Capitão

14:00 Invited speaker: **Hugues Renault (IBMP - University of Strasbourg)**
A glimpse of plant adaptation to land through the biopolymer lens

14:30 Invited speaker: **Andrea Ramirez (Stanford University)**
A comparative study of adaptive stress tolerance in the Brassicaceae family

SESSION 8 – STRUCTURE/METABOLISM II
Chairs: Christiane Nawrath and Yifat Quan

15:00 Invited speaker**: Bénédicte Bakan (INRAE)**
Architecture of the cuticular biocomposite: challenges, news and prospects

15:30 **Alice Berhin (Uclouvain - Belgium)**
GPAT4, GPAT6, and GPAT8 are required for suberin deposition in roots of Arabidopsis seedlings with non-redundant functions to GPAT5 and GPAT7

15:50 **Jessica Sinka (University of Western Ontario)**
Metabolic flux analysis during wound-healing in potato tubers

16:10 – 18:30 TEA AND POSTER SESSION

Symposium Banquet at the

Malmaison Hotel

19:00

followed by a Scottish Ceilidh

DAY 3: THURSDAY 15TH SEPTEMBER

SESSION 9 – DEVELOPMENTAL EVENTS II
Chairs: Sarah McKim and Linsan Liu

9:00 **Christopher Grefen (Ruhr University Bochum)**
Lack of GDSL-motif containing proteins increases drought tolerance via modulation of the stomatal cuticle

9:20 **Trisha McAllister (University of Dundee)**
Identification of a key regulator controlling cuticular wax in barley

9:40 **Johann Petit (INRAE Bordeaux)**
The SlSHN2 transcription factor is essential for cuticle formation and epidermal patterning in tomato fruit

10:00 TEA

SESSION 10 – STRUCTURE/METABOLISM III
Chairs: Mark Bernards and Jessica Sinka

10:30 **Tonni Grube Andersen (Max Planck Institute for Plant Breeding Research)**
Walking the line - whole-plant effects of enhanced Casparian strip formation under natural conditions

10:50 **Olga Serra (University of Girona)**
The apoplastic barriers of potato roots: a tale of the suberin function in exodermis

11:10 **Nicolas Reynoud (INRAE-BIA)**
Architectural dynamics of the tomato cutin polymer matrix over fruit development

11:30 **Rochus Benni Franke (University of Bonn)**
Feeding a cross-linker – the metabolic control of suberin deposition

11:50 *Closing Remarks, EDI Statement, Survey*

12:00 LUNCH AND FAREWELL

Identification of a polarly localized oi1 barrier regulated by temperature and promoting dormancy in Arabidopsis seeds

Lena Hyvärinen, Anne Utz-Pugin, Christelle Fuchs, Lara Demonsais, Sylvain Loubéry and Luis Lopez-Molina

Department of Plant Biology, University of Geneva, Geneva, Switzerland

Seeds are highly resistant capsules sheltering the plant embryo and favoring plant dispersion and colonization of new habitats. Newly produced seeds exhibit primary dormancy, a trait whereby germination is repressed even under favorable germination conditions. Dormancy facilitates seed dispersion and improves reproductive success by promoting seedling formation during a season favorable for seed production. Accumulation of oxidative events in seeds reduces their dormancy whereas cold temperatures during seed development increase seed dormancy. The seed coat biophysical properties have a profound influence on seed dormancy most likely by limiting oxygen entry and, furthermore, there is strong evidence that cold enhances dormancy by modifying apoplastic barriers. The best documented case is that of flavonoid-derived tannins, which promote dormancy and whose levels increase during seed development in response to cold temperatures. Tannins accumulate in the inner integument 1 (ii1) cell walls that form a reticulated continuous structure surrounding the seed's living tissues. The role of other apoplastic barriers is less understood.
We found that cold induces the formation of a previously uncharacterized polar seed coat apoplastic barrier located on the external side of the outer integument 1 (oi1) cells. I will present our current understanding of its developmental origin, its composition, the transcription factors regulating its formation in response to cold and its role in seed physiology.

Seed apoplastic barriers ensure seed viability and seedling establishment

Joan Renard[1,2], Eduardo Bueso[2], Isabel Molina[3], Jekaterina Truskina[1], Nicolas M Doll[1] and Gwyneth Ingram[1]

[1]Laboratoire Reproduction et Développement des Plantes, ENS de Lyon, France
[2]Instituto de Biología Molecular y Celular de Plantas, Universitat Politècnica de Valènica, Spain
[3]Department of Biology, Algoma University, Canada

The *Arabidopsis thaliana* seed is a complex plant organ made from three genetically different tissues. Apoplastic modifications within the seed protect against oxygen penetration and damage and prevent cotyledon dehydration during germination. Moreover, they also facilitate slippage between tissues during embryo growth and germination. Of particular interest in this context are the embryo cuticle, the cuticle of the inner integument, and the suberin layer within the seed coat.
Regarding seed coat apoplastic barriers, we have found that the transcription factors COG1 and AtHB25 positively regulate suberin and/or cutin deposition in the seed coat by regulating genes encoding peroxidases and lipid polyester biosynthetic enzymes, respectively. Peroxidases have an essential role in lignin polymerization but may also be involved in suberin polymerization. AtHB25-regulated lipid polyester biosynthetic enzymes include LACS2 and the glycerol kinase NHO1. COG1 and AtHB25 control seed coat polyester layers important for seed longevity, likely in response to environmental cues such as light and temperature.
In contrast to seed coat apoplastic modifications, the embryonic cuticle, formed de novo on the embryo surface, needs to maintain permeability to allow embryo nutrition. The GSO1/GSO2 signaling pathway detecting the endosperm-processed peptide TWS controls embryo cuticle integrity. However, it remains still unclear how this is achieved mechanistically. Altering levels of reactive oxygen species in the apoplastic space between the embryo and the endosperm, where the embryo cuticle is deposited, leads to embryonic cuticle defects. NADPH oxidases may be necessary to allow the precise cuticle deposition needed during the cuticle gap-filling process.

Dirigent protein complex regulates monolignol polymerization and deposition at Casparian strip

Yiqun Gao[1], Jinquan Huang[2], Guilhem Reyt[3], Daiyin Chao[2], David Salt[1]

[1]Future Food Beacon of Excellence & School of Biosciences, University of Nottingham, Sutton Bonington, UK
[2]Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China.
3. The Laboratory of Plants Microbes and Environment Interactions, INRA-CNRS, Auzeville Tolosane, France.

The Casparian strip (CS) and suberin lamellae constitute the diffusion barrier in roots. Lignin polymerization and deposition are the core processes of CS development. Current knowledge shows peroxidases and RBOHF are essential during CS lignin polymerization. ESB1, a CS localized dirigent protein, also plays a vital role in lignin deposition, but the biochemical function of ESB1 in lignin deposition at CS is still unknown. Here, we show that ESB1-like dirigent protein family contains six members, classified into three subgroups. Proteins from all three subgroups are localized at the CS but show different localization features. The phenotype analysis of the double mutant of each subgroup showed all three subgroups are essential for CS integrity and correct lignin deposition at the CS. Interestingly, we find that the CS localization of CASP1 is dependent on the ESB1-like dirigent proteins. Moreover, these dirigent proteins appear to interact with each other to the potential for a complex. Our preliminary results from an *in vitro* lignin polymerization assay, suggest these dirigent proteins may regulate monolignol polymerization, and all the three subgroups of dirigent proteins seem essential for fulfilling this function. Overall, we identified the critical role a family of dirigent-like proteins plays in the formation of the CS .

From Phellogen to Phellem, a tale of MYBs Transcription Factors

David Molina[1], Tonni Grube Andersen[2], Wie Xiao[1], and Laura Ragni[1].

[1]ZMBP -The Center for Plant Molecular Biology, University of Tübingen, Germany
[2]Max Planck Institute for Plant Breeding Research, Germany

Plants have developed specialized tissues and polymers to keep their homeostasis and face several environmental stresses. In the root, at early developmental stages, the endodermis protects and regulates nutrient and gas exchange, while during secondary growth the predominant barrier that protects the plant against stresses is the periderm. The periderm is a multilayer barrier, comprising a meristematic tissue: the phellogen, the phelloderm, and the suberized/lignified phellem. The regulatory network underlying phellem differentiation is still largely unknown and only a few regulators have been identified. Based on transcriptomic data from several plant species such as poplar, potato, and Arabidopsis we selected a clade of MYB transcription factors that is expressed in the phellem of many species. Here, we show that this MYB TF clade has a key role in regulating suberin deposition in the phellem. Furthermore, transcriptomic analysis showed that these MYBs activate GDSL-lipases that are essential for suberin formation. In addition, the overexpression of these MYBs triggers the consumption of the phellogen, limiting phellogen divisions and root radial growth. Our results demonstrate a dual role for MYB TFs in the periderm, on one hand they promote suberin deposition in the phellem, while on the other hand they regulate cork cambium proliferation, shedding light on the mechanisms balancing stem cell proliferation and differentiation.

Apoplastic lignin-based barrier spatially restricts invading pathogens and cell death in plant immunity

Myoung-Hoon Lee, Hwi Seong Jeon, Eunjeong Jang, Seu Ha Kim, Ohkmae K. Park

Department of Life Sciences, Korea University, Korea

Plants are exposed to bacterial pathogens that have the ability to invade plant tissues and proliferate in the extracellular space or apoplast. Plants have evolved the immune system to recognize and limit the growth of pathogens. In spite of considerable progress in the study of plant immunity, the mechanism how plants restrict pathogen growth remains unclear. In this study, we report that lignin plays an essential role in plant immunity. We demonstrate that lignin deposition readily occurs during incompatible plant-pathogen interactions and this process requires Casparian strip membrane domain protein (CASP)-like proteins (CASPLs). CASPs are known to be the organizers of the lignin-based Casparian strip, which functions as a diffusion barrier in roots. The spread of bacterial pathogens is allowed and disease resistance is decreased by defects in lignin deposition. Moreover, the motility of pathogenic bacteria is negatively affected by lignin accumulation. These results suggest that the lignin-deposited structure functions as a physical barrier, similar to the Casparian strip, trapping pathogens and thereby terminating their growth. Moreover, our recent data show that lignin deposition is dependent on autophagy. I will provide evidence suggesting that autophagy regulates the transport of monolignols, building blocks of lignin, required for lignin barrier construction and disease resistance.

The impact of drought on phellem development: assessing morpho-physiological adaptations and gene expression dynamics in cork oak stems

Pedro M. Barros, Helena Sapeta, Diogo Lucas, Hugo Rodrigues, M. Margarida Oliveira

GPlantsS Unit, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Oeiras, Portugal

The longevity and high activity of the phellogen in cork oak (*Quercus suber*) are the cornerstones for the sustainable exploitation of cork (phellem), a unique raw material enriched in suberin. With extreme drought events imposing a negative impact on cork productivity and tree vitality, novel forest management strategies are required to shape the resilience and productivity of cork oak stands. This work aimed to characterize the molecular adaptations occurring in cork oak stems in adaptation to drought, and identify key genetic pathways regulating phellem development. One-year-old cork oak plants were grown for 6 months under well-watered (WW) or water-deficit (WD) conditions and main stems were targeted for histological characterization, transcriptomic analysis, and chemical profiling. WW treatment resulted in a 2-fold increase in stem diameter when compared to WD, with an increase in phellogen activity to cope with stem enlargement. Following a tissue-specific approach, we analyzed the transcriptional changes imposed by WD in phellem, phloem, and xylem, and found a global downregulation of genes related to cell division, cell wall biogenesis, lignin and/or suberin biosynthesis. Phellem and phloem showed a concerted upregulation of photosynthesis-related genes, suggesting a determinant role of stem photosynthesis in the adaptation of young plants to long-term drought. Based on gene co-expression analysis and literature search, we identified important genetic modules and candidate regulators of phellogen activity and phellem development, which are modulated by drought. The data gathered will be important to further harness the diverse genetic background of this species for the development of optimized management practices.

Engineering structural defense responses in tomato for resistance against the bacterial wilt

Álvaro Luis Jiménez-Jiménez[1], Anurag Kashyap[1,2], Montserrat Capellades[1,3], Weiqi Zhang[1] Sumithra Srinivasan[4], Anna Laromaine[4], Olga Serra[5], Mercè Figueras[5], Jorge Rencoret[6], Ana Gutiérrez[6], Marc Valls[1,7], Nuria S. Coll[1,3]

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, 08193 Bellaterra, Spain
[2]Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam 785013, India
[3]Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain,
[4]Institute of Material Science of Barcelona (ICMAB), CSIC, Campus UAB, Bellaterra, Spain
[5]Laboratori del Suro, Biology Department, Universitat de Girona, Campus Montilivi, Girona, Spain,
[6]Institute of Natural Resources and Agrobiology of Seville (IRNAS), CSIC, Seville, Spain
[7]Department of Genetics, Universitat de Barcelona, Barcelona, Spain

Vascular pathogens cause devastating diseases for plants, and are an important threat for several crops of great agronomic importance. These pathogens adopt different strategies to make their way into the xylem, and when this system is reached, they multiply massively, clogging the vessels, blocking water and nutrient transport, causing wilting of the infected plants and eventual death of the organism. Interestingly, resistant plants have evolved molecular mechanisms in the xylem vasculature to perceive pathogens and mount an array of defense responses against these aggressors. One of the most effective strategies described so far is the generation of physico-chemical barriers upon pathogen perception, which avoid the entry and multiplication of the pathogen in the vasculature. Recently, these molecular barricades were characterized in the pathosystem tomato-*Ralstonia solanacearum*, finding that cell wall reinforcements composed of lignin and suberin polymers were deposited in the xylem vasculature specifically in resistant cultivars upon infection. Taking advantage of this knowledge, we set out to generate commercial tomato cultivars resistant to vascular wilt diseases by engineering the metabolic pathways controlling the production of these barriers in the xylem vascular system.

CASPs: MARVELous proteins shaping and sealing the Casparian Strip

Inês C. R. Barbosa[1], Damien De Bellis[1], Isabelle Flückiger[1], Etienne Bellani[1], Mathieu Grangé-Guerment[1], Kian Hématy[1,2], Niko Geldner[1]

[1] Department of Plant Molecular Biology, University of Lausanne, Switzerland
[2] Institut Jean-Pierre Bourgin, INRAe, Université Paris-Saclay, France

The Casparian Strip is an extracellular barrier of the root endodermis, analogous to animal epithelia tight-junctions. It consists of a modified membrane-cell wall domain, where the plasma membrane of unique protein composition is tightly attached to the lignin-impregnated cell wall. The CASP1-5 (CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS), plant-specific MARVELs homologues to tight-junction occludins, were the first proteins identified to localize at the CS membrane domain (CSD), from where most proteins are excluded. Genetically, at least two CASPs are required for a continuous and functional CS, but how exactly remained unclear. Here, we isolated and characterized the CASP1-5 quintuple mutant, *caspQ.* We found CASPs are not required for localized lignification, since aligned lignin spots with lignin-polymerizing enzymes still occur in the mutant. Ultra-structurally, these spots appear as uncontrolled secretion hotpots and cell wall outgrowths. On the membrane side, *caspQ* is unable to exclude proteins from CSD, to form a membrane-wall attachment and has impaired exocyst dynamics. Complementation revealed CASP1-5 are partially redundant, and a minimum of three CASPs is required for full CS function. Finally, proximity labelling identified Rab-GTPases, known activators of the exocyst, as potential CASP-interactors. Rab-GTPases vesicles associate to the CS in a dynamic and CASP-dependent manner, resemblant of the exocyst complex. Altogether, our work reveals CASPs are required for CS controlled secretion, domain formation and membrane-wall attachment. We propose a model where CASP-microdomains displace initial secretory foci by excluding vesicle tethering factors, thereby promoting CS microdomain fusion into a membrane-cell wall band that seals the extracellular space.

The role of SlEXO1 and SlEXO2 genes in controlling the exodermis lignification

Concepcion Manzano[1], Kevin Morimoto[1], Lidor Shaar-Moshe[1], Alex G. Mason[1], Alex Canto-Pastor[1], Mona Gouran[1], Damien De Bellis[2], Robertas Ursache[2], Kaisa Kajala[3], Niko Geldner[2], Juan Carlos Del Pozo[4], Siobhan M. Brady[1]

[1]*University of California at Davis, USA*
[2]*University of Lausanne, Switzerland*
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Plant roots have developed root barriers to control soil interaction. One of these barriers is the endodermis, that when differentiated forms the Casparian strip (CS). The CS is a ring-like structure made of lignin that surrounds the endodermis cell. In a survey with more than 200 species of angiosperms, the authors found that almost 90% of them developed an exodermis that invariably has Casparian strips or suberin lamellae. The exodermis is the outermost cortex layer underneath the epidermis. In Solanum Lycopersicum (tomato) the exodermis is lignified and suberized. Here, we present 2 genes SlEXO1 and SlEXO2 that participates in the exodermis lignification in tomato. We have obtained CRISPR-Cas9 mutants and Overexpression lines for these genes and prove the function of these genes as repressors. Transcriptomic analyses from these mutant backgrounds show an over-representation of phenylpropanoid, lignin, and secondary cell wall biosynthesis genes among others. In addition, by analyzing the ionomic profile of these slexo1 and slexo2 CRISPR-Cas9 mutants and the slmyb36 CRISPR-Cas9 mutant, that has defects in the endodermis CS, we were able to analyze the consequences for the ionomic leaf profile of having defects in the lignin barriers in the exodermis, endodermis, and both cell layers.

Cutin polymerization in tomato is coordinated through spatiotemporal control by diverse GDSL-hydrolase enzymes

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The epidermis of the aerial organs of land plants is covered by a hydrophobic cuticle that plays many critical protective roles, most notably in preventing desiccation. This layer is structurally complex and highly dynamic, and must remain both flexible and intact, during organ growth and differentiation, but also retain its barrier properties. The mechanisms of cuticle assembly are not well understood, but tomato fruit development provides an excellent model to study such processes. Members of the *CUTIN SYNTHASE* (*CUS*) gene family, which represent a subfamily of GDSL-motif lipases, have been shown in tomato (*Solanum lycopersicum*) to polymerize cutin, a lipidic polyester that forms the major component of plant cuticles. However, based on spatio-temporal gene expression patterns and predicted protein structures, GDSL-lipase enzymes from other clades may also be involved in the maintenance of cutin integrity. We are testing the hypothesis that some GDSL-lipases contribute to the dynamic assembly of the cuticle by evaluating their activities through *in vivo* and *in vitro* enzymatic studies. In addition, to determine the biological functions of these proteins, we generated and characterized tomato CRISPR/*Cas9* lines of several GDSL-motif genes, including *CUS* genes and members of the *CUTICLE DEFECTIVE* (*CDEF*) gene family, which have been associated with cutinase activity. These investigations have led to a model of cutin deposition coordinated by GDSL-lipase enzymes in response to developmental cues or environmental demands.

Between a cell and a hard shell: how the toughest PADiBa, the pollen wall, is made

Teagen Quilichini

National Research Council Canada

The cell wall that encapsulates pollen grains is fortified by sporopollenin, a durable biopolymer contributed by the surrounding sporophytic tapetum. The inert nature of sporopollenin facilitated the terrestrial progression of plant life by enabling spore and pollen dispersal, but has challenged efforts to resolve its composition. This talk will outline major advances made in a century long effort to decipher the secrets of sporopollenin and how our understanding of this recalcitrant biopolymer has been shaped by cross-disciplinary research in molecular genetics, structural biology and chemistry. Insights provided by our research on the trafficking of sporopollenin from tapetal cells to the outer pollen wall in Arabidopsis will be presented, followed by a working model for the molecular structure of sporopollenin and its assembly into this complex and fascinating plant cell wall.

How plants modify their fruit epicarps to generate structural color

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The vast majority of plant species have a simple epicarp structure in their fruits: a cuticular layer surrounding the cell wall of the epicarp cells. These components provide structural integrity and protection from water loss, among other functions. However, some plant species have modified this typical epicarp to produce novel architectures that interfere with light to reflect color, a phenomenon known as structural color. These novel architectures are known as "photonic structures" (due their ability to reflect colorful light) and are constructed of a variety of materials in different plant species that result in a blue coloration to the fruit or seed. Several species (*Pollia condensata, Margaritaria nobilis*) reflect polarized blue light through modified cell wall, using helicoidal cellulose nanofibers. Several other species use lipids (*Viburnum tinus, Lantana strigocamara*) arranged in a multilayered structure embedded in the epicarp cell wall. In the case of *Lantana strigocamara,* the photonic structure is chemically and morphologically consistent with cuticle, suggesting the possibility that it has modified the production of cuticle to serve a secondary function of reflecting blue light to enhance seed dispersal. Here, we discuss the different types of modifications to the epicarp structure that result in distinct structural colors in fruits across plant species, and what we can learn about their evolution from their chemistry and nano-architecture.

Understanding the significance of adaptive suberin plasticity

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Due to sessile living conditions, plants forage the soil to acquire nutrients necessary for their growth and development. In this process, the root endodermis forms a checkpoint controlling the transport of nutrients through two distinct differentiation states: Casparian strips, wood-like barriers blocking the apoplastic pathway, and suberin, a cork-like substance, coating endodermal cells controlling the transcellular pathway. With these two distinct barriers roots can fine-tune nutrient uptake and adjust nutrition to stress conditions. In particular suberin lamellae formation is highly in response to nutrient availability, biotic and abiotic stresses, but the function of this plasticity in plant adaptation remains unclear.

In order to decipher suberin function in plant adaptation we developed high-throughput suberin patterning analysis in roots in order to screen for suberin variations in Arabidopsis accessions. For this purpose, we collected 284 natural accessions of Arabidopsis from the 1001 genome projects and screened for suberin variants. Combined with ionomic analysis these analyses provide a large dataset allowing to uncover the correlations between adaptive suberization and nutrients uptake. Moreover, genome-wide association was studied to identify the associated polymorphisms which would further help us understand how adaptive suberization was achieved at the molecular level. Overall this analysis of natural variation for suberin will pave the way for deciphering suberin function in plant adaptation.

Genetic and physiological analyses of a barrier that restricts radial oxygen loss and prevents the entry of phytotoxins into the root

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Some wetland plant species [e.g., rice (*Oryza sativa*)] form apoplastic barriers at the outer cell layers of their roots, and these barriers reduce radial oxygen loss (ROL) to the rhizosphere and prevent toxic compounds from entering the root. So far, the molecular mechanism of ROL barrier formation has not been well understood; to understand this mechanism, we used *Zea nicaraguensis*, a wild relative of maize (*Z. mays ssp. mays*), as a genetic resource. *Z. nicaraguensis* is known to tolerate soil waterlogging, attributed to the formation of a large volume of aerenchyma and a strong ROL barrier in the roots, which cooperatively enhance oxygen diffusion from shoot to root tips under waterlogged soil conditions. We recently demonstrated that the short arm of chromosome 3 in *Z. nicaraguensis* is responsible for the formation of a tight ROL barrier in the roots. We conducted genetic and physiological analyses to identify the gene controlling the ROL barrier formation. We produced a maize near isogenic line carrying the *Z. nicaraguensis* gene responsible for ROL barrier formation. Using the near isogenic line, we isolated the outer cell layers of the roots via laser microdissection and extracted RNA and then conducted RNA-sequencing analysis to identify the genes involved in ROL barrier formation. On the bases these results, we present the recent advances we have made in understanding the mechanisms of ROL barrier formation.

The rice wax synthesis-related gene Leaf Gas Film-1 (LGF1) is involved in the formation of the radial oxygen loss barrier

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Efficient oxygen transport via enhanced root aerenchyma and a barrier to impede radial oxygen loss (ROL) from roots to rhizosphere is essential for plants inhabiting wetland conditions. When grown in stagnant conditions, a rice mutant drp7 (dripping wet leaf 7), lacking a functional Leaf Gas Film-1 (LGF1) gene, forms poor aerenchyma spaces and a weak barrier to ROL in roots; whereas its wild-type (cv. Kinmaze) develop high root aerenchyma spaces and a tight barrier to ROL in roots. LGF1-overexpression lines in the drp7 mutant background were developed. The formation of a tight barrier to ROL was recovered in the overexpression lines when grown in stagnant conditions. In contrast, the aerenchyma formation was only partly recovered. Transgenic rice introducing the LGF1 promoter::GUS gene was used to visualize tissue-specificity of gene expression in roots. The GUS expression profiles indicated higher activity of the LGF1 gene in the hypodermal/exodermal cell layers near the root tips in stagnant but not in control conditions. Chemical characterization of enzymatically separated root hypodermal/exodermal cell layers indicated that the amount of suberin associated waxes was significantly higher in the wild-type genotype in comparison to the mutant line. The LGF1-overexpression lines showed significant increase in the amount of these suberin associated waxes in roots, in comparison to the mutant line. The identification of the LGF1 gene as a gene that is responsible for the development of tight barriers to ROL and increased suberin associated waxes in rice roots represents a major advance in the development of waterlogging tolerant plants.

Cutin depolymerisation generates oligomeric structures able to trigger plant immunity in Arabidopsis thaliana

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The acquisition of the cuticle constitutes one of the most important evolutionary adaptations of land plants. The lipid-based barrier protects against several threats, including pathogen attack. Though, microbial pathogens can breach the cuticular barrier to invade plant tissues, the plant senses the barrier degradation since some cutin-derived molecules act as signals to activate plant defences. In fact, cutin monomers are described in diverse plant species as initiators of plant responses to biotic attack. Oligomers (i.e. esterified cutin-based molecules), instead of monomers are more likely to be released during infection. How these bigger molecules impact plant response constitutes an important unresolved question. Cutin, which was isolated using an ionic liquid extractant that preserves the chemistry of the polymer, was used to generate cutin oligomeric mixtures (COMs). The COMs were subsequently screened for the ability to act as elicitors of plant immunity, monitoring calcium influx and MAP kinase activation, and transcriptional reprogramming as well. Under the test conditions, COMs induced a strong and reproducible calcium response and activate MAP kinases in Arabidopsis thaliana. On the contrary, cutin-monomers did not show any effect on calcium influx and MAPK activation. The transcriptional profile of the plant's response to COMs presents high similarity to other well described plant immune elicitors, as well as a certain level of uniqueness. These results are suggestive that COMs constitute a novel family of plant damage associated molecular patterns (DAMPs). Ongoing studies using MS-based methodologies and solution NMR are unravelling the identity of the oligomeric elicitors.

Cell walls at plasmodesmata and the regulation of intercellular transport

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A major route for cell-to-cell signaling in plants is mediated by cell wall-embedded pores termed plasmodesmata that forms the symplasm as a route for molecular diffusion. Plasmodesmata regulate plant development and responses to the environment by controlling the transport of essential metabolites, RNA molecules and multiple transcription factors. Cell walls offer physical constrictions to plasmodesmata aperture affecting their permeability thus cell-to-cell signalling. We have carried out a meta-analysis to identify proteins that regulate cell wall composition at plasmodesmata sites. Conditions were identified to induce the expression of these genes / proteins and we hypothesized these conditions likely affect symplasmic communication. We have experimentally tested this hypothesis by studying plasmodesmata response to osmotic stress as a condition affecting the expression of key plasmodesmata genes. In this talk I will present recent data mechanistically linking a member of the PDCB (Plasmodesmata- callose binding protein) family in controlling plasmodesmata in response to these osmotic stress conditions. I will also talk about our most recent advances in understanding the role of cell walls components on the regulation of plasmodesmata mechanics.

Novel insights into the mechanism of secondary plasmodesmata formation for intercellular communication

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Plasmodesmata (PD) are membrane-bound cytoplasmic bridges that span plant cell walls to form direct connections between neighbouring cells. They are essential for plant growth, development, and defence, as they allow metabolite exchange and signalling between cells. PD can form in already existing cell walls, allowing communication across tissues, as in between cell files in roots, and the epidermis and mesophyll in leaves. The mechanisms mediating the addition of these so-called secondary PD to existing cell walls have been elusive. Using several advanced imaging approaches, we have recently made significant progress into understanding the mechanism of secondary PD formation. While PD are essential to plant survival, they also serve as direct routes for the cell-to-cell spread of plant viruses during infection. Plant viruses are known to increase trafficking through PD in a process referred to as gating. Gating increases PD permeability, allowing viral genomes in association with host and viral proteins to move from infected to uninfected cells. Gating also increases the intercellular trafficking of fluorescent dyes and proteins used as markers for PD function. Our data reveal that viruses induce the formation of secondary plasmodesmata, reiterating that plant viruses are adept at manipulating PD, co-opting plant developmental programs for their success. We are currently using viruses as tools to further interrogate how PD form across existing cells walls.

How does the Nepenthes trap rim get its ridges? Common processes in a new combination create a complex hierarchical pattern

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The hierarchical pattern of radial grooves, ridges, and overlapping steps found on the trap rim (peristome) of carnivorous *Nepenthes* pitcher plants is unlike any other leaf. The unique surface topography renders the peristome highly wettable and slippery to insects when wet. Whilst the structure and function of the mature peristome are well documented, virtually nothing is known about how the peristome's characteristic microtopography is formed inside the developing pitcher bud. We hypothesise that conserved developmental processes are employed and combined by *Nepenthes* in a unique way to produce the intricate surface patterning of the mature peristome. Using scanning electron microscopy, we elucidated the peristome surface development for the first time. Strictly oriented cell divisions were followed by a sequence of distinct epidermal patterning events (conical cell formation, papillate cell outgrowth, and cell elongation) which are common across the plant kingdom. Our findings suggest that during peristome development, *Nepenthes* employ conserved patterning pathways for the formation of an entirely novel surface structure. In order to probe the genetic underpinnings of this series of events, we identified external morphological markers on the growing pitcher bud and linked these to a timeline of internal peristome development. This will allow us to target specific stages of surface patterning with confidence, which is crucial for investigating gene expression patterns and gene function during development.

Potato/potahto, tomato/tomahto: biological inspiration for the design of protective barrier materials

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Staple crops such as potato tubers and tomato fruits have versatile protective skins that mitigate environmental stresses from water loss, bruising, UV radiation, and microbial pathogens, also providing inspiration for the design of sustainable barrier materials for applications such as food packaging. We used the principal constituents of potato skins to develop self-assembling lamellar composites that have antimicrobial potential, and we blended tomato processing waste, algal polysaccharides, and beeswax to fabricate mechanically robust polymeric films that resist water uptake and transmission. These plant-inspired composites and bioplastics use feedstocks derived from abundant, renewable staple crops and ocean-based algae; it is estimated that they could replace as much as 90% of their persistent and hazardous petroleum-based homologs, though their scale-up would involve intensified farming that could compete with food production and require fertilizers that augment the production of greenhouse gases. Nonetheless, scientific studies of such plant-inspired composite materials lay the groundwork for repurposing abundant waste products generated during industrial food processing and for developing sustainable food packaging that can avoid the deleterious environmental impacts of petroleum-based plastics.

A glimpse of plant adaptation to land through the biopolymer lens

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Upon transition from water to land half a billion years ago, pioneer plants were exposed to the challenging terrestrial conditions. The ability to build extracellular protective barriers has most likely been a critical adaptation of land plants, as they shield cells from damaging environmental insults and allow the formation of specialized structures required for water management (e.g., cuticle). In vascular plants (i.e. tracheophytes), these barriers are essentially comprised of four hydrophobic biopolymers – cutin, suberin, sporopollenin and lignin – that reinforce and waterproof the cell wall. Based on angiosperm models, the understanding of genetic, biochemical and cellular determinants of biopolymer-mediated apoplastic barrier formation has made tremendous progress over the last decade. However, the evolutionary mechanisms that led to the emergence of land plant biopolymers are still unknown. To fill this gap, we implement multidisciplinary research in bryophytes, a group of non-vascular land plants reportedly able to produce only two biopolymers - cutin and sporopollenin. The presentation shall illustrate the power of this approach to elucidate the origin and early functions of biopolymers in the context of plant adaptation to land.

A comparative study of adaptive stress tolerance in the Brassicaceae family

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Plants often face adverse environmental conditions such as drought, flooding, and soil salinity due to their sessile lifestyle. Salt stress, in particular, poses a major threat to crop yield around the world. Roots respond to increases in soil salinity by modifying their anatomy and ensuring selective uptake of nutrients while excluding toxic ions. However, much of our understanding of root anatomy and function in response to salt stress comes from the stress-sensitive model organism *Arabidopsis thaliana* thus preventing the identification of adaptive traits that confer stress tolerance. The Brassicaceae family contains, apart from *Arabidopsis thaliana*, stress tolerant species such as *Eutrema salsugineum* and *Schrenkiella parvula*. In this study, we will use a comparative phenomics approach to understand the physiological and genetic mechanisms that contribute to the diversity in stress tolerance in species of the Brassicaceae family. In particular, I have identified striking differences in the anatomical features of the endodermal cell layer that may contribute to differences in solute retention or exclusion. The results obtained from this research will be important for future crop breeding strategies to make plants more stress resilient.

Architecture of the cuticular biocomposite : challenges, news and prospects

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Cuticles are hydrophobic supramolecular assemblies covering all plant surfaces. Cuticles fulfil multiple crucial biological functions by enabling terrestrial plant growth and resistance to biotic (pathogens) and abiotic (heat, drought, mechanical injury) stresses. In the context of plant adaptation to climate changes and reduction of pesticide, determining the structure and architecture of plant cuticles is an essential prerequisite to understanding and controlling their functionalities for sustainable crop production.

The cuticle can be considered as a polymeric composite displaying spatial heterogeneity. Indeed, the cuticle is composed of cutin polymer, an insoluble polyester of ω- and mid-chain hydroxy C16 and/or C18 fatty acids, which forms a matrix coated and filled by waxes. Glycerol and phenolics, although minor components, are also involved in the polymeric scaffold and its properties. The cuticle also comprises a significant amount of cutin-embedded polysaccharides (CEP). An unexpected discovery was that CEP exhibit specific chemical and structural features. While much progress has been made in understanding the composition of the cuticle, the fine structure and detailed architecture of the CEP-cutin network is just beginning and should further our understanding of the contribution of cuticle building blocks. Open questions include how these specific polymer systems evolve during the plant development and how it affects cuticle properties. Our knowledge of the architecture of cuticles is rapidly progressing thanks to the development of physical instrumentation and in the future, probably with the development of correlative investigations.

GPAT4, GPAT6, and GPAT8 are required for suberin deposition in roots of Arabidopsis seedlings with non-redundant functions to GPAT5 and GPAT7.

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A family of closely related land plant-specific glycerol-3-phosphate acyltransferases (GPATs) play a key role in the precursor synthesis of cutin and suberin strengthening the diffusion barrier properties of cell walls in specific cell types. GPATs have an acyltransferase and a phosphatase domain, although the site conferring phosphatase activity is only conserved in the GPAT4/6/8-clade that had been associated with cutin formation up to now. Arabidopsis *gpat4 gpat6 gpat8* triple mutant seedlings forming suberin in the endodermis exhibit a 75% reduction in the suberin polyester, affecting aliphatic monomers of all chain lengths as well as of ferulate. In contrast, the *gpat5 gpat7* double mutant seedlings exhibit a 50% reduction in suberin, affecting only aliphatic monomers, predominantly of C18-C24 in length. In the *gpat4 gpat6 gpat8* mutant endodermal suberin forms a continuous layer that is partially detached from the cell wall. In contrast, in the *gpat5 gpat7* mutant amorphous globular polyester deposits are formed in the apoplast that are not integrated into a lamellae structure despite the normal amounts of ester-bound hydroxycinnamic acids. While the phosphatase domain of GPAT4, GPAT6 and GPAT8 is required for high level suberin deposition, different lines of evidence suggest that the loss of phosphatase is essential for the formation of well-structured suberin lamellae. Thus, during evolution the accumulation of mutations in the phosphatase domain might have been central for the formation of suberin having specialized functions.

Metabolic flux analysis during wound-healing in potato tubers

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Suberin is a cell wall associated biopolymer that forms a protective barrier, providing structural integrity and limiting water loss, in roots, tubers, bark and specialized cells. Suberin is also deposited at the wound-site as an innate defence mechanism against threats to the newly exposed tissue. The chemical composition of suberin is well defined however the overall macromolecular structure remains unresolved. Despite this ambiguity, it is accepted that suberized cells possess both poly(phenolic) and poly(aliphatic) elements. Metabolic studies during the early stages of wound-induced suberization revealed highly coordinated, temporal changes between phenolic and aliphatic metabolisms, supporting the deposition of distinct phenolic and aliphatic domains as the tissue suberizes. To better understand the temporal pattern of suberin monomer biosynthesis and deposition our objective was to quantify the allocation of carbon between phenolic and aliphatic metabolisms. Through flux analysis, based on stable isotope labeling, metabolism leading to phenolic and aliphatic monomers was tracked over seven days in a potato model system using 'proxy' metabolites to measure phenolic and aliphatic destined carbon. Additionally, suberin polymer composition was assessed seven days post wounding to establish the incorporation pattern of heavy carbon into the suberin polymer. Quantification of the proxy metabolites suggests carbon is preferentially partitioned into suberin phenolics early-on during wound-induced metabolism, before being evenly partitioned between phenolic and aliphatic monomer biosynthesis. Greater understanding of suberin and suberization could lead to the production of enhanced crops (e.g., bolstering innate resistance to drought, pathogens, and improving storability) by informing strategies for genetic engineering and/or marker-assisted breeding.

Lack of GDSL-motif containing proteins increases drought tolerance via modulation of the stomatal cuticle

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Stomatal opening and closing underlies complex regulation that facilitate gas exchange across the impermeable cuticle of the plant surface. To control this gas exchange, guard cells undergo reversible changes in their shape and volume to regulate the dynamic of the stomatal pore, underpinning the importance of their cell wall structure. We have functionally characterized CGM4, a meristemoid expressed GDSL lipase and direct target of SPEECHLESS. Double knockout mutants of CGM4 and its closest sequence homolog CGM3 result in aberrant stomatal pore morphology. This causes a significant reduction of the stomatal aperture index – independent of ABA – and leading to increased ROS production, decreased transpiration rate, and enhanced drought tolerance. The cgm3cgm4 guard cells have an altered wax and cell wall composition. We hypothesize that the lack of flexibility of the stomata cell walls which is caused through these alterations impairs guard cell function.

Identification of a key regulator controlling cuticular wax in barley

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Land plants cover their epidermis with a specialised protective layer called the cuticle, a feature which is crucial for surviving the perils of a terrestrial environment, including desiccation, UV damage and pathogen attack, dangers which may worsen with our escalating climate crisis. Several cereal staples such as barley and wheat show further epidermal specialisation with a distinctive, glaucous wax bloom on reproductive stage tissues, a feature associated with improved drought tolerance. Understanding the mechanisms underpinning wax bloom development may help breed more climate resilient varieties. To date, studies on barley eceriferum mutants have identified several enzymes involved in cuticular wax biosynthesis. However, little is known about the upstream regulation of this process. My research addresses this knowledge gap by exploiting genetic resources to identify a key regulatory player in barley cuticle development. Here, I will discuss how I used high density genotyping, comparative gene expression and wax compositional analyses to identify a gene essential for wax bloom formation and the expression of cuticular metabolic genes in barley.

The SlSHN2 transcription factor is essential for cuticle formation and epidermal patterning in tomato fruit

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Tomato (*Solanum lycopersicum*) is an established model for studying plant cuticle because of its thick cuticle covering and embedding the epidermal cells of the fruit. In this study, we screened an EMS mutant collection of the miniature tomato cultivar Micro-Tom for fruit cracking mutants and found a mutant displaying a glossy fruit phenotype. By using an established mapping-by-sequencing strategy, we identified the causal mutation in the *SlSHN2* transcription factor that is specifically expressed in outer epidermis of growing fruit. The point mutation in the *shn2* mutant introduces a K to N amino acid change in the highly conserved 'mm' domain of SHN proteins. The cuticle from *shn2* fruit was strongly deficient in cutin (~5-fold reduction) while waxes were barely affected. In addition to alterations in cuticle thickness and properties, epidermal patterning and polysaccharide composition of the cuticle were changed. RNAseq analysis further highlighted the deregulation of hundreds of genes in the fruit skin of *shn2*, including genes associated with cuticle and cell wall formation, hormone signaling and response, and transcriptional regulation. In conclusion, we showed that a point mutation in the transcriptional regulator *SlSHN2* is sufficient to induce major changes in fruit cuticle formation and its coordination with epidermal patterning.

Walking the line - whole-plant effects of enhanced Casparian strip formation under natural conditions

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In roots, formation of the lignin-based Casparian strip (CS) prevents unwanted diffusion across the endodermis and allow the plant to control flow of nutrients and water into the vasculature. CS function is under tight spatial control by the master regulator MYB36 and the surveillance system termed the SCHENGEN pathway (SGN). Mutants in these genes typically have defective CS or activated compensatory mechanisms . Therefore, it remains difficult to untangle the direct function of the CS. - Especially when it comes to whole-plant effects under natural conditions, which can display a range of pleiotropic effects. In this work we employ MYB36 in a feed-back based expression system to create plants with earlier and wider CS restricted to their native pattern. We found that leads to improved abiotic stress tolerance in vitro, but severe growth retardation and dysbiosis in the microbial communities of both roots and shoots under natural conditions. With basis in a set of -omics and physiological analyses, we propose that this is due to a repression of immunity signals as a consequence of the stronger physical barrier, possibly through long-distance signaling from roots to shoots. Combined, our work provides important novel insights into the role of root barriers on a whole-plant level in a natural environment and highlights feedback-driven endogenous pattern overexpression as a powerful tool for the study of root barriers.

The apoplastic barriers of potato roots: a tale of the suberin function in exodermis

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The roots uptake water and nutrients from the soil and translocate them to the shoot. While doing this, they adjust the development of their apoplastic barriers, endodermis and exodermis, to restrict or allow the radial transport and thus better adapt to drought stress, flooding or nutrient scarcity and toxicity. Endodermis has been extensively studied in Arabidopsis and the role of suberin in selectively blocking nutrients and its plastic behavior is gaining knowledge. However, such progress for exodermis is still lacking, despite exodermis being present in around 90% of angiosperms including many crop plants. Potato (*Solanum tuberosum* L.), even though is the third most important crop in the world, behind only rice and wheat, has almost no references on the development and function of their root apoplastic barriers.

To make progress on that we first histologically identified the potato root apoplastic barriers. Although we observed variability in the overall root suberization pattern in different varieties or growing conditions, the suberin was first and mainly deposited in exodermis. To uncover the function of the exodermal suberin, we grew a root suberin-deficient mutant under the growing conditions in which the root prioritizes the exodermal suberization. The ionome analysis at two different stages of development revealed that the exodermal suberin blocks selectively the transport of nutrients, contributing to the shoot and root ionome homeostasis. We also obtained evidences that a deficiency in exodermal suberin decreases the plant water content and impacts on plant growth.

Architectural dynamics of the tomato cutin polymer matrix over fruit

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Emergence of land plant is concomitant with the specialization of plant epidermis through the deposition of a complex polymer matrix, i.e., the plant cuticle, that protects all aerials organs, fulfils multiple role in plant-environment interactions and ensure plant development. Such functions are inherent to the structural features of the cuticle and deciphering its dynamics upon organ expansion is one of the critical issues in our understanding of architecture-properties relationships of plant cuticle. Combining Raman imaging, multivariate analyses and biochemical analyses, we delineated the evolution of the cuticle's architecture of the cherry tomato model from the early cell expansion phase to the red-ripe stage. Theses approaches revealed a clear chemical clustering within the cutin polymer matrix. In particular, we demonstrated that these clusters are finely tuned during fruit development including compositional and macromolecular rearrangements. Besides, an unexpected dynamic remodelling of the cutin-embedded polysaccharides was highlighted. These results indicate a fine structural cluster-tuning of the cutin-polysaccharides continuum, consistent with the need of an architectural plasticity of cuticle to ensure fruit growth while maintaining a protective barrier. This study provides new insights into the plant cuticle architecture and in particular into the dynamic organization of the epidermal cell wall-cuticle upon development.

Feeding a cross-linker – the metabolic control of suberin deposition

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Critical for the root's function in water and selective nutrient transport are two apoplastic diffusion barriers in the endodermis, Casparian strips (CS) and suberin lamellae. Whereas the CS is dominated by the aromatic polymer lignin, suberin consists predominantly of lipids and only 1-5% of aromatics. Multiple genes in the biosynthesis and regulation of aliphatic suberin constituents have been functionally characterized in the past decade. However, little is known about the structural and physiological importance of aromatics in suberin lamellae formation and function.

We investigated the effect on endodermal barriers in several phenylpropanoid biosynthesis mutants. Consistent with the importance of the phenylpropanoid pathway (PPP) for lignin biosynthesis, some PPP mutants exhibited severe CS defects. Surprisingly, in two of these mutants suberin deposition was also delayed and they did not show the ectopic suberin deposition expected from the CIF/SNG surveillance pathway typically compensating CS deficiencies. The parallel characterisation of a mutant in an endodermis expressed gene putatively involved in phenylpropanoid cross-linking revealed a very similar delayed suberin development. Subsequent suberin analysis discovered oxidatively cross-linked aromatic dimers that were reduced in this mutant and the above PPP mutants. Furthermore, transmission electron microscopy revealed that the highly structured, lineally deposited suberin lamellae between the plasma membrane and the primary cell wall are undulated when phenylpropanoid cross-linking is inhibited. Together, our data indicate that an unrestricted metabolic flux through the PPP and the cross-linking of phenylpropanoid metabolites are required for the deposition and barrier function of developmental and CIF/SNG-induced responsive suberin.

1. Lack of GDSL-motif containing proteins increases drought tolerance via modulation of the stomatal cuticle

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Stomatal opening and closing underlies complex regulation that facilitate gas exchange across the impermeable cuticle of the plant surface. To control this gas exchange, guard cells undergo reversible changes in their shape and volume to regulate the dynamic of the stomatal pore, underpinning the importance of their cell wall structure. We have functionally characterized CGM4, a meristemoid expressed GDSL lipase and direct target of SPEECHLESS. Double knockout mutants of CGM4 and its closest sequence homolog CGM3 result in aberrant stomatal pore morphology. This causes a significant reduction of the stomatal aperture index – independent of ABA – and leading to increased ROS production, decreased transpiration rate, and enhanced drought tolerance. The cgm3cgm4 guard cells have an altered wax and cell wall composition. We hypothesize that the lack of flexibility of the stomata cell walls which is caused through these alterations impairs guard cell function.

2. An evolutionary role for aliphatic omega-hydroxylases in extracellular hydrophobic polymer emergence

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Land plants emerged from freshwater algae around 500 million years ago. They faced a new, harsh environment, including desiccation, high ultraviolet radiations, lack of buoyancy, and new pathogens. These environmental challenges provided a driving force for the acquisition of specialized metabolic innovations. Among them, the ability to build apoplastic barriers was one of the key innovations that allowed pioneer plants to adapt to terrestrial conditions. Seminal studies in angiosperms have shown that polymers shaping apoplastic barriers, i.e. cutin, suberin, and sporopollenin, are composed of a large fraction of aliphatic monomers. Moreover, compositional and enzymatic commonalities between these apoplastic polymers have recently led to hypothesize that they might have derived from a single ancestral polymer. However, the origin and emergence process of extant apoplastic polymers are unknown. To address this question, we focus on aliphatic omega-hydroxylases from to the cytochrome P450 superfamily. These enzymes catalyze the addition of a hydroxyl group at the terminal position of free fatty acids, a perquisite for the formation of ester bonds required for polymerization. Omega-hydroxylase emergence may be therefore regarded as a critical step in the evolutionary history of apoplastic polymer. We will present data from the functional analysis of genes encoding aliphatic omega-hydroxylase in two bryophyte species, the moss *Physcomitrium patens* and the liverwort *Marchantia polymorpha*, which represent the non-vascular stage of land plant evolution.

3. The function and regulation apoplastic barriers in the tomato root via single cell transcriptomics and physiological analyses of mutants

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Plants have evolved complex cell type regulatory processes to respond and adapt to dynamic environments. Changes in plant cell wall composition allow plants to adapt to external stresses. The composition of this matrix determines the diffusion and transport characteristics of the cell wall, and in turn, regulates how communication and exchange with the environment occurs. Some cell layers form additional diffusion barriers via the deposition of polymers such as suberin. The best studied example is the endodermis, which controls the diffusion of molecules in and out of the vasculature. However, many plant species also contain an additional barrier cell type right underneath the outer epidermis known as the exodermis. Exodermal differentiation and its ability to dynamically adapt to external conditions have not been formally characterized, nor the genetic and molecular mechanisms that govern them.

By leveraging single cell transcriptional sequencing of tomato (*Solanum lycopersicum*) roots, we have identified the expression dynamics during exodermal differentiation and have uncovered many of the genes that are activated during suberin deposition. We have validated many of these candidates using CRISPR-Cas9 mutagenesis. Finally, we have focused on two tomato mutants have significantly impaired suberin deposition in roots. We have exposed these mutants to water deficit and have explored the effects on relevant physiological traits. Understanding how these processes are regulated is critical to a plant's ability to adapt to changes in water or nutrient availability; and will enable the breeding of crops with higher resistance to drought and other stresses.

4. Comparing anatomy, chemical composition, and water permeability of suberized organs in five plant species: wax makes the difference

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Suberized cell walls formed as barriers at the plant/soil or plant/atmosphere interface in various plant organs (soil-grown roots, aerial roots, tubers, and bark) were enzymatically isolated from five different plant species (*Clivia miniata, Monstera deliciosa, Solanum tuberosum, Manihot esculenta, and Malus domestica*). Anatomy was investigated by microscopy and chemical composition was characterized by analytical chemistry. Barrier properties of the different suberized cell wall samples were quantified by measuring transpiration kinetics of water and permeances (speed of water loss in m s^{-1}) were calculated. Results clearly indicated that there was no correlation between barrier properties of the suberized interfaces and the number of suberized cell layers, the amount of soluble wax and the amounts of suberin. Suberized interfaces of *C. miniata* roots, *M. esculenta* tubers, and *M. domestica* bark periderms formed poor or hardly any transpiration barrier. Permeances varying between 1.1 to 5.1 · 10^{-8} m·s^{-1}, were very close to the permeance of water (7.4 · 10^{-8} m·s^{-1}) evaporating from a water/atmosphere interface. Suberized interfaces of aerial roots of M. deliciosa and tubers of *S. tuberosum* formed reasonable transpiration barriers with permeances varying between 7.4 · 10^{-10} to 4.2 · 10^{-9} m·s^{-1}, which were similar to the upper range of permeances measured with isolated cuticles (10^{-9} m·s^{-1}). Upon wax extraction permeances of *M. deliciosa* and *S. tuberosum* increased nearly by 10-fold. This proves that the transpiration barrier of a suberized cell wall is established by the wax molecules sorbed to the suberin polymer and not by the suberin polymer itself.

5. Lignin-based barrier restricts pathogens to the infection site and confers resistance in plants

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Pathogenic bacteria invade plant tissues and proliferate in the extracellular space. Plants have evolved the immune system to recognize and limit the growth of pathogens. Despite substantial progress in the study of plant immunity, the mechanism by which plants limit pathogen growth remains unclear. Here, we show that lignin accumulates in Arabidopsis leaves in response to incompatible interactions with bacterial pathogens in a manner dependent on Casparian strip membrane domain protein (CASP)-like proteins (CASPLs). CASPs are known to be the organizers of the lignin-based Casparian strip, which functions as a diffusion barrier in roots. The spread of invading avirulent pathogens is prevented by spatial restriction, which is disturbed by defects in lignin deposition. Moreover, the motility of pathogenic bacteria is negatively affected by lignin accumulation. These results suggest that the lignin-deposited structure functions as a physical barrier similar to the Casparian strip, trapping pathogens and thereby terminating their growth.

6. Pathogen-induced autophagy regulates monolignol transport and lignin formation in plant immunity

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The evolutionary plant-pathogen arms race has equipped plants with the immune system that can defend against pathogens. Pattern-triggered immunity and effector-triggered immunity are two major branches of innate immunity that share immune responses, including oxidative bursts, transcriptional reprogramming, and cell wall modifications such as lignin deposition. In a previous study, we reported that lignin rapidly accumulates in pathogen-infected Arabidopsis leaves and acts as a mechanical barrier, spatially restricting pathogens and cell death. Lignin deposition into the cell wall is a three-step process: monolignol biosynthesis, transport, and polymerization. While monolignol biosynthesis and polymerization are relatively well understood, the mechanism of monolignol transport remains unclear. In this study, we show that macroautophagy/autophagy modulates pathogen-induced lignin formation. Lignification and other immune responses were impaired in autophagy-defective atg (autophagy-related) mutants. In microscopy analyses, monolignols formed punctate structures in response to pathogen infection and colocalized with autophagic vesicles. Furthermore, autophagic activity and lignin accumulation were both enhanced in dnd1 (defense, no death 1) mutant with elevated disease resistance but no cell death and crossing dnd1-1 with atg mutants resulted in a lignin deficit, further supporting that lignin formation requires autophagy. Collectively, these findings demonstrate that lignification, particularly monolignol transport, is achieved through autophagic membrane trafficking in plant immunity.

7. An atypical lignin is deposited in the outer cell wall of the Arabidopsis root epidermis

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During land colonization, plants developed diffusion barriers to isolate themselves from the outside environment. In several species, including onion and soybean, modifications of the outer cell wall of the root epidermis with a “diffuse”, non-lamellated form of suberin that can be stained with lipid dyes, such as fluorol yellow (FY), has been described.
Here we report that in Arabidopsis the outer epidermal wall is reinforced by molecules that can be stained with Auramine O, but not with FY. Pharmacological inhibition of the phenylpropanoid pathway reduces the intensity of AO staining, while exogenous application of lignin monomers and other phenylpropanoids lead to increased AO staining of the outer cell wall. *PAL2* and *CH4*, key genes of the phenylpropanoid pathway, are expressed in the root epidermis, as well as several *RBOH* genes that are required the generation of reactive oxygen species. Mutations in *RBOH* genes and application of the peroxidase inhibitor SHAM lead to a reduction in AO staining. Furthermore, the quintuple mutant *cad4 cad5 f5H1 f5h2 ccr1* that is knocked out in several genes of the monolignol biosynthesis pathway shows reduced AO staining. These findings point to the deposition of a lignin-like polymer in the outer root epidermis. Since the lignin stain Basic Fuchsin does only weakly stain the outer root epidermal cell wall, but Safranine O stains as strongly as Auramine O, we hypothesize that an atypical lignin is deposited in the outer cell wall of the root epidermis of Arabidopsis.

8. Coordination of physical barriers and the glucosinolate-myrosinase defence system in Arabidopsis thaliana roots

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The accumulation of toxic glucosinolate (GSL) breakdown products constitutes a major chemical defense – or barrier system in Brassicales species including the model plant *Arabidopsis thaliana*. In contrast to our understanding of the spatiotemporal formation of physical barriers within the root, we know very little of where GSL are produced and deployed underground. However, evidence is emerging that the establishment of GSL-mediated protection becomes imminently vital for root regions which inherently have weak or no physical barriers. Intriguingly, this suggests an yet to be characterized interplay between chemical and physical root barriers that may be important under natural conditions.

Armed with CRISPR/Cas9-based genome editing, mass spectrometry, microdialysis-based exudate analysis, and advanced long-term *in vivo* root imaging we intend to systematically dissect how and where the GSL machinery is coordinated with physical root barriers under laboratory and – more importantly - under natural conditions in presence of microbial communities. We aim to shed light on the underlying plant processes that govern the regulation of synthesis, distribution and secretion of GSLs in coordination with the deployment of physical root barriers in the context of root microbiota and edaphic adaptations. A deeper understanding of the crosstalk between chemical and physical defense strategies and how these reshape the rhizosphere when grown in the wild could contribute to a multitude of potential agricultural implementations.

9. Interplay in the control of trichome development and cuticle formation in Nicotiana tabacum

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Trichomes and cuticle are protective epidermal specializations that help plants cope with challenging environmental conditions. Plants have developed gene networks to efficiently connect the build-up of these two epidermal protective layers. We were primarily interested in studying the development of glandular trichomes in *Nicotiana tabacum*. However, the study of several mutants and overexpressing lines of transcription factors from the MYB, ZINC FINGER and MADS family revealed a clear cuticle phenotype.

Detailed characterization of the transcriptional targets of these transcription factors could shed some light on the genetic interplay existing between trichome and cuticle formation in *Nicotiana tabacum*. Controlling trichome development, the biosynthesis of trichome-derived specialized metabolites as well as cuticle biosynthesis and deposition processes can be viewed as different aspects of a common defensive strategy adopted by plants to protect themselves from environmental stresses. Given the existence of several trichome developmental pathways depending on the plant species and the types of trichomes, genetic interactions between cuticle formation and trichome development are complex to decipher and to generalize. In addition to fundamental new insights on the regulation of these processes, identifying key transcriptional switches controlling both processes could also facilitate more applied investigations aiming at improving crop resistance to environmental stresses.

10. Regulation of endodermal suberization

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Suberin is a hydrophobic biopolymer deposited as a secondary cell wall of endodermal cells protecting the plant vasculature against stresses but also controlling water and nutrient acquisition. Endodermal suberization is highly plastic being regulated by both environmental and developmental cues. Previous work reported increased endodermal suberization in presence of ABA and an ethylene mediated decreased suberization. On the other hand, endodermal suberization is also induced upon Casparian strip defects through the endodermal integrity control system SCHENGEN3 (SGN3) and its ligand CIF1/2 (Casparian strip Integrity Factors 1/2). Although suberin plasticity is increasingly studied and appears to be a hallmark of endodermal suberization, the molecular players involved remain largely unknown. Recent work in the lab demonstrated that suberin induction through ABA and SGN3/CIFs involves independent pathways. Moreover, four different MYB transcription factors were associated with endodermal suberization. Indeed, MYB41, MYB53, MYB91 and MYB92 are expressed in endodermal cells and their expression is induced by suberin-inducing conditions. Additionally, all are sufficient to induce endodermal suberization. A simultaneous mutation of these four MYBs led to a with drastic reduction in endodermal suberization. More importantly, the quadruple mutant displays almost no suberin induction in the presence of ABA and CIF1/2. Although severely diminished, suberin is still present in some endodermal cells, indicating that the involvement of more factors in the regulation of suberin deposition in the endodermis is highly likely. In this context, more factors controlling endodermal suberization are being identified through gene expression analysis, genetic tools and bioinformatic data.

11. The putative role of Casparian strip membrane domain-like (CASPL) proteins in suberized tissues

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During the radial vascular growth occurring in mature organs that undergo secondary growth, like stems and roots of angiosperms and gymnosperms, protective primary barriers fail to expand in response to the internal pressures and break. Subsequently, a new protective barrier, the phellem or cork, forms in inner tissues that, as the organ increases in girth, will become the outer shell, sloughing off the tissues left outward phellem. The phellem, like endodermis, deposits lignin and suberin in their cell wall following a spatio-temporal pattern and prevent the organs from dehydration and pathogen attack. Transcriptomic studies in cork of *Quercus suber* (cork oak) highlighted a multigenic family of *Casparian strip membrane domain* (CASP)-like (CASPL) proteins, homologous to CASPs and upregulated in cork. Through forming membrane domains, the CASPs recruit the enzymatic machinery and guide the cell wall lignification to form the Casparian strip in Arabidopsis endodermal cells. Since CASPLs have predicted plasma membrane localization, it is suggested that this family could guide the cell wall modification in phellem cells, such as suberization. We first identified the CASPL orthologs in Arabidopsis and confirmed their promoter activation in root phellem and endodermis using GUS transcriptional reporter lines in native and inductive suberization conditions. We also described the putative subcellular localization using a translational reporter line. To unravel their function, we used a multiplex CRISPR-Cas9 system to simultaneously mutate four CASPLs. CASPL expression, CASPL localization and phenotypic results of the mutants will be discussed.

12. Functional characterization of a non-specific Lipid Transfer Protein putatively involved in suberization

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Suberin plays a key role in the protective function of phellem, a plant tissue present in stems and roots that undergo radial growth. Suberin is a hydrophobic biopolymer constituted by monomers derived from fatty acid precursors. These are synthesized in plastids, and they are elongated, oxidized and glycerilated in the endoplasmic reticulum and finally polymerized in the inner face of the polysaccharide primary cell wall. Non-specific Lipid Transfer Proteins (nsLTP) are proteins with a hydrophobic cavity able to transport hydrophobic molecules, such as fatty acids and derivatives. The role of nsLTPs in suberization is still unclear but they have been related with two scenarios: apoplastic proteins able to transfer the monomers to polymerization sites or intracellular proteins able to transport monomers within hydrophilic compartments, such as cytoplasm.

We first identified a nsLTP gene highly upregulated in suberized tissues of different plant species. Using a heterologous system in *Nicotiana benthamiana*, we assessed its subcellular localization using confocal imaging. The observations were compatible with a protein intracellular localization, despite the specific organelle were the protein is located was unclear. To reveal the function of this nsLTP, we identified the corresponding knock-out (ltp) Arabidopsis mutants and analyzed the phenotypic effects in root suberized tissues. We performed fluorol yellow staining and suberin chemical compositional analyses using gas chromatography. Considering that some nsLTP family members have been related with callose deposition, we also stained the callose deposits in root suberized tissues. Phenotypic data will be discussed.

13. Analysis of cuticle development and functional diversity reveals a key role for wax esters in the water barrier function of adult maize leaf cuticles

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The cuticle, a hydrophobic layer of cutin and waxes synthesized by epidermal cells, has many functions in protection of shoot tissues including restricting water loss. Transpiration across the cuticle (cuticular conductance, gc) is the major source of water loss at night and in water-limiting conditions when stomata are closed. gc is therefore a target trait for efforts to improve maize drought tolerance. Multiple lines of evidence support the conclusion that wax esters have a key function in restricting the evaporation of water across adult maize leaf cuticles. (1) Analysis of cuticle maturation along the developmental gradient of partially expanded maize leaves showed that maturation as a water barrier coincides with a replacement of alkanes with wax esters as the dominant cuticular wax class. (2) Natural variation in adult leaf gc values across the Wisconsin Diversity Panel is associated with the abundance of several wax esters; collectively, this group of waxes explains about 30% of the variance in gc. (3) A genome-wide association study identified a predicted Rab-GAP gene as a determinant of gc. The same gene was identified in a transcriptome-wide association study (TWAS) searching for genes whose transcript abundance in the cuticle maturation zone is associated with the abundance of ~50 cuticular wax components. Specifically, this Rab-GAP gene was associated by TWAS with six wax esters. In addition to strengthening the evidence of a role for wax esters in water barrier function, these findings implicate this predicted regulator of vesicle targeting in cuticle maturation as a functional water barrier.

14. Understanding the evolutionary and biosynthetic determinants of cuticle emergence in plants through the investigation of CUTIN SYNTHASES in Physcomitrium patens

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About 500 million years ago, an ancestor of embryophytes emerged from freshwater algae and started to colonize lands. This event, called plant terrestrialization, was one of the most formative evolutionary steps, paving the way for the development of nowadays terrestrial ecosystems. Upon the water-to-land transition, plants were exposed to many new constraints such as increased solar radiations, fluctuating temperatures, desiccating atmosphere, new pathogens or lack of buoyancy. A key adaptation of plants' life out of water has been the ability to synthesize a cuticle, a protective hydrophobic layer consisting of a polymer, cutin, covered with waxes. In angiosperms, cutin synthases (CUS) from the GDSL enzyme superfamily are involved in the polymerization of fatty acid monomers to form the cutin polyester. Most recent phylogenomic data indicate an emergence of *CUS* genes in the common ancestor of embryophytes, concomitant to cuticle appearance. In this context, we set out to perform a functional analysis of CUS homologs in the moss Physcomitrium patens that belongs to bryophytes, a group of non-vascular embryophytes. Combining molecular genetics, biochemistry, microscopy and analytical chemistry, we show that CUS function is conserved in embryophytes, therefore suggesting that the emergence of the CUS family was instrumental in the ability to form cuticle and in the adaptation to terrestrial conditions.

15. Heavy metal-associated isoprenylated plant protein 7 regulates plasmodesmata

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Plants rely on the interconnection of neighbouring cells to share resources and signals to coordinate developmental, physiological and pathogen defence responses of cells within a tissue. The plant cell wall is a barrier to connection overcome by membrane-lined intercellular channels called plasmodesmata. The overall structure of plasmodesmata has been determined, however, many molecular components remain unidentified. The mechanisms for plasmodesmata regulation are not fully understood and do not reach the level of sophistication expected of a system so essential to plant life. Here, a novel plasmodesmata regulating factor is introduced; Heavy metal-associated isoprenylated plant protein 7 (HIPP7). HIPP7 has been previously localised to plasmodesmata and ongoing work continues its characterisation to analyse HIPP7-mediated regulation of cell-to-cell connectivity. Current investigations involve the use of fluorescent tracers, callose quantification, confocal microscopy, and interactor screens. This includes use of the proximity-ligation system TurboID. Our characterisation of HIPP7 intends to provide more insight into the regulation of plasmodesmata.

16. Biosynthesis of fatty acid-based polymers in tobacco stigmas

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Plant stigmas defined as “wet” secrete a very abundant sticky exudate that is involved in pollen trapping and possibly plays a role in pollen-pistil recognition and defense against pathogens. In Solanaceae species, such as tobacco *(Nicotiana tabacum*), stigma exudates are rich in ω-hydroxy fatty acids. These ω-hydroxy fatty acids are present as polyesters (estolides) of glycerolipids, namely triacylglycerol, diacylglycerol and polar lipid fractions. A series of chloroform-extractable tetra- through heptaacyl glycerides has been previously identified in the tobacco stigma exudates. To gain insight into the mechanism of assembly and secretion of these novel lipids we have characterized their composition across tobacco stigma development and monitored their biosynthesis through [^{14}C]-acetate and [^{14}C]-glycerol radiolabeling using a novel *in planta* method. The distribution of TAG-estolides between the internal tissues and surface exudate implies that the polyesters assemble in the glandular tissue and are then exported to the stigma surface. Depolymerization of isolated TAG-estolides followed by gas chromatography analysis of released monomers confirmed that estolide chains are end-capped with a non-hydroxy fatty acid. Mild-transmethylation analysis showed that estolides can be attached to all sn-positions of glycerol, and that each TAG-estolide included a mixture of isomers of multiacyl glycerides. Progress on the characterization of the labeled polyester products and kinetics of their accumulation will be also presented.

17. Novel regulators of epidermal permeability

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The uncontrolled loss of water from plant aerial organs is restricted by the epidermis which is covered by a hydrophobic layer called cuticle. The cuticle is formed by epidermal cells and limits the diffusion of gases. Because of these properties, the majority of plant gas exchange occurs through stomata. Stomata are epidermal pores surrounded by two guard cells that control the stomatal aperture in response to multiple environmental factors and air pollutants such as ozone. Ozone is a powerful oxidant that penetrates plant epidermis via stomata and decomposes to reactive oxygen species (ROS) in the apoplast. Accumulation of ROS in the apoplast of guard cells initiates a signaling cascade leading to stomatal closure. Plants with high epidermal permeability, or those in which ozone-induced stomatal closure is impaired, receive high doses of ozone and develop easy-to-score leaf lesions allowing for identification of mutants. Based on this mechanism we have conducted a forward genetics screen aiming at the identification of novel regulators of epidermal permeability and stomatal function. Next to the ozone susceptibility, we have performed gas exchange-based assays to investigate stomatal responses. Additionally, genomic regions encoding known stomatal regulators have been sequenced to avoid re-discoveries. The screen yielded approximately 76 mutants impaired in stomatal closure and 20 mutants exhibiting high epidermal permeability. The causative mutations in four mutants exhibiting permeable epidermis were mapped to two genes previously implemented in cell wall biogenesis. Our current efforts aim at elucidating the role of these proteins in the determination of epidermal integrity.

18. The roots of the halophytic date palms exhibit a unique pattern of lignified diffusion barriers

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In the Middle East, the date palm (*Phoenix dactylifera*) is one of the most important crops. Since this plant is adapted to arid environments and produces vital fruits in desert regions, it was already called the "tree of life" in the Bible. In addition to the extreme conditions of heat and drought stress, this plant survives and thrives at beaches with direct root contact to highly saline seawater. However, date palms do not possess visible morphological adaptations like salt glands or salt bladders typically found in other halophytes. The underlying mechanism(s) of this exceptional high salt tolerance is so far unknown and revealing it could help to make other crops more salt resistant.
For this work, a hydroponic system was developed to cultivate date palm seedlings quickly and efficiently, allowing easy and reproducible quantitative investigations of salt tolerance-related seedlings and root phenotypes.
In this study, developing roots were investigated in terms of growth phenotypes and the formation and differentiation of the ion barriers Casparian strip (CS) and Suberin lamellae (SL) under control conditions and 200 mM NaCl stress. As a result, the root growth is inhibited under saline conditions. Histochemical staining shows that only the formation of SL but not the CS in the endodermis is developed closer to the root tip under NaCl influence. The SL and lignification of the exodermis, as well as the CS of the endodermis are not influenced by salt exposure and are formed at the same distance from the root tip. Notably, we discovered an early and apparently constitutively lignifying root epidermis in date palms, which could function as an additional ion barrier that is unique to date palms and may provide the basis for the exceptional salt tolerance.

19. Survey of root barriers in the legume family

Leonardo Jo, Rianne M. Kluck, Kaisa Kajala

Utrecht University, Netherlands

The root exodermis is characterized by the deposition of two secondary cell wall polymers, suberin and lignin in the outermost cortex layer. Many plant species can dynamically develop the exodermis in response to abiotic stresses. The phytohormone abscisic acid (ABA) is a core bridge between the abiotic stress perception and the exodermis differentiation program. Yet very little is known on how the ABA signal is integrated into the exodermis regulatory network. Our work aims to investigate the ABA regulatory network involved in exodermis differentiation of roots in different plant species. We focus on the legume family (Fabaceae) as it contains a diversity of exodermis forms with a wide range of developmental responses to ABA.

We initially surveyed for exodermal traits in different legume species. We identified few species with constitutively suberized and lignified exodermis, while others showed the unique ability to promote the exodermal differentiation only in the presence of ABA. Interestingly, we observed that the pattern of suberin deposition in the cortex differs across distinct legume species in response to ABA. While a few species showed a more diffuse deposition of suberin in the cortex, other species promoted the accumulation of suberin specifically in the outermost layer of the cortex. Our results have shown a wide range of exodermal responses to ABA in different legume species. Our next steps are building gene regulatory network models of the outermost cortex layer across the legume family and surveying the exodermal ABA responses across angiosperms.

20. Separation of cork and vascular cambia identities from a single cell

Jennifer López Ortiz[1,2], Hiroyuki Iida[1,2] and Ari Pekka Mähönen[1,2]

[1]University of Helsinki, Institute of Biotechnology, HiLIFE, Finland
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When organs undergo secondary growth, cork and vascular cambia are established as lateral meristems in most dicots. The vascular cambium is nested inside the cork cambium and these cambia provide thickness to stems and other organs. The vascular cambium is the inner lateral meristem and produces the conductive tissues –secondary xylem and phloem. The cork cambium provides the protective barrier, the cork layer, against abiotic and biotic stresses in organs undergoing secondary growth. In Arabidopsis roots, the vascular cambium emerges from procambial cells; the cork cambium arises from pericycle cells. Recently, from lineage tracing analysis, we have discovered that the xylem pole pericycle (XPP) cell contributes to the formation of both vascular and cork cambia. The XPP cell makes it possible to form a radially symmetric pattern in secondary tissue from a bisymmetric pattern in the primary root vasculature. When the XPP cells fail to produce either cambium, secondary tissue formation is severely disturbed. Therefore, the contribution of XPP cell lineage to both cambia is critical for the proper patterning. However, little is known about how cork and vascular cambia identities are diverged in the XPP cell lineage. By reporter analysis, we found that this identity separation happens in the early stage of secondary growth initiation. In this presentation, I will present how these identities are diverged in the XPP cell lineage and discuss potential mechanisms underlying the separation processes.

21. Arabidopsis thaliana cotyledon cuticle: forward genetic screen

Lucia Arenas Alfonseca, Christelle Fuchs, Christian Megies, Luis Lopez-Molina

Department of Botany and Plant Biology, University of Geneva, Switzerland

The plant cuticle is a hydrophobic layer of lipids, mainly cutin and waxes, that covers primary plant organs and forms a diffusion barrier between the plant and the surroundings. An embryonic cuticle is established early during embryogenesis and is present in the embryos of mature seeds. Nevertheless, this preliminary cuticle is still not -well sealed as in seedlings and is progressively acquiring low permeability during the embryo-to-seedling transition, as it is shown by toluidine blue O (TBO) uptake assays (De Giorgi et al., 2015) as well as in other approaches. Grafting experiments have shown that this sealing mechanism is a non-autonomous process that depends on the mature endosperm. Sulfated peptides released by the endosperm promotes cuticle restructuration in the epidermis of embryos. However, available evidence suggest that endosperm releases additional and unknown activities promoting seedling cuticle formation. To identify these activities, we developed a forward genetic screen to find mutants bearing defective cuticle. This approach allowed us to identify several loci, including characterized cutin biosynthesis genes, which establish the validity of the screen, but also previously uncharacterized genes.

22. Walking a waxy path: molecular characterisation of barley Eceriferum-yy

Chiara Campoli[1], Miriam Schreiber[2], Zenith Tandukar[3], Alasdair Iredale[1], Gary Muehlbauer[3], Micha Bayer[2], Sarah M. McKim[1] and Robbie Waugh[1,2]

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Improving crop productivity within our rapidly changing climate is pivotal to sustainably enhance agriculture. Cuticles cover all plant aerial organs, to protect the plant against a range of stresses from the atmosphere and terrestrial pests. Cuticles comprise a hydrophobic matrix of cutin polyester, embedded and covered with waxes. Synthesis and composition of cuticles varies within species and across plant organs and is controlled by environmental cues. Unravelling the genetic control of cuticle synthesis represent a promising route to manipulate plant adaptation to changing environments.

Barley is the fourth most cultivated cereal worldwide and an excellent model to study the genetic basis of crop adaptation to the environment. Barley *eceriferum* (*cer*) mutants showing glossy appearance of the leaves, leaf-sheaths or spikes, represent an attractive tool to dissect the molecular mechanisms underlying wax synthesis and deposition in cereals.

Here, I will describe the characterisation of *Cer-yy*, a dominant, organ-specific suppressor of wax accumulation present in wild and cultivated barley accessions. Composition and crystal structure of cuticular waxes showed that the non-glaucous appearance of the mutant spikes is due to the absence of β-diketones. An RNA-seq analysis revealed that *Cer-yy* mutants strongly down-regulated the expression of the *Cer-cqu* cluster, a key element for β-diketones synthesis, suggesting *Cer-yy* as major regulator of β-diketones accumulation in spikes. We fine-mapped the mutation to a sub-centimorgan region on barley 1H and identified a deletion associated with the mutation. I will present the major findings of this research and outline future developments aimed at identifying the *Cer-yy* gene.

23. Exploring endodermal translatomic in response to ABA

Laura Pérez-Martín, Kevin Robe, Vinay Shukla, Linnka Lefebvre-Legendre, Marie Barberon

Department of Botany and Plant Biology. University of Geneva, Switzerland

Suberin is a polyester deposited as a secondary cell wall of endodermal cells, forming a diffusion barrier for the transcellular pathway. Suberin is highly plastic and regulated independently by developmental and environmental cues. Suberin can be degraded by ethylene and induced by abscisic acid (ABA) or independently by the leucine-rich-repeat receptor-like kinase SCHENGEN3/GASSHO1 and its ligands CASPARIAN STRIP INTEGRITY FACTORS 1/2 (SNG3-CIF1/2) signaling. In addition, the transcription factors MYBs (41-53-92-93) are involved in the control of endodermal suberization in response to ABA or SGN3/CIF pathways.

However, the molecular mechanisms involved in the regulation of endodermal suberization are still largely unknown. In particular, the spatio-temporality of endodermal responses upon suberin-inducing stresses remains elusive. In this context, we aim to reconstitute the gene regulatory network controlling endodermal suberization. To this end, we are examining the impact of ABA in the endodermis by performing a time-course experiment using cell-type-specific ribosome pull-down and RNA sequencing.

Genes obtained from this translatomic analysis will be selected for their ontologies and specificity in the endodermis. Candidate genes will be validated through overexpression, knock-out, and promoter-reporter lines. Different suberin patterning and nutrient mineral content will be the key factors for the selection and validation of candidate genes which will be characterized in greater detail. Research on this topic will help reconstitute the chronology of endodermal responses, from ABA sensing to endodermal suberization We can predict that this work will identify new genes involved in this series of events, including new suberin regulators.

24. SlABCG36 and SlABCG42 act largely redundant in tomato fruit cutin formation

Yifat Quan[1], Aurore Guerault[1], Damien de Bellis[1,2] and Christiane Nawrath[1]

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Cuticle formation requires the export of cutin precursors by ATP-binding cassette family G (ABCG) transporters. *SlABCG36* and *SlABCG42* are two homologs of Arabidopsis AtABCG32 and encode closely related full-size ABCG transporters in tomato plants. Their down-regulation by a RNAi-approach had led to reduced cutin amounts and thinner fruit cuticles. Since both genes were simultaneously and partially silenced, the exact contribution of each gene and a full knockout of these transporters remained still to be elucidated. *SlABCG36* and *SlABCG42* knockout single and double mutants were generated by the CRISPR technology in the Micro Tom variety. Despite the high expression of *SlABCG36* in wild type (WT) tomato fruits, the single *slabcg36* mutant exhibited only a 20-30% reduction in cutin amount and no reduction in cuticle thickness at 20 dpa. No changes in cutin amount and cuticle thickness could be observed at 50 dpa, nor changes in epidermal cell shape. The double mutant however had a strong reduction in cuticle thickness and a flatter epidermal cell shape compared to the WT and the single mutants highlighting that both *SlABCG36* and *SlABCG42* act largely redundant in cutin formation. The absence of severe phenotypes, such as organ fusions, suggests that other export mechanisms, likely by ABCG-half transporters, also contribute to tomato fruit cuticle formation. Further investigations will be performed to shed light on potential compensatory mechanisms in *slabcg36/42* double mutants. Furthermore, the relation between cutin composition/amount and cutin structure as well as biophysical properties of the cuticle will be investigated.

25. Thickening the cuticular skin – The contribution of a novel eceriferum gene to the biosynthesis of cuticular waxes in Hordeum vulgare

Yannic Müller[1], Payal Patwari[2], Tyll Stöcker[3], Viktoria Zeisler-Diehl[4], Ulrike Steiner[3], Chiara Campoli[5], Ivan Acosta[6], Heiko Schoof[3], Lukas Schreiber[4], Peter Dörmann[1]

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Changing environmental conditions demand the expansion of the genetic resources to enable the rapid adaptation and development of novel crop species. The cuticle as direct interface between plants and their environment is thereby of special interest. Our study aimed to contribute to the knowledge about the genetic background of the cuticular wax biosynthesis in *Hordeum vulgare* in leaves. We characterized the cuticular wax compositions of wax-deficient eceriferum mutants and performed a BSR-Seq with these plants to identify a novel *eceriferum* gene in *H. vulgare*. The expression pattern of the gene was investigated and the gene-product was subcellular localized. Expression in heterologous host systems confirmed a function as fatty acyl-CoA reductase with a putative substrate specificity. Our results confirm that the identified gene locus contributes significantly to the biosynthesis of cuticular waxes in *H. vulgare*.

26. Regulation of endodermal barrier and its consequences for oxygen diffusion.

Vinay Shukla[1] and Marie Barberon[2]

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Oxygen availability inside plant tissues depends on its diffusion from photosynthesizing organs or uptake from surrounding environment. Reduced oxygen diffusion induces the formation chronic hypoxic niches, even when plants grow under aerobic conditions. Oxygen diffusion is affected by metabolic rates, cell proliferation, and physical barriers. The latter consists of secondary cell walls in which gas-impermeable polymers are secreted outside of the plasma membrane or primary cell wall. For example, in the root, endodermal cell walls are characterized by suberin lamellae that control the passage of nutrients and pathogens inside the vasculature. Endodermal suberin is regulated independently by developmental and exogenous signals to fine-tune suberin deposition in roots. We found a set of four MYB transcription factors (MYB41, MYB53, MYB92, and MYB93), each of which is individually regulated by these two signals and is sufficient to promote endodermal suberin. Mutation of these four transcription factors simultaneously through genome editing leads to a dramatic reduction in suberin formation in response to both developmental and environmental signals. Additionally, my observations indicate that this suberin deposition during the primary growth of plant in fact affects the oxygen in endodermis and the tissues surrounded by the endodermis. Therefore, I have set out to characterize the establishment of such chronic hypoxia in the root and investigate the effect of active hypoxia signalling components on the molecular regulation of suberin biosynthesis and deposition. I am combining genetic tools and microelectrodes to define the relationship between endodermis suberization, the generation of oxygen gradients in root.

27. Identification of a crosstalk between flavonoid biosynthesis pathways and apoplastic barrier formation in the Arabidopsis seed coat

Lena Hyvärinen[1], Anne Utz-Pugin[1,2], Christelle Fuchs[1], Lara Demonsais[1,2], Sylvain Loubéry[1,2] and Luis Lopez-Molina[1]

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The seed is composed of an embryo which is surrounded by two different tissues: the endosperm and the seed coat. In mature seeds, the seed coat is a dead tissue originating from maternal outer and inner integuments of the ovule. The seed coat confers mechanical resistance to the seed, limits oxidative damage and promotes dormancy. Thus, the seed coat enhances seed longevity and successful dispersion. In this context, the seed coat flavonoid-derived tannins play a major role as shown by *transparent testa* mutants, deficient in flavonoid synthesis. Tannins accumulate in the inner integuments (ii) of the seed coat.

Interestingly, by investigating histologically *transparent testa* mutant seeds produced in standard conditiond, we observed a reinforcement of the polar outer integument 1 (oi1) barrier compared to WT seed (cf Prof. Lopez-Molina's presentation). This reinforcement is similar to the one observed in seeds developed under cold temperature compared to seeds developed in standard conditions. This indicates the occurrence of safeguard mechanisms in the developing seed coat that compensate for the lack of tannins, which act as antioxidants and confer mechanical resistance, by promoting apoplastic barrier formation in other seed coat tissues.

In this poster, we present data showing evidence for the crosstalk between the flavonoid biosynthesis pathway and the pathway controlling the formation of the polar oi1 barrier. Furthermore, different hypotheses regarding the nature of this crosstalk and the approaches to investigate them will be presented.

28. Engineering plant extracellular vesicles to enhance growth and defence

Josh T Farley[1], Mahmoud K Eldahshoury[1], Vincent Postis[2], Carine de Marcos[3]

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Extracellular vesicles (EVs) are nanosized vesicles secreted by all cells. Similar to mammalian EVs, plant EVs are characterised by various sizes, density, specific protein markers and content. Three main types of apoplastic vesicles have been discovered: exosomes, microsomes and EXPO vesicles. It is suggested that all three EVs types can cross biological barriers and transfer sRNA in a cross-kingdom interaction to prevent pathogen infection. Our laboratory is starting to explore the role of EVs in plant communication and growth as well as the use of engineered siRNA-loaded EVs in spray induced gene silencing. We have first optimised the purification yield of EVs from leaf apoplastic fluids of Nicotiana Benthamiana. We then characterised further the EVs with Nanoparticle tracking, negative Stain EM and with specific protein markers. We confirmed the presence of exosomes in our fraction and identified a new soluble marker for exosomes. These results suggest the successful purification of exosomes from *Nicotiana benthamiana*. Future work will focus on translating exosomes purification protocols to other crop species as well as understanding exosome biogenesis for engineering purposes.

29. Biokinetics of foliar-applied pesticides: the role of the plant cuticle

Aline Xavier de Souza, David Stafford, Karen Meade, George Carter, Emily Reeves, Ellie Payne, Giovambattista Depietra

Syngenta Crop Protection, Jealott's Hill International Research Centre, Bracknell, UK

Foliar applied pesticides must be absorbed into plants and may also require their movement from the leaf surface and reach the target site to be effective.

However, their first site of contact is protected by the plant cuticle, a continuous hydrophobic membrane covering the outer surface of primary aerial organs. This outermost layer plays a critical role in the interaction of plants with its surrounding abiotic and biotic environment, comprising the main barrier for diffusion of water and uptake of solutes into the leaf tissues. Thus, the absorption and movement of the active ingredient (AI) across the cuticular wax barrier is a critical step for the performance of surface applied pesticides.

Due to their chemical nature, lipophilic compounds are rapidly absorbed by the plant cuticle. Hydrophilic formulations, on the other hand, move slower into the cuticular waxes, and pesticide efficacy is often enhanced with adjuvants that help to increase their cuticle penetration. Our cross-indication team is focused on further understanding the uptake, movement, and distribution of AIs within the plant body. For that, we use quantitative and qualitative methods (e.g., SEM images of the leaf surface, ^{14}C images, LC-MS analysis) that provide data on the speed of the AI movement and its accumulation in different leaf compartments. In short, we were able to show AI recovery over time for the leaf epicuticular surface, cuticular wax mixture and leaf tissue for different crop species, contributing to unveil specific features related to the biokinetics of agricultural compounds.

30. Conserved signalling components coordinate epidermal patterning and cuticular deposition in barley

Linsan Liu[1], Sarah B. Jose[2], Chiara Campoli[1], Micha M. Bayer[3], Miguel A. Sánchez-Diaz[1], Trisha McAllister[1], Yichun Zhou[1], Mhmoud Eskan[1], Linda Milne[3], Miriam Schreiber[3], Thomas Batstone[2], Ian D. Bull[4], Luke Ramsay[3], Penny von Wettstein-Knowles[5], Robbie Waugh [1,3], Alistair M. Hetherington[2], and Sarah M. McKim[1]

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Land plants seal their aerial epidermis with the waxy cuticle to prevent from water loss and other threats. To permit gas exchange and transpiration, the epidermis also forms specialised epidermal cell pairs that surround adjustable pores, called stomata. Other specialised cell types like hair cells also develop on the epidermis for defence and other functions. However, the mechanisms coordinating epidermal cell patterning and cuticle deposition to form a coherent surface remain poorly understood. In this study, we used two barley *eceriferum* (*cer*) mutants, *cer-g* and *cer-s*, which show defects in both stomatal patterning and cuticular deposition to investigate this knowledge gap. We found both mutants also show abnormal patterning of prickle hair cells and silica-cork cells, as well as cuticular defects on the grain that weakened grain-hull adhesion. We discovered that *CER-G* encodes a MAPKKK while *CER-S* encodes a BREVIS-RADIX-Like (BRX) domain protein. Our analyses suggest that these two genes interact and regulate a similar transcriptome associated with epidermal differentiation and cuticle formation in the leaf and gene expression linked to cuticular deposition and cell wall formation in the grain. We further show that these factors are land-plant specific, and revealed *CER-G* haplotypes with non-synonymous variation specific for cultivated barley. Taken together, our work suggests that these two factors may interact to link epidermal patterning with cuticle formation and also influence cuticular development in the grain, which may represent varied mechanisms coordinating epidermal features in contexts of different organs of barley.

31. Suberin monomers : in search of their itinerary to the apoplast and their fate

Etienne Bellani, Niko Geldner, Damien De Bellis

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Plants have developed several mechanisms to cope with fluctuations in water and nutrient availability, including formation of diffusion barriers. Among them, we find polyphenolic polymer and aliphatic polyester such as lignin and cutin, respectively, or combination of both in the form of poly(acylglycerol) polyester named suberin. It is established that main components of suberin are fatty acids, glycerol and ferulic acid. Suberin monomers formation go through a large set of modifications. Indeed, from synthesizing C16 /18 fatty acids to the production of acyl-glycerol-esters or ferulic acid esterifiation to w-hydroxyacids, many steps are required. Localized between the primary cell wall and the plasma membrane in Arabidopsis endodermal cells, suberin monomers have to be transported to the apoplast, where they are polymerized by a set of GDSL/GELP enzymes. The precise mechanism of transport of these monomers to the apoplast remains unclear. ATP-binding cassette transporters of the clade G (ABCG) have been proposed to actively transport suberin monomers, this includes ABCG1/2/6/16/20 in Arabidopsis. However, phenotypes observed in available mutants suggest they are either functionally redundant or additional ones are involved. Despite the involvement of the GDSL/GELP enzymes, the precise process and the cell wall remodeling occurring in the apoplast to form the complex suberin structure is unknown. In my project, I will try to elucidate the putative specific role of a set of ABCG transporters in the endodermis suberization based on current datasets available as well as reverse genetics, by generating high order mutants, and structure analysis through electron microscopy. Further, chemical analysis of the suberin will be performed. In parallel, the involvement of reactive oxygen species (ROS) in suberin formation will be investigated.

32. The CASPL2 family of Arabidopsis contributes to cuticle formation and stomata development

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CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS (CASP) and related CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS-like (CASPL) proteins are required for the deposition of lignin in Casparian strips and at other specific locations. Co-expression data indicate a possible link of *CASPL2A1* and *CASPL2C1* to cuticle formation. Despite both genes being epidermis-specific expressed, only the knockout of *CASPL2A1,* but not of *CASPL2C1* changes the composition of cuticular components. The *caspl2a1* mutant shows an increase in leaf waxes due to an augmentation in very-long chain fatty acids, and a reduction in unsubstituted fatty acids in cutin. Some other *CASPL2* family members contribute to cuticle formation since the permeability of the cuticle is only increased in sextuple mutant knocking out the entire *CASPL2* family (*caspl2S*). In addition, the stomata index as well as the number of stomata satellites were increased in leaves of the *capsl2S* mutants, but their leaf temperature was not changed. *CASPL2* family members contribute thus to different processes in cuticle formation and stomata development.

33. The effect of K deficiency on apoplastic barrier development in soil-grown maize roots

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Potassium (K) is the second most required mineral nutrient in plants and plays a versatile role in a plethora of physiological processes including water and nutrient uptake relations. In plant roots, the formation of suberin and Casparian bands in the endo- and exodermis can limit the apoplastic transport of water and mineral nutrients. However, there is little knowledge about how suberin and Casparian bands develop under K deficiency. Unfortunately, model plants such as *Arabidopsis thaliana* develop apoplastic barriers only in the endodermis and completely lack an exodermis. Therefore, we studied the effect of K deficiency on the formation of apoplastic barriers in soil-grown maize roots developing both endodermis and exodermis to better understand the relationship between K nutrition and apoplastic barriers.

We investigated changes in anatomical parameters of primary and seminal roots by histochemistry and microscopy, quantitative and qualitative suberization, and nutrient contents in different root developmental zones. Our results indicate that K deficiency remarkedly influences mineral nutrient uptake and apoplastic barrier development. We hypothesize that the sealed apoplast reduces the uncontrolled backflow of water and nutrients under K deficiency.

34. Investigating Plant Foliar Uptake of Herbicides

Emily Prince, Emily Stratford, Melissa Brazier-Hicks, Giovambattista Depietra

Syngenta Crop Protection, Jealott's Hill International Research Centre, Bracknell, UK

Herbicidal compounds are primarily sprayed onto plants and need to be absorbed into leaf tissue to reach their target and control weeds. The bioavailability (availability of compound at the target site) is influenced by absorption, distribution and metabolism. While the level of uptake will vary between different compounds with varying physical chemical properties, a single compound can also show variation in level of foliar uptake between different plant species. This can influence the overall level of weed control and the selectivity (the capacity of an herbicide to control weeds in a crop without harming the crop itself). In cases where there is higher uptake into weeds than crops this can contribute to selectivity. The level of uptake also varies with different formulations – formulation design can be a balance between getting enough compound into weeds without getting too much uptake into crops. We will illustrate how we use LCMS based techniques with non-labelled compounds to determine uptake profiles of AIs into weeds and crops to explain and optimise herbicide efficacy and selectivity.

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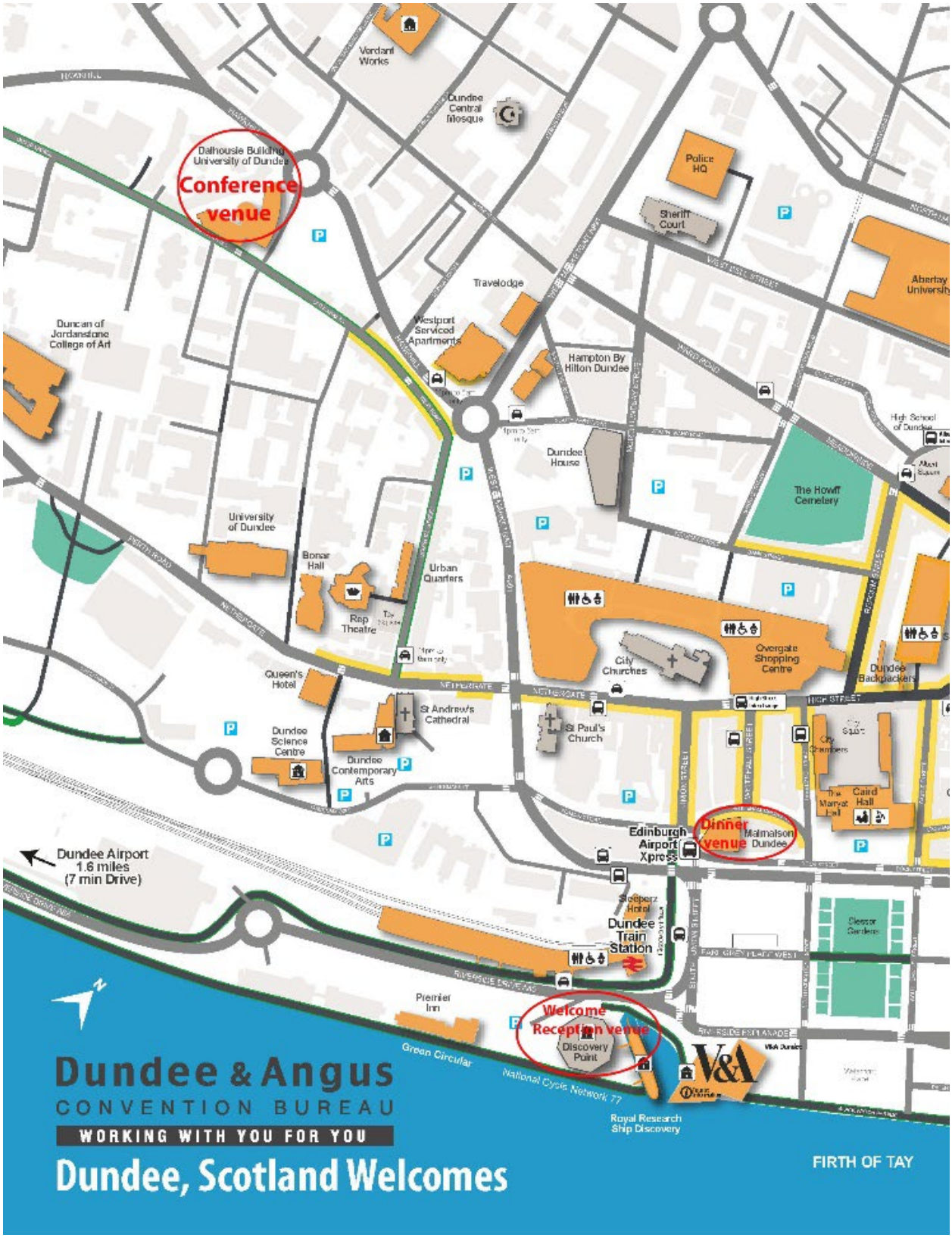
FINDING THE VENUE

The symposium will take place at
Dalhousie Building,
Old Hawkhill, Dundee City DD1 4HE

We are delighted to invite you to a
Welcome Reception
Tuesday evening at 17:30
at Discovery Point

The Symposium Banquet will take place
Wednesday evening at 19:00 at the
Malmaison Hotel
followed by a Scottish Ceilidh


Verdant Works
Dundee Central Mosque
Dalhousie Building University of Dundee
Conference venue
Police HQ
Sheriff Court
Travelodge
Westport Serviced Apartments
Hampton By Hilton Dundee
Duncan of Jordanstone College of Art
Dundee House
The Howff Cemetery
University of Dundee
Bonar Hall
Rep Theatre
Urban Quarters
Queen's Hotel
City Churches
Overgate Shopping Centre
Dundee Backpackers
St Andrew's Cathedral
St Paul's Church
Dundee Science Centre
Dundee Contemporary Arts
Caird Hall
Dinner venue
Malmaison Dundee
Edinburgh Airport Xpress
Dundee Airport 1.6 miles (7 min Drive)
Dundee Train Station
Premier Inn
Welcome Reception venue
Discovery Point
V&A
Green Circular
National Cycle Network 77
Royal Research Ship Discovery
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