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# **SCIENCE ACROSS BOUNDARIES ABSTRACTS (CELL AND PLANT BIOLOGY)**

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# PC1 PLANT CELL BIOLOGY

**ORGANISED BY:** PANAGIOTIS MOSCHOU (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), STEPHANIE ROBERT (UMEÅ PLANT SCIENCE CENTRE, SWEDEN) AND ALYONA MININA (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN)

**SESSION SPONSORED BY:** SWETREE TECHNOLOGIES

## PC1.1 DIVERSIFICATION OF MEMBRANE TRAFFICKING PATHWAYS DURING LAND PLANT EVOLUTION

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 TAKASHI UEDA (NATIONAL INSTITUTE FOR BASIC BIOLOGY, JAPAN)

@ TUEDA@NIBB.AC.JP

The membrane trafficking system responsible for transporting proteins, lipids, and polysaccharides plays pivotal roles in various plant functions including development, defense responses, intercellular communication, and cell wall biogenesis. This system involves evolutionarily conserved machinery components such as RAB GTPases and SNARE proteins, which regulate tethering and membrane fusion, respectively, between transport vesicles and destination membranes. It is reported that the numbers of genes for these machinery components have been increased during land plant evolution, which could be associated with diversification of membrane trafficking pathways and neofunctionalization and/or acquisition of organelles. For insights into diversification of the membrane trafficking system during land plant evolution, we are conducting comparative analyses of membrane trafficking pathways between *Arabidopsis* and the liverwort, *Marchantia polymorpha*. We have systematically identified RAB GTPases and SNARE proteins in *M. polymorpha*. Some of these molecules exhibited intriguing distribution and dynamics, which suggested that they act in unique secretion-related trafficking. Our latest results in this topic will be presented.

## PC1.2 DISSECTION OF A NOVEL PLANT CELL POLARITY PATHWAY

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 MARITZA VAN DOP (WAGENINGEN UNIVERSITY AND RESEARCH, NETHERLANDS), SAIKO YOSHIDA (NATIONAL INSTITUTE FOR BASIC BIOLOGY, JAPAN), DOLF WEIJERS (WAGENINGEN UNIVERSITY AND RESEARCH, NETHERLANDS)

@ MARITZA.VANDOP@WUR.NL

In plants, cell polarity is a crucial feature involved in development, defense, reproduction and transport. Proteins are targeted to specific domains of the cell to carry out local functions. We discovered a family of proteins in *Arabidopsis thaliana* with unique and robust polar localization throughout plant development. SOKs contain a well-conserved N-terminal region that we found to be a DIX domain. The DIX domain is a protein-protein interaction module known from the Dishevelled polarity regulator in animals. We found that the SOK DIX domain can self-interact and is necessary for polar clustering and biological activity. Here, we have used a biochemical approach to identify the context of these novel polar proteins. In immunoprecipitation / mass spectrometry experiments, we identified common and distinct interactors for three family members. We found that the DIX domain is required for protein complex formation. At least two of the interactors are directly recruited to polar sites by SOK proteins. We are currently exploring the molecular and cellular function of this novel polarity module. Taken together, our work showed that both plants and animals use the DIX domain in the context of cell polarity. Furthermore, SOK proteins allow exploring mechanisms underlying plant cell polarity establishment.

### PC1.3 TOWARDS STRUCTURAL INSIGHT INTO THE ENDOCYTIC TPLATE ADAPTOR COMPLEX

MONDAY 3 JULY, 2017

09:55

DANIEL VAN DAMME (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), KLAAS YPERMAN (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), JIE WANG (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), JOANNA WINKLER (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), EVELIEN MYLLE (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), MICHAEL VANDORPE (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM)

@ DANIEL.VANDAMME@PSB.VIB-UGENT.BE

The plant plasma membrane (PM) contains a wide range of receptors, channels and other integral membrane proteins that mediate communication of the cell with the outside world. Modulation of signalling pathways starting from the PM requires control over the PM proteome. While anterograde secretory pathways deposit PM proteins, their removal depends on retrograde transport by endocytosis, in which PM material and extracellular ligands are predominantly internalized using coated vesicles.

Clathrin-mediated endocytosis (CME), defined by the involvement of the scaffold protein clathrin to form the cage around the invaginating membrane, is the best characterized endocytic pathway in eukaryotes. Initiation of CME relies on adaptor proteins, which precisely select the cargo to be internalized, recruit the clathrin cage and facilitate membrane curvature.

The identification of the TPLATE complex (TPC) as a novel adaptor complex regulating CME in plants challenges the general belief that CME is highly conserved in eukaryotes. TPC is claimed to represent an evolutionary ancient adaptor module which is lost completely in the lineage leading to animal and fungal cells.

Structural modeling and identification of specific protein domains led to a theoretical model of the TPC. This model shows that the TPC shares many features with the evolutionary conserved AP-2 and COPI complexes, but also has distinct differences. Subunit co-interaction assays in yeast and *N. benthamiana* confirmed the structural predictions of the model and revealed that the TPC is likely a hexameric core complex which associates with its two peripheral subunits, forming the full octameric TPC at the PM.

### PC1.4 INVESTIGATING THE ROLE OF THE *ARABIDOPSIS THALIANA* GOLGIN AT GOLGIN-84B IN GOLGI BODY STRUCTURE AND FUNCTION

MONDAY 3 JULY, 2017

10:25

ANNE OSTERRIEDER (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), ALEX WISTOW (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM)

@ A.OSTERRIEDER@BROOKES.AC.UK

The Golgi apparatus is an intriguing organelle. Its seemingly simple morphology, a polarised stack of flattened cisternae, depends on complex cascades of tethering events. Tethering factors regulate the trafficking of cargo and the structural integrity of the Golgi

stack. Plant Golgi bodies move with or over the surface of the endoplasmic reticulum in an actin-dependent manner, which adds an additional layer of tethering complexity. Golgins are a subgroup of tethering factors, localising to Golgi membranes and containing long coiled-coil domains. These reach into the cytoplasm to bind to membranes, small GTPases and other tethering factors. Mammalian Golgin-84 is involved in the regulation of retrograde COPI-mediated transport and maintenance of the Golgi 'ribbon'. Two *Arabidopsis* Golgin-84 homologues, AtGolgin-84A and AtGolgin-84B have been identified, but their roles remain largely unknown.

Here we present novel data on AtGolgin-84B. Fluorescent fusions of AtGolgin-84B and the Golgi marker STtmd were transiently expressed in tobacco leaf epidermal cells and visualised using confocal laser scanning microscopy. Over expression of GFP-AtGolgin-84B resulted in increased clustering of Golgi bodies. Upon treatment with the secretory inhibitor Brefeldin A (BFA), GFP-AtGolgin-84B dissociated off Golgi membranes before STtmd-mRFP. After prolonged BFA treatment, GFP-AtGolgin-84B appeared to label the cytoplasm and surprisingly, in many cells, microtubules. Our data suggest that AtGolgin-84B is involved in retrograde transport and structural maintenance of plant Golgi bodies. This opens up new and exciting questions around the relationship between Golgi body structure and function, as well as about the nature of interactions between Golgi bodies and microtubules.

### PC1.5 S-ACYLATION: WHAT THE FLS2 IS GOING ON?

MONDAY 3 JULY, 2017

10:40

CHARLOTTE H HURST (UNIVERSITY OF DUNDEE AT THE JAMES HUTTON INSTITUTE, UNITED KINGDOM), PIERS A HEMSLEY (UNIVERSITY OF DUNDEE AT THE JAMES HUTTON INSTITUTE, UNITED KINGDOM)

@ C.H.HURST@DUNDEE.AC.UK

Receptor-Like Kinases (RLK) are single pass transmembrane proteins required to transmit extracellular signals into cells, allowing cells to respond and adapt to environmental changes. FLS2, the most widely-studied plant RLK, is the receptor for the bacterial protein flagellin and we have shown that FLS2 is S-acylated. S-acylation is a reversible and dynamic post-translational protein modification whereby fatty acids are added to cysteine residues but the effects of S-acylation on protein function are largely unknown. Here, using FLS2 as a model, we describe the effects of S-acylation on RLK function.

In plants treated with bacterial flagellin the amount of S-acylated FLS2 rapidly increases. To identify where and when S-acylation is occurring various mutants in the FLS2 signalling pathway were tested for flagellin-mediated increases in FLS2 S-acylation. Loss of components required for activation (co-receptor BAK1) or attenuation (E3 ubiquitin ligases PUB12/13) prevented the flagellin-mediated increase in FLS2 S-acylation. However, loss of components required for endocytosis (DYNAMIN-RELATED PROTEIN B) did not prevent flagellin-mediated increases in FLS2 S-acylation. Furthermore, it appears that S-acylated FLS2 accumulates in drp2B mutants. This suggests that S-acylation occurs after ubiquitination but before endocytosis of activated FLS2 and we hypothesise that S-acylation is required for efficient endocytosis.

We have identified the sites of S-acylation within FLS2 and found that they are conserved throughout the RLK superfamily. Based on these data, we currently hypothesise that S-acylation is an entirely novel means to regulate RLK function.

## PC1.6 VACUOLE BIOGENESIS – PUMPING UP THE VOLUME

📅 MONDAY 3 JULY, 2017 ⌚ 14:00

👤 KARIN SCHUMACHER (CENTRE FOR ORGANISMAL STUDIES (COS) HEIDELBERG UNIVERSITY, GERMANY), FALCO KRÜGER (CENTRE FOR ORGANISMAL STUDIES (COS) HEIDELBERG UNIVERSITY, GERMANY), JANA ASKANI (CENTRE FOR ORGANISMAL STUDIES (COS) HEIDELBERG UNIVERSITY, GERMANY), UPENDO LUPANGA (CENTRE FOR ORGANISMAL STUDIES (COS) HEIDELBERG UNIVERSITY, GERMANY)

@ KARIN.SCHUMACHER@COS.UNI-HEIDELBERG.DE

Plant architecture follows the need to collect CO<sub>2</sub>, solar energy, water and mineral nutrients via large surface areas. It is by the presence of a central vacuole that fills most of the cell volume that plants manage to grow large at low metabolic cost. In addition vacuoles buffer the fluctuating supply of essential nutrients and help to detoxify the cytosol when plants are challenged by harmful molecules. Despite their large size and multiple important functions, our knowledge of vacuole biogenesis and the machinery underlying their amazing dynamics is still fragmentary. In my presentation, I will try to reconcile past and present models for vacuole biogenesis and report on our recent findings concerning the pathways and the molecular machinery driving vacuole biogenesis and fusion.

## PC1.7 PROTEIN STORAGE VACUOLES ORIGINATE BY REMODELLING OF PRE-EXISTING VACUOLES IN *ARABIDOPSIS THALIANA*

📅 MONDAY 3 JULY, 2017 ⌚ 14:40

👤 MISTIANNE FEENEY (WARWICK UNIVERSITY, UNITED KINGDOM), MAIKE KITTELMANN (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), RIMA MENASSA (AGRICULTURE AND AGRI-FOOD CANADA, CANADA), CHRIS HAWES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), LORENZO FRIGERIO (WARWICK UNIVERSITY, UNITED KINGDOM)

@ M.FEENEY@WARWICK.AC.UK

Protein storage vacuoles (PSV) are the main repository of protein in dicotyledonous seeds. Little is known about the origins of these transient organelles. During seed maturation PSV are hypothesized to arise either *de novo* or to originate from the pre-existing embryonic vacuole (EV). We have tested these hypotheses by studying PSV formation in *Arabidopsis* embryos at different stages of seed maturation and have recapitulated this process in *Arabidopsis* leaves reprogrammed to an embryogenic fate by inducing expression of the *LEC2* transcription factor. Confocal and immunoelectron microscopy indicate that both seed storage proteins and tonoplast proteins typical of PSV are delivered to the pre-existing EV or to the lytic vacuole in embryo and leaf cells, respectively. In addition, serial sectioning through entire embryos at several developmental stages using serial block face scanning electron microscopy revealed the 3D architecture of forming PSV. Our results suggest that in *Arabidopsis* the pre-existing vacuole is reprogrammed to become the PSV.

## PC1.8 CELL-LAYER SPECIFIC ANALYSES OF THE ENDOMEMBRANE SYSTEM AND ESCRT-III IN BARLEY ENDOSPERM

📅 MONDAY 3 JULY, 2017 ⌚ 14:55

👤 VERENA IBL (UNIVERSITY OF VIENNA, AUSTRIA), JULIA HILSCHER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), AZITA SHABRANGI (UNIVERSITY OF VIENNA, AUSTRIA), ROLAND BERDAGUER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), ELSA ARCALIS (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), EVA STOEGER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA)

@ VERENA.IBL@UNIVIE.AC.AT

The barley endosperm tissue comprises transfer cells, embryo surrounding tissue cells, aleurone and starchy endosperm. The endomembrane system of endosperm tissue is characterized by a high structural plasticity and endosomal activity. Protein trafficking in these cells is complicated by the presence of several different storage organelles including dynamic protein storage vacuoles (PSVs) and protein bodies (PBs), the latter derived from the endoplasmic reticulum (ER). Recently we could show by live cell imaging, that the endomembrane system is capable of massive reorganization during barley endosperm development and germination.

The Endosomal sorting complex required for transport (ESCRT) consists of four subunits 0, I, II, and III and is responsible for endocytic recycling of membrane proteins. ESCRT-III is highly conserved and is responsible for membrane deformation. In detail, ESCRT-III mediates the biogenesis of multivesicular bodies (MVBs) where the sorted proteins are accumulated before they enter the next step of the pathway within the endomembrane system.

Recently we have identified barley ESCRT-III members in the (model) crop *Hordeum vulgare* (Hv) and show that all known members are expressed in developing barley endosperm. Here, we will present cell biological, molecular biological, bioinformatic and biochemical results indicating that ESCRT-III is possibly involved in functions that are barley endosperm specific, depending on the cell-layer. These results are accompanied by the spatio-temporal endomembrane rearrangement in developing barley endosperm. This outcome highlights the importance to be more specific and that it is indispensable to study the ESCRT-III complex in various plants and in different cell-layers in the specific tissue.

## PC1.9 CellularGA DISTRIBUTION GRADIENTS IN ARABIDOPSIS HYPOCOTYLS AND ROOTS

📅 MONDAY 3 JULY, 2017 ⌚ 15:10

👤 ANNALISA RIZZA (SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ANKIT WALIA (SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ALEXANDER JONES (SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

@ ANNALISA.RIZZA@SLCU.CAM.AC.UK

Gibberellin (GA) promotes cell elongation and plays a key role during different stages of plant development, such as seed germination, hypocotyl and root elongation, and transition to flowering. Studying how GA is distributed in specific tissues and cells and how GA concentrations vary over time is crucial to further understanding how GA regulates these processes. In order to visualize and quantify GA at the cellular level, we are using GPS1 (Gibberellin Perception Sensor 1), the first FRET biosensor which allows for high-resolution GA measurement in vivo. Using GPS1, we have discovered GA distribution gradients in multiple tissues such as Arabidopsis hypocotyls and roots and this distribution correlates with the rate of cell elongation. We have observed a reduced GA level in dark grown hypocotyls of a phytochrome interacting factor (PIF) quadruple mutant and an increased GA level in light grown hypocotyls of a phytochrome double mutant, indicating that PIFs promote GA level in the dark and that phytochrome mediated degradation of PIFs could lower GA in the light. In growing Arabidopsis roots, we have observed low GA levels in the root division zone grading to higher levels in the elongation zone. What establishes this gradient and how it is maintained remain unclear. We present evidence that patterned GA import and/or catabolic activity could contribute to the formation of the root GA gradient. We are now investigating how an ensemble of GA biosynthetic, catabolic, and transport activities together determine the differential GA distribution among the cells of root tip and hypocotyls.

## PC1.14 CELL SIZE DETERMINATION AND DIFFERENTIAL GROWTH REGULATION

📅 MONDAY 3 JULY, 2017 ⌚ 16:10

👤 JÜRGEN KLEINE-VEHN (UNIVERSITY OF NATIONAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA)

@ JUERGEN.KLEINE-VEHN@BOKU.AC.AT

Multicellular plants require particularly defined cellular strategies for tissue patterning and expansion, because the encapsulating cell wall literally binds neighbouring cells to each other. This interdependency limits cellular migration and, therefore, imposes outstanding importance to cell size determination and supracellular growth regulation. Phytohormones are central to these regulations and the unravelling of their subcellular working mechanisms bares high biotechnological potential. My lab is combining cell biological, physiological and developmental genetics approaches to decipher growth regulation on a sub-cellular, tissue and organ level (e.g. Barbez et al., Nature 2012; Ruiz Rosquete et al., Curr Biol 2013; Löffke et al., eLife 2015; Scheuring et al., PNAS 2016). On a subcellular level, we are particularly interested in cellular

organelles, such as the endoplasmic reticulum or the vacuole, and their mechanistic contribution to growth regulation. To address cell size determination and tissue growth in plants, we are utilizing epidermal cell files, displaying shorter (tricho-) and longer (atrachoblast) cell files. This cell biological model system allows us to identify cellular effectors controlling cellular expansion.

## PC1.15 GETting TO THE ROOT (HAIR) OF IT – INSERTION OF SNARE PROTEINS IN ARABIDOPSIS

📅 MONDAY 3 JULY, 2017 ⌚ 16:40

👤 CHRISTOPHER GREFEN (UNIVERSITY OF TÜBINGEN, GERMANY), SHUPING XING (UNIVERSITY OF TÜBINGEN, GERMANY), DIETMAR MEHLHORN (UNIVERSITY OF TÜBINGEN, GERMANY), LISA ASSECK (UNIVERSITY OF TÜBINGEN, GERMANY), MARKUS SCHWARZLÄNDER (UNIVERSITY OF BONN, GERMANY), GUIDO GROSSMANN (UNIVERSITY OF HEIDELBERG, GERMANY)

@ CHRISTOPHER.GREFEN@UNI-TUEBINGEN.DE

The Guided-Entry of Tail-anchored proteins (GET) pathway is viewed as textbook example of TA protein insertion into the ER membrane. In yeast, the cytosolic ATPase ScGET3 shuttles nascent TA proteins to the ER receptors ScGET1/2 for membrane insertion. Loss of ScGET function is dispensable and only a limited number of TA proteins have so far been tested to depend on GET for membrane insertion, leaving the question unresolved which alternative pathway(s) is(are) likely responsible for posttranslational insertion of most TA proteins.

We have identified and characterised the main orthologues of a putative GET pathway in plants and uncovered that GET *loss-of-function* lines in *Arabidopsis* show shorter root hairs, while otherwise growing normally. While this defect can at least in part be attributed to reduced abundance of an important plasma membrane SNARE (SYP123), lack of a more pronounced phenotype and identification of TA proteins that do not bind to AtGET orthologues suggest existence of alternative insertion pathways. Moreover, aberrant expression of the cytosolic AtGET3a in the *Atget1* line leads to a range of severe phenotypes from seedling lethality to reduced growth and fertility suggesting presence of alternative insertion pathways while highlighting an intricate involvement for the GET pathway in cellular homeostasis of plants.

See also: Xing et al. 2017, Proc Natl Acad Sci USA 114:E1544-E1553 (doi: 10.1073/pnas.1619525114)

## PC1.16 THE AUXIN-REGULATED CrRLK1L KINASE ERULUS CONTROLS CELL WALL COMPOSITION DURING ROOT HAIR TIP GROWTH

MONDAY 3 JULY, 2017 16:55

SEBASTJEN SCHOENAERS (UNIVERSITY OF ANTWERP, BELGIUM), DARIA BALCEROWICZ (UNIVERSITY OF ANTWERP, BELGIUM), GORDON BREEN (UNIVERSITY OF BRISTOL, UNITED KINGDOM), KRISTINE HILL (UNIVERSITY OF TÜBINGEN, GERMANY), MALGORZATA ZDANIO (UNIVERSITY OF ANTWERP, BELGIUM), GRÉGORIE MOUILLE (INRA, FRANCE), TARA J. HOLMAN (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JAESUNG OH (NATIONAL FUSION RESEARCH INSTITUTE, KOREA (SOUTH)), MICHAEL H WILSON (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), RANJAN SWARUP (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), WINNOK DE VOS (UNIVERSITY OF ANTWERP, BELGIUM), ISABEL PINTELOON (UNIVERSITY OF ANTWERP, BELGIUM), DIRK ADRIAENSEN (UNIVERSITY OF ANTWERP, BELGIUM), CLAIRE GRIERSON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), MALCOLM J BENNETT (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), KRIS VISSERBERG (UNIVERSITY OF ANTWERP, BELGIUM)

@ SEBASTJEN.SCHOENAERS@GMAIL.COM

Root hair (RH) morphogenesis is an auxin-regulated process ultimately dependent on synthesis, secretion and modification of the apical cell wall (CW). However, the link between auxin and CW dynamics remains elusive.

We characterized ERULUS (ERU), an auxin-regulated *Arabidopsis* receptor-like kinase from the *Catharanthus roseus* RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) subfamily of putative CW sensor proteins. *Eru* (-/-) RHs are short, swollen, and show irregular and slower growth. ERU transcription is confined to trichoblasts and commences before bulge formation. The ERU promoter contains AUXIN RESPONSE FACTOR (ARF) binding sites, suggesting auxin-dependent transcription. qPCR and micro-array data of control and *arf7/arf19* mutant roots treated with auxin confirmed the latter. ChIP-qPCR showed that the ERU promoter is a direct target of ARF7 and ARF19.

During RH growth, CW turnover is focused at the tip. Functional ERU-GFP localizes to the secretory pathway and the apical plasma membrane throughout RH development. Micro-Fourier Transform-Infrared (FT-IR) spectroscopy revealed compositional CW changes in *eru* mutant RHs. Immunolocalization of CW components, in vivo visualization of pectin Ca<sup>2+</sup> egg-box oscillations and determination of pectin methyltransferase (PME) activity lead to the conclusion that ERU regulates tip-growth through modulation of CW pectin dynamics by negatively regulating PME activity. In addition, ERU transcription was altered specifically in pectin-perturbed mutants, suggesting an ERU/CW feedback mechanism. We conclude that ERU, as a first, provides a direct link between ARF7/ARF19-mediated auxin signaling and cell wall dynamics during RH morphogenesis.

## PC1.17 DESIGNER ORGANELLES: SUBVERTING THE PEROXISOMAL IMPORT PATHWAY

MONDAY 3 JULY, 2017 17:10

ALISON BAKER (UNIVERSITY OF LEEDS, UNITED KINGDOM), LAURA L CROSS (UNIVERSITY OF LEEDS, UNITED KINGDOM), RUPESH PAUDYAL (UNIVERSITY OF LEEDS, UNITED KINGDOM), YASUKO KAMISUGI (UNIVERSITY OF LEEDS, UNITED KINGDOM), ALAN BERRY (UNIVERSITY OF LEEDS, UNITED KINGDOM), ANDREW C CUMING (UNIVERSITY OF LEEDS, UNITED KINGDOM), STUART L WARRINER (UNIVERSITY OF LEEDS, UNITED KINGDOM)

@ A.BAKER@LEEDS.AC.UK

The development of 'designer' organelles could be a key strategy to enable foreign pathways to be efficiently controlled within eukaryotic biotechnology. A fundamental component of any such system will be the implementation of a bespoke protein import pathway that can selectively deliver constituent proteins to the new compartment in the presence of existing endogenous trafficking systems. Here we show that the protein-protein interactions that control the peroxisomal protein import pathway can be manipulated to create a novel pair of interacting partners that still support protein import in vivo in moss cells but are orthogonal to the naturally occurring pathways. In addition to providing a valuable experimental tool to give new insights into peroxisomal protein import, the variant receptor-signal sequence pair forms the basis of a system in which normal peroxisomal function is downregulated and replaced with an alternative pathway, an essential first step in the creation of a 'designer' organelle.

## PC1.18 INTER-ORGANELLAR COMMUNICATION AND AUTOPHAGY DURING INNATE IMMUNITY

TUESDAY 4 JULY, 2017 10:30

SAVITHRAMMA DINESH-KUMAR (UC DAVIS, UNITED STATES), EUNSOOK PARK (UC DAVIS, UNITED STATES), JONGCHAN WOO (UC DAVIS, UNITED STATES), UGRAPPA NAGALAKSHMI (UC DAVIS, UNITED STATES), NEERAJ LAL (UC DAVIS, UNITED STATES), AMUTHA SAMPATH KUMAR (UNIVERSITY OF DELAWARE, UNITED STATES), ALI ALQARNI (UNIVERSITY OF DELAWARE, UNITED STATES), ALEXANDER NEDO (UNIVERSITY OF DELAWARE, UNITED STATES), JEFFREY CAPLAN (UNIVERSITY OF DELAWARE, UNITED STATES)

@ SPDINESHKUMAR@UCDAVIS.EDU

The innate immune system of both plants and animals employs cell-surface and intracellular receptors to detect pathogens and trigger defenses. Emerging evidence suggests that chloroplasts play an important function during innate immunity and they also have a central role in the production of immune signals. Our recent findings demonstrated that chloroplasts dynamically change their morphology by sending out stroma-filled tubular projections known as stromules during immune responses. Interestingly, stromules form complex associations with the nuclei

and subsequent clustering of chloroplasts around nuclei during immune response. I will discuss these findings and our recent results on the role of cytoskeleton on stromule formation and chloroplast association with nuclei during plant innate immunity.

Macroautophagy, hereafter referred to as autophagy, is a dynamic process that is conserved across eukaryotes and entails the engulfment of cellular components or cargoes in double membrane vesicles called autophagosomes. Autophagosomes are then targeted to the vacuole/lysosome for degradation or recycling. It has been well established that recycling of long-lived cellular proteins and organelles by autophagy is an important adaptive response to nutrient deprivation. However, recent studies have revealed that autophagy participates in other diverse biological processes including innate immunity and programmed cell death (PCD). I will discuss emerging perspectives on autophagy, cell death, and innate immunity. In addition, we will discuss strategies to identify small-molecule regulators of autophagy for disease control.

### PC1.19 ORCHESTRATION OF THE OXIDATIVE BURST IN ELICITOR-INDUCED IMMUNITY REQUIRES THE MULTIPLE ORGANELLE-TARGETED ARABIDOPSIS NPK1-RELATED PROTEIN KINASES (ANPS)

TUESDAY 4 JULY, 2017 11:10

DANIEL V SAVATIN (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), LUCIA MARTI (SAPIENZA - UNIVERSITY OF ROME, ITALY), NORA GIGLI BISCEGLIA (NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, NORWAY), VALERIA DE TURRIS (ISTITUTO ITALIANO DI TECNOLOGIA, ITALY), FELICE CERVONE (SAPIENZA - UNIVERSITY OF ROME, ITALY), EUGENIA RUSSINOVA (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), GIULIA DE LORENZO (SAPIENZA - UNIVERSITY OF ROME, ITALY)

KDASAV@PSB.VIB-UGENT.BE

Recognition at the plasma membrane of danger signals (elicitors) belonging to the classes of the microbe/pathogen- and damage-associated molecular patterns is a key event in pathogen sensing by plants. Triggering of appropriate downstream defense-related responses is achieved through rapid production and activation of signaling molecules as well as inter-compartmental communication inside the cell. Arabidopsis NPK1-related Proteins (ANPs) are mitogen-activated protein kinase kinase kinases previously shown to have role in immunity. In this paper, we studied the in vivo dynamic of ANP1- and ANP3-GFP fusions and found that in physiological conditions these proteins localize in the cytoplasm, while ANP3 shows also localization in mitochondria. After elicitor perception, both proteins localize also into plastids and nucleus, revealing a localization pattern that is so far unique. The N-terminal region was responsible for mitochondria and plastid localization of the proteins. Moreover, we found that sites of elicitor-induced ROS accumulation and ANP localization coincide and that plants lacking the ANP function do not produce intracellular ROS. Our results suggest that ANPs are required both for ROS generation and ROS signaling in those organelles, pointing to ANPs as central hubs in the orchestration of ROS accumulation and signaling.

### PC1.20 AUTOPHAGY IS REQUIRED FOR GAMETE DIFFERENTIATION IN THE MOSS *PHYSCOMITRELLA PATENS*

TUESDAY 4 JULY, 2017 11:25

VICTORIA SANCHEZ-VERA (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), CHANDRA SHEKAR-KENCHAPPA (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), KATARINA LANDBERG (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), SIMON BRESSENDORFF (UNIVERSITY OF COPENHAGEN, DENMARK), STEFAN SCHWARZBACH (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), TOM MARTIN (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES UPPSALA UNIVERSITY, SWEDEN), JOHN MUNDY (UNIVERSITY OF COPENHAGEN, DENMARK), MORTEN PETERSEN (UNIVERSITY OF COPENHAGEN, DENMARK), MATTIAS THELANDER (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), EVA SUNDBERG (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN)

VICTORIA.SANCHEZ@SLU.SE

Autophagy in plants has been widely related to cope with biotic and abiotic stresses, as well as to programmed cell death, promotion of lifespan or starch degradation. Much less is known regarding its role in plant cell differentiation. In our work we show that macroautophagy is highly active during germ cell differentiation in the early diverging land plant *Physcomitrella patens*. Our data provide evidence that suppression of ATG5-mediated autophagy results in reduced density of the egg cell-mediated mucilage that surround the mature egg, pointing towards a potential role of autophagy in extracellular mucilage formation. In addition, we found that ATG5- and ATG7-mediated autophagy is essential for the differentiation and cytoplasmic reduction of the flagellated motile sperm and hence for sperm fertility. Recently, similar results have been described in ATG7-knockout spermatozooids in mouse. These similarities strongly points towards an ancestral function of autophagy in gamete differentiation.

### PC1.21 DEGRADATION OF CELLULAR COMPONENTS BY AUTOPHAGY: FROM MOLECULES TO ORGANELLES

TUESDAY 4 JULY, 2017 11:40

DIANE C BASSHAM (IOWA STATE UNIVERSITY, UNITED STATES)

BASSHAM@IASTATE.EDU

Autophagy is a macromolecule degradation pathway in which cellular components are transported to the vacuole to recycle nutrients during nutrient deficiency and senescence and to clear damaged molecules and organelles during environmental stress. Autophagy therefore contributes to plant survival and growth during adverse environmental conditions. Autophagy can be non-selective, indiscriminately degrading cellular components, or selective, in which substrates for autophagy are recognized by receptor proteins and targeted for degradation. Autophagy is active at a low level under normal conditions for homeostasis and



is upregulated by many different stresses. We are analyzing the function and regulation of autophagy under different conditions, including its role in homeostatic rRNA degradation under normal growth conditions and in degradation of the endoplasmic reticulum during ER stress. Upstream regulators of autophagy activation have been identified, some of which are common to multiple stresses and some are specific to individual stresses.

### PC1.22 REGULATION OF UBIQUITIN-DEPENDENT TRANSPORT AND DEGRADATION OF MEMBRANE PROTEINS IN PLANTS

📅 TUESDAY 4 JULY, 2017 ⌚ 12:10

👤 ERIKA ISONO (UNIVERSITY OF KONSTANZ, GERMANY), KAMILA KALINOWSKA (TECHNICAL UNIVERSITY OF MUNICH, GERMANY), MARIE-KRISTIN NAGEL (UNIVERSITY OF KONSTANZ, GERMANY)

@ ERIKA.ISONO@UNI-KONSTANZ.DE

Plasma membrane receptors and transporters are important factors in various signaling pathways in plants that translate extracellular stimuli into intracellular signaling cascades. The abundance of signaling receptors at the plasma membrane is key for the regulation of downstream pathways and therefore underlies multiple layers of control both at the transcriptional and post-translational level. The ubiquitin-dependent degradation of plasma membrane proteins in the vacuole enables fast removal of target proteins and, as a consequence, rapid attenuation of downstream signaling events. In the past years, multiple plasma membrane proteins in plants were reported to be ubiquitinated and more and more molecular details of their regulation has been revealed. However, the molecular framework of the regulation of ubiquitin-dependent vacuolar degradation is still not completely elucidated. Our group is interested in the molecular mechanisms of the recognition of ubiquitinated cargos and the regulation of their transport to the vacuole. Our data show that many of the components required for this pathway are essential for growth and development of plants and that multiple ubiquitin binding proteins as well as deubiquitinating enzymes play central roles in the ubiquitin-dependent degradation of membrane cargos.

### PC1.23 MOLECULAR MACHINES UNDER TENSION: HOW KINESINS GET TO THE MICROTUBULE END AND POSITION THE PLANT CELL DIVISION PLANE

📅 TUESDAY 4 JULY, 2017 ⌚ 13:40

👤 ERIK SCHÄFFER (UNIVERSITY OF TÜBINGEN, GERMANY)

@ ERIK.SCHAEFFER@UNI-TUEBINGEN.DE

During cell division in plants, a specialized cytoskeletal structure, the phragmoplast, aids in the formation and orientation of the cell plate. The cell plate nucleates in the cytoplasm and grows towards the cell wall, where it merges with the cell membrane dividing the cell. The large motor proteins Phragmoplast Orienting Kinesin (POK) 1 and 2, belonging to the kinesin-12 class, are essential for

the guidance and orientation of the phragmoplast. Therefore, POKs are key to a proper cell-division-plane orientation and subsequent plant growth. However, little is known about the molecular mechanism of how POK motors operate. Here, we investigated the motor functionality using single-molecule fluorescence and force measurements. We found that GFP-tagged, truncated POK2 motor proteins - comprising the motor domain and the subsequent first coiled-coil domain - are moderately-fast, processive, dimeric kinesins which move towards the plus-end of microtubules. Surprisingly, the motor often switched between a processive and diffusive state. Furthermore, optical tweezers measurements show that POK2 stalls at forces below 1 pN making it a very weak kinesin. The weakness may be caused by switching to the weakly-bound, diffusive state. The mechanical properties indicate a complex interplay between the dynamics and localization of motors for phragmoplast guidance in plants.

### PC1.24 ROLE OF MICROTUBULES IN *ARABIDOPSIS THALIANA* POLLEN TUBE GROWTH

📅 TUESDAY 4 JULY, 2017 ⌚ 14:20

👤 LUCIE RIGLET (ENS DE LYON, FRANCE), FRÉDÉRIQUE ROZIER (ENS DE LYON, FRANCE), CHIE KODERA (ENS DE LYON, FRANCE), THIERRY GAUDE (ENS DE LYON, FRANCE), ISABELLE FOBIS-LOISY (ENS DE LYON, FRANCE)

@ LUCIE.RIGLET@ENS-LYON.FR

In plants, the female reproductive organ is covered with epidermal cells, named the stigmatic papillae, capable of trapping the male partner (pollen). After adhesion to the stigma, pollen germinates and produces a pollen tube that grows towards the ovules to transport the male gametes. During its growth through the cell wall of stigma papillae, the pollen tube applies an external pressure. Such physical forces are known to reorganize the cytoskeleton in plant cells. To investigate the potential role of stigmatic microtubules (MTs) in pollen tube growth, we examined *Arabidopsis* mutants impaired in MT pattern and dynamics. Using scanning electron microscopy, we observed that wild-type (wt) pollen tubes generally grow straight on the papilla surface of wt stigmas. By contrast, in one of the MT mutants, wt pollen tubes twist around the stigma papillae before reaching the underlying tissue. Application of the drug oryzalin, which destabilizes MTs, copies the defect observed in the mutant. Unexpectedly, these results suggest that MTs from the stigma papilla can orientate the path of the pollen tube growing at its surface. We are currently investigating the potential implication of a crosstalk between MTs and cell wall deposition in this guidance function.

## PC1.25 CONTROL OF PLANT DEVELOPMENT BY THE KINESIN-SEPARASE COMPLEX IN COORDINATION WITH UBIQUITIN-PROTEASOME SYSTEM IN ARABIDOPSIS

📅 TUESDAY 4 JULY, 2017 ⌚ 14:35

👤 CHEN LIU (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), PANAGIOTIS N. MOSCHOU (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN)

@ CHEN.LIU@SLU.SE

Plant growth and development are modulated by the interdependent cellular processes of cell expansion and cell division, but the feedbacks that link these processes remain poorly understood. The microtubule cytoskeleton and the ubiquitin-proteasome system (UPS) play a key role in these feedbacks (Moon et al., 2004) (Liu & Moschou, 2017). The microtubule-based kinesin-separase complex (KISC), comprising of three microtubule motors of the kinesin (Kin) 7.3-clade and the protease separase, regulates growth by executing the proteolytic processing of as yet unidentified targets (Moschou et al., 2016). Here, we report the identification KISC physical partners in an attempt to determine KISC proteolytic targets. We conducted a KISC interactome study using leaves from Arabidopsis plants expressing one of the three kinesin KISC components, the Kin7.3, as a translational fusion with Tandem Affinity Purification (TAP) at its carboxyl terminus. Kin7.3 showed enrichment of proteins that are components of the UPS. In accordance, a triple Kin7.3-clade loss-of-function mutant showed defects reminiscent of UPS mutants, such as, narrow and twisted cotyledons and leaves, aberrant venation patterns, and abnormal shoot development. We propose that KISC may optimize UPS activity to regulate cell division and expansion.

## PC1.26 DECIPHERING MOLECULAR COMPOSITION OF STRESS GRANULES IN ARABIDOPSIS THALIANA THROUGH ISOLATION OF TSN-INTERACTING PROTEINS

📅 TUESDAY 4 JULY, 2017 ⌚ 14:50

👤 EMILIO GUTIERREZ BELTRAN (INSTITUTE OF PLANT BIOCHEMISTRY AND PHOTOSYNTHESIS (IBVF)- NATIONAL RESEARCH COUNCIL (CSIC), SPAIN), PANAGIOTIS N MOSCHOU (DEPARTMENT OF PLANT BIOLOGY UPPSALA BIOCENTER SLU, SWEDEN), PETER V BOZHKOV (DEPARTMENT OF MOLECULAR SCIENCE UPPSALA BIOCENTER SLU, SWEDEN)

@ EMGUBE@GMAIL.COM

Efficient adaptation to stress depends on the availability of energy resources. Stress drives cells to an energy crisis whereupon they have to reduce energy expenditure in order to survive. To this end, eukaryotic cells compartmentalize specific mRNAs and proteins in cytoplasmic ribonucleoprotein complexes (mRNP) known as stress granules (SGs). In these structures mRNA molecules are stored, degraded or kept silent in order to prevent energy expenditure on producing useless, surplus or even harmful proteins under stress conditions. Molecular composition, structure, and function of SGs

in plants are largely unknown. Recently, we have revealed that Tudor staphylococcal nuclease (TSN) is essential for the integrity and function of SGs in Arabidopsis thaliana. Yet, TSN is stably associated with SGs, suggesting that it may serve scaffolding role to recruit other proteins to the mRNP complexes. Therefore we used TSN as bait in tandem affinity purification of SG-associated proteins. Localization of identified proteins to SGs in vivo has been further verified using a combination of biochemical and live imaging techniques. As a result, we have produced a list of SG-associated proteins. Some of these proteins have previously been found in animal and/or yeast stress-induced mRNP complexes, while others appear to be novel or plant-specific SG components.

## PC1.31 ORGANIC ELECTRONICS TO RECORD AND REGULATE PLANT PHYSIOLOGY

📅 TUESDAY 4 JULY, 2017 ⌚ 16:00

👤 MAGNUS BERGGREN (LINKÖPING UNIVERSITY, SWEDEN), ELENI STAVRINIDOU (LINKÖPING UNIVERSITY, SWEDEN), DANIEL SIMON (LINKÖPING UNIVERSITY, SWEDEN), ROGER GABRIELSSON (LINKÖPING UNIVERSITY, SWEDEN), DAVID POXSON (LINKÖPING UNIVERSITY, SWEDEN), XAVIER CRISPIN (LINKÖPING UNIVERSITY, SWEDEN), ELIOT GOMEZ (LINKÖPING UNIVERSITY, SWEDEN), OVE NILSSON (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), MICHAEL KARADY (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), AZIZ ALKATTAN (LINKÖPING UNIVERSITY, SWEDEN), ANNA GUSTAFSSON (UMEÅ UNIVERSITY, SWEDEN), SIAMSA DOYLE (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), STEPHANIE ROBERT (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), KARIN LJUNG (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), MARKUS GREBE (UMEÅ UNIVERSITY, SWEDEN)

@ MAGNUS.BERGGREN@LIU.SE

Organic electronic materials and devices can process and transport both chemical and electronic signals. This provides us with a technology platform that can translate signals in between biology, such as plants, and electronics. With sensors and delivery devices, based on organic electronics, we aim at developing a technology to record and regulate the physiology of plants in a highly accurate and feedback manner. Organic electronic devices and systems have been manufactured within or applied to plants systems. Within vivo-manufacturing protocols, both analogue and digital circuits have been established in the stems and leaves of living plants. Further, an organic electronic ion pump, which converts an electronic addressing signal into the delivery of phytohormones, has been applied to regulate the growth speed of the root hairs of Arabidopsis thaliana seedlings. With this novel technology, we explore organic electronics as sensor-actuator systems and as energy converting technology in plants.

### PC1.32 MODULATING AUXIN GRADIENTS IN *ARABIDOPSIS* WITH ORGANIC ELECTRONICS

TUESDAY 4 JULY, 2017 16:30

● MICHAL KARADY (UMEÅ PLANT SCIENCE CENTRE (UPSC) DEPARTMENT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), DAVID J POXSON (LABORATORY OF ORGANIC ELECTRONICS DEPARTMENT OF SCIENCE AND TECHNOLOGY LINKÖPING UNIVERSITY, SWEDEN), ROGER GABRIELSSON (LABORATORY OF ORGANIC ELECTRONICS DEPARTMENT OF SCIENCE AND TECHNOLOGY LINKÖPING UNIVERSITY, SWEDEN), AZIZ Y ALKATTAN (DEPARTMENT OF PHYSICS CHEMISTRY AND BIOLOGY LINKÖPING UNIVERSITY, SWEDEN), ANNA GUSTAVSSON (UMEÅ PLANT SCIENCE CENTRE (UPSC) DEPARTMENT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN), SIAMSA M DOYLE (UMEÅ PLANT SCIENCE CENTRE (UPSC) DEPARTMENT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), STEPHANIE ROBERT (UMEÅ PLANT SCIENCE CENTRE (UPSC) DEPARTMENT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), KARIN LJUNG (UMEÅ PLANT SCIENCE CENTRE (UPSC) DEPARTMENT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), MARKUS GREBE (INSTITUTE OF BIOCHEMISTRY AND BIOLOGY PLANT PHYSIOLOGY UNIVERSITY OF POTSDAM, GERMANY), DANIEL T SIMON (LABORATORY OF ORGANIC ELECTRONICS DEPARTMENT OF SCIENCE AND TECHNOLOGY LINKÖPING UNIVERSITY, SWEDEN), MAGNUS BERGGREN (LABORATORY OF ORGANIC ELECTRONICS DEPARTMENT OF SCIENCE AND TECHNOLOGY LINKÖPING UNIVERSITY, SWEDEN)

@ MICHAL.KARADY@SLU.SE

The organic electronic ion pump (OEIP) provides flow-free and accurate delivery of small organic and inorganic compounds at high spatiotemporal resolution. To date, the application of OEIPs has been limited to the delivery of non-aromatic or inorganic molecules to mammalian systems, particularly for neuroscience applications. Here, we demonstrate the use of an OEIP for plant hormone auxin *in vivo* delivery to the root tissues of *Arabidopsis thaliana* seedlings. This delivery was monitored in real time through dynamic fluorescent auxin-response reporters and induced specific modulation of plant physiology. Electronically regulated transport of aromatic structures such as auxin in an organic electronic device was achieved by synthesis of a previously unidentified class of dendritic polyelectrolyte. Our results provide a starting point for technologies enabling direct, rapid and dynamic electronic-based interaction with the biochemical regulation systems of plants.

### PC1.33 MULTICHANNEL AFM CHARACTERIZATION OF PLANT CELL WALLS AT THE NANOSCALE

TUESDAY 4 JULY, 2017 16:45

● KIRSTIN CASDORFF (ETH ZURICH EMPA DÜBENDORF, SWITZERLAND), TOBIAS KEPLINGER (ETH ZURICH EMPA DÜBENDORF, SWITZERLAND), INGO BURGERT (ETH ZURICH EMPA DÜBENDORF, SWITZERLAND)

@ KCASDORFF@ETHZ.CH

The macroscopic properties of the hierarchical material wood originate from the nanoscale structure and its chemical components. Revealing structure-property relationships of wood beyond SEM, TEM or nanoindentation requires the application of multichannel characterization at the nanometer scale. By using Atomic Force Microscopy (AFM) it is possible to push the limit of resolution down to the cell wall level in terms of imaging the topography. Furthermore, applying a mapping approach with force-distance curves in every pixel, like in Quantitative Imaging mode, allows for a mechanical characterization in terms of Young's Modulus, adhesion, and work of adhesion. We have developed a protocol for AFM to characterize the cell wall mechanically on the nanometer level, without embedding the samples. Thereby, it is possible to distinguish between structural features of the cell wall layers (compound middle lamella,  $S_1$ ,  $S_2$  and  $S_3$ ) of spruce wood on the basis of their Young's Moduli. Additionally, a change in cellulose microfibril angle in the transition zone,  $S_{12}$ , could be proven, by determining the stiffness gradient. Furthermore, it was shown that when tilting the orientation of the sample in the transverse plane from  $0^\circ$  to  $30^\circ$  the visualization of the  $S_2$  changes from a network to concentric parallel lamellas. The gained basic knowledge is crucial for our understanding of wood properties and their improvement in a controlled and desired fashion.

### PC1.10 PROBING THE EDGE PROTEOME: INVESTIGATING THE MECHANISM OF RAB-A5C ACTION IN *ARABIDOPSIS*

TUESDAY 4 JULY, 2017 POSTER SESSION

● LIAM ELLIOTT (UNIVERSITY OF OXFORD, UNITED KINGDOM), MONIKA KALDE (UNIVERSITY OF OXFORD, UNITED KINGDOM), IAN MOORE (UNIVERSITY OF OXFORD, UNITED KINGDOM)

@ LIAM.ELLIOTT@PLANTS.OX.AC.UK

Rab GTPases are regulators of membrane identity and vesicle trafficking in eukaryotes. In *Arabidopsis*, plant-specific RAB-A5c localises to a discrete population of vesicles at the geometric edges of cells in developing organ primordia. It has previously been shown that proper function of RAB-A5c is essential for anisotropic cell growth in these tissues (Kirchhelle et al., 2016). Evidence suggests that this cell edge compartment lies on an exocytic pathway however, the mechanism by which RAB-A5c acts on cell growth from this position remains unknown and no cargoes have been identified. We have taken a proteomics-based approach to investigate the function of RAB-A5c at the cell edges of developing primordia in *Arabidopsis*. A quantitative co-immunoprecipitation protocol using YFP:RAB-A5c generated a ranked list of specific candidate interactors of RAB-A5c. From this, 20 candidate interactors have

been selected for molecular characterisation. This has begun with analysis of the sub-cellular localisation of candidates to test for co-localisation with RAB-A5c at cell edges. Here we will present evidence that an uncharacterised LRR-containing protein is an interactor of RAB-A5c. Further work will investigate the effect of dominant-negative RAB-A5c on the localisation of this LRR protein. The impact of loss-of-function insertional mutants of this uncharacterised protein on RAB-A5c localisation will also be assessed. Through this approach, molecular insights will be gained into the function of the essential plant GTPase RAB-A5c, and edge-directed vesicle trafficking, in anisotropic cell growth and morphogenesis.

### PC1.11 RECEPTOR-LIKE CYTOPLASMIC KINASE PERSEUS IS AN IMPORTANT REGULATOR OF TIP GROWTH IN *ARABIDOPSIS THALIANA*

TUESDAY 4 JULY, 2017 POSTER SESSION

DARIA M BALCEROWICZ (UNIVERSITY OF ANTWERP, BELGIUM), SÉBASTJEN SCHOENAERS (UNIVERSITY OF ANTWERP, BELGIUM), GORDON BREEN (UNIVERSITY OF BRISTOL, UNITED KINGDOM), FLORIS VAN EYGEN (UNIVERSITY OF ANTWERP, BELGIUM), THANAA DOUBBO (UNIVERSITY OF ANTWERP, BELGIUM), KRISTINE HILL (UNIVERSITY OF TÜBINGEN, GERMANY), TARA HOLMAN (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JAESUNG OH (NATIONAL FUSION RESEARCH INSTITUTE, KOREA (SOUTH)), RANJAN SWARUP (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), MICHAEL WILSON (UNIVERSITY OF LEEDS, UNITED KINGDOM), MARIOS N MARKAKIS (UNIVERSITY OF ANTWERP, BELGIUM), IVE DE SMET (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), LAM DAI WU (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), MALCOLM J BENNETT (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), CLAIRE GRIERSON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), KRIS VISSENBERG (UNIVERSITY OF ANTWERP, BELGIUM)

@ DARIA.BALCEROWICZ@UANTWERPEN.BE

We describe identification and characterization of an *Arabidopsis thaliana* class VII receptor-like cytoplasmic protein kinase (RLCK) which we named PERSEUS. Its role in plant development was investigated using genetic, molecular and cell biological approaches. Promoter-reporter studies showed that PERSEUS is exclusively expressed in root hairs and pollen (tubes), both tip growing cell types. *Perseus* mutants revealed a 34% reduction in root hair length, a 30% reduction in RH growth velocity and aberrant root hair morphologies. Moreover, *perseus* pollen exhibited 80% reduced germination and 27% shorter pollen tubes *in vitro*, although no alterations were seen in fertilization efficiency. PERSEUS expression can be induced by auxin and depends at least on the presence of auxin response factors ARF7 and ARF19, both transcription factors. Chromatin immunoprecipitation followed by gene-specific qPCR showed that ARF19 binds directly to the promoter region of PERSEUS *in vivo*. Functional GFP-PER localized to the cytoplasm, nucleus and/or ER in *Arabidopsis* seedlings. *Perseus* RH cell walls seem not affected (FT-IR), yet changes are detected in the responsiveness to alterations in the pH of the growth medium and supplementation with fusicoccin and DCCD (which both alter H<sup>+</sup>-ATPase activity). (Phospho) proteome analysis showed that PERSEUS function could be related to water stress regulation. Hence, *perseus* root tips exhibit a 7-fold decrease in phosphorylation of

the GTL1 transcription factor at serine 17, and the RESPONSE TO DESSICATION2 at serine 22. In addition, *perseus* roots show a 14-fold decrease in calreticulin 1b abundance. These proteins have been related to water stress management in *Arabidopsis*.

### PC1.12 PECTIN MODIFICATION AND PROMOTION OF ROOT ELONGATION BY ALUMINUM IN *CAMELLIA SINENSIS* L. SEEDLINGS

TUESDAY 4 JULY, 2017 POSTER SESSION

MASOUMEH SAFARI (TARBIAT MODARES UNIVERSITY, IRAN), FAEZEH GHANATI (TARBIAT MODARES UNIVERSITY, IRAN)

@ M.SAFARY@MODARES.AC.IR

Aluminum (Al) toxicity is a major limiting factor for crop productivity in acidic soils. Al bound to the cell wall of sensitive plants and negatively affects wall structure and function by increasing its rigidity and reducing its expansibility. Different from most of terrestrial plants, the growth of tea (*Camellia sinensis* L.) plant is stimulated by Al. In this study the effects of Al on root elongation, mechanical extensibility of the cell wall, and modification of pectin fractions were examined. Ten day old tea seedlings were treated with or without 400 μM Al in a modified Hoagland solution, for 8 days. Mechanical changes in elongation zone of root apex were monitored by freeze-thawing method. Cell wall pectins were localized via immunofluorescence of monoclonal antibodies JIM5 and JIM7. Uronic acid content and the activity of PME were measured as well. Exposure to Al significantly promoted root cell extensibility and increased cell elongation of tea seedlings. Immunofluorescence studies revealed bright fluorescence of antibody JIM7 in Al-treated root apices demonstrating high-methyl-ester pectins, whereas the fluorescence intensity of JIM5 was high in control seedlings. Al treatment significantly decreased PME activity but increased the ratio of total sugars to uronic acids. The result suggest that structural modifications of pectin in elongation zone of tea roots prevent insertion and accumulation of Al and its inhibitory role. However the mechanism by which elongation is triggered is yet to be clarified.

### PC1.13 MAGIC ARABIDOPSIS AND SEARCH FOR GENETIC REGULATION OF STOMATAL RESPONSES TO RISING CO<sub>2</sub>

TUESDAY 4 JULY, 2017 POSTER SESSION

• HANNAH SEWELL (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), DAVID BEERLING (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), PAULA KOVER (UNIVERSITY OF BATH, UNITED KINGDOM), JULIE GRAY (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), STUART CASSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

@ HEGSEWELL1@SHEFFIELD.AC.UK

Rising atmospheric CO<sub>2</sub> has been shown to influence development of stomata of many plant species, leading in many cases to lower stomatal densities (SD) in elevated CO<sub>2</sub>. This reduction in stomata can be an advantage to the plant, through a reduction in water loss and increased water use efficiency, or a hindrance, as a reduction in water loss can lead to elevated leaf temperatures and heat stress.

Despite this response to CO<sub>2</sub> being well known, relatively little is known about the genes involved in the changes that lead to altered SD in elevated CO<sub>2</sub>. To fill more of the gaps, Multiparent Advanced Generational Inter-Cross (MAGIC) Arabidopsis, a series of recombinant inbred lines, are being used in this project to attempt to identify Quantitative Trait Loci (QTL).

A QTL analysis has been run on over 400 of the MAGIC lines, highlighting regions of the genome controlling stomatal density in different CO<sub>2</sub> concentrations. Work is currently being carried out to identify the gene controlling the trait in each region and how they effect this developmental pathway.

### PC1.27 THE ROLE OF HYDROGEN PEROXIDE IN ELONGATION DYNAMICS OF THE FIRST INTERNODE OF DEEP-SOWN WHEAT, *TRITICUM AESTIVUM* TIR

TUESDAY 4 JULY, 2017 POSTER SESSION

• ASKIM H SEKMEN (EGE UNIVERSITY FACULTY OF SCIENCE, TURKEY), TOLGA YALCINKAYA (EGE UNIVERSITY, TURKEY), AZIME GOKCE (EGE UNIVERSITY, TURKEY), YIGIT AKYOL (EGE UNIVERSITY, TURKEY), ISMAIL TURKAN (EGE UNIVERSITY, TURKEY)

@ HEDIYE.SEKMEN@EGE.EDU.TR

Deep sowing tolerance is the one of the most efficient way to avoid drought, which can be described as the ability of the elevation of the shoot apical meristem above the soil surface by elongation of mesocotyl or first internode. In the literature, there are only a few reports concerning the first internode elongation. Moreover, there is no study that covers the potential roles of reactive oxygen species (ROS), especially H<sub>2</sub>O<sub>2</sub>, in first internode elongation under deep seeding conditions. For this aim, in the present study, H<sub>2</sub>O<sub>2</sub> level, the activities of NADPH oxidase, superoxide dismutase (SOD), cell wall peroxidase (CWPOX), POX and ascorbate peroxidase (APX) and the expressions of cell wall loosening-related genes (Glucanase EI and TaEXPB23) were measured in the first internode of 10d-old TIR seedlings which were grown in shallow (2 cm) and deep sowing

(10 cm) conditions. The length of the first internode was increased in the H<sub>2</sub>O<sub>2</sub>-treated wheat seedlings at the depth of 10 cm. As seen by quantitative RT-PCR, H<sub>2</sub>O<sub>2</sub> up-regulated cell wall loosening-related genes (Glucanase EI and TaEXPB23) in the first internode of "Tir" under deep sowing condition (10 cm). Increased NOX (up-regulated RBOHD and RBOHF) and CWPOX, unchanged APX and SOD induced the wall loosening reaction by means of increased accumulation of H<sub>2</sub>O<sub>2</sub> at a depth of 10 cm. To our knowledge, this is the first study that H<sub>2</sub>O<sub>2</sub> could cause first internode elongation by both expansin/glucanase- and OH<sup>-</sup> mediated cell wall loosening, thereby promoting the deep-sowing tolerance of "Tir" wheat cultivar.

### PC1.28 STRESS ACTIVATED KINASES IN THE REGULATION OF LIGHT INDUCED CHLOROPLAST MOVEMENTS

TUESDAY 4 JULY, 2017 POSTER SESSION

• OLGA SZTATELMAN (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND), EWA SITKIEWICZ (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND), MICHAŁ DADLEZ (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND), GRAŻYNA DOBROWOLSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND)

@ OLGA.SZTATELMAN@IBB.WAW.PL

Light induced chloroplast movements are a mechanism to optimize light utilization by plants. Chloroplasts in weak light gather at the cell walls perpendicular to light direction in order to maximize light absorption. In strong light they move to the cell walls parallel to light direction to avoid stress caused by excess light. Several proteins involved in the movements have been identified to date. One of the potential regulators of those proteins are SNF1-related kinases 2 (SnRK2s). SnRK2s are plant specific protein kinases regulating responses to adverse environmental conditions, such as salt or drought. They are rapidly activated upon stress and phosphorylate downstream targets leading to plant acclimation. Phosphoproteomic studies identified a number of potential targets of these kinases, including proteins involved in light induced chloroplast movement. In order to assess the link between salt stress and chloroplast movements, we examined the phosphorylation of some of known proteins involved in the movements by SnRK2s. All tested proteins were phosphorylated by several SnRK2 kinases in vitro. Phosphorylation sites were mapped using mass spectrometry and confirmed by directed mutagenesis approach. The sites were compared with available phosphoproteomic data. The Bimolecular Fluorescence Complementation assay showed that selected proteins interact with kinases in *planta* indicating that indeed they could be *bonafide* SnRK2s' substrates. Treating leaves with NaCl lead to decrease in chloroplast movements capacity. We propose that SnRK2s may act as negative regulators of light induced chloroplast movements, leading to the inhibition of this response during salt stress.

## PC1.29 THE OPTIMIZATION OF ANTIBODY EXPRESSION IN TOBACCO BY-2 CELLS USING TRANSIENT ASSAY

TUESDAY 4 JULY, 2017 POSTER SESSION

ZUZANA POBORILOVA (INSTITUTE OF EXPERIMENTAL BOTANY AS CR, CZECH REPUBLIC), HELENA PLCHOVA (INSTITUTE OF EXPERIMENTAL BOTANY AS CR, CZECH REPUBLIC), NOEMI CEROVSKA (INSTITUTE OF EXPERIMENTAL BOTANY AS CR, CZECH REPUBLIC), JAKUB DUSEK (INSTITUTE OF EXPERIMENTAL BOTANY AS CR, CZECH REPUBLIC), TOMAS MORAVEC (INSTITUTE OF EXPERIMENTAL BOTANY AS CR, CZECH REPUBLIC)

@ POBORILOVA@UEB.CAS.CZ

Over the last decades, plant-produced antibodies have received considerable interest. Today increasing attention is paid to plant cell suspension cultures as an alternative bioproduction platform. Using the approaches of synthetic biology and combinatorial cloning it is relatively simple to assemble a large numbers of multigene expression constructs including multiplex CRISPR/Cas9 constructs; however it might be very difficult to quantitatively assess their functionality. One possibility to overcome these obstacles and test up to hundreds of gene constructs in a relatively short time is to combine the advantages of transient expression assays with those of plant cell suspension cultures. Here we present data on optimization of antibody production in tobacco BY-2 cells employing co-expression of two genes using both replicating geminiviral vectors (pRIC derivatives) and non-replicating CPMV based vectors (pEAQ derivatives).

## PC1.30 ROLE FOR PHOSPHOLIPASE D (PLD) IN THE ER-STRESS RESPONSE IN ARABIDOPSIS

TUESDAY 4 JULY, 2017 POSTER SESSION

RENGIN OZGUR UZILDAY (EGE UNIVERSITY, TURKEY), BARIS UZILDAY (EGE UNIVERSITY, TURKEY), ASKIM H. SEKMEN (EGE UNIVERSITY, TURKEY), TEUN MUNNIK (UNIVERSITY OF AMSTERDAM, NETHERLANDS), ISMAIL TURKAN (EGE UNIVERSITY, TURKEY)

@ RENGINOZGUR@GMAIL.COM

Accumulation of unfolded proteins caused by inefficient chaperone activity in 'Endoplasmic Reticulum (ER)' is called 'ER Stress' and this triggers 'Unfolded Protein Response (UPR)', which increases protein folding capacity. Besides proteins, lipids are also synthesized at the ER. In animals, the lipid, phosphatidic acid (PA) acts as a signalling molecule in the ER. In plants, PA is a lipid second messenger too, but its role in UPR is completely unknown. Phospholipase D (PLD) is one of major enzymes producing PA, hydrolyzing structural phospholipids into PA and choline.

To understand the relationship between PLD and ER stress in plants, we used both chemical inhibitor of PLD (1-butanol) and different genotypes deficient in *PLD* genes (*plda1*, *plda2*, *plda3*, *pld5* and *plda1/a3/5*) of *Arabidopsis thaliana*. The effects of ER stress (tunicamycin induced) on *pld* mutants were evaluated by phenotyping their root growth. Moreover, the response

to ER stress of these mutant were determined by measuring the transcript abundance of ER stress-related genes using Q-PCR incl. *bZIP28*, *IRE1A*, *BiP3*, *CNX*, *HRD1*, *DER1*, and *UBC32*.

When PLD activity was inhibited with 1-butanol expressions of genes related to UPR were decreased indicating a role for PA for induction of UPR. Among the twelve PLD genes *Arabidopsis* has, we obtained genetic evidence that *PLDα2* is likely involved in the ER-stress response, as *plda2* mutant seedlings exhibited reduced root growth when grown on agar plates containing TM. In this study we demonstrate that PLD signaling is a key factor for ER stress in plants for the first time.

## PC1.34 ELECTROPHORETIC PROFILE AND HERITABILITY OF PEROXIDASE ACTIVITIES IN THE TOLERANCE OF THEOBROMA CACAO AGAINST PHYTOPHTHORA MEGAKARYA, THE MOST AGGRESSIVE AGENT OF BLACK POD DISEASE

TUESDAY 4 JULY, 2017 POSTER SESSION

CLAUDE SIMO (DEPARTMENT OF PLANT BIOLOGY FACULTY OF SCIENCE P.O. BOX 24 157 DOUALA CAMEROON, CAMEROON), FRANÇOIS P DJOUGOUE (DEPARTMENT OF PLANT BIOLOGY FACULTY OF SCIENCE P.O. BOX 812 YAOUNDÉ, CAMEROON), EMILE MINYAKA (DEPARTMENT OF BIOCHEMISTRY FACULTY OF SCIENCES P.O. BOX 24157 DOUALA, CAMEROON), DENIS N OMOKOLO (LABORATORY OF PLANT PHYSIOLOGY DEPARTMENT OF BIOLOGICAL SCIENCES HIGHER TEACHER'S TRAINING COLLEGE, CAMEROON)

@ SIMOCLAUDE@UNIV-DOUALA.COM

Black pod disease caused by *Phytophthora megakarya* is the main limiting factor in the production of cocoa. Plants exposed to biotic stresses exhibit changes in their physiology and metabolism. The study focused on the heritability and activity of peroxidases (POX) in two hybrid populations F13 (♀SNK13 x ♂T79/467) and F79 (♀T79/467 x ♂SNK13). The results show that more tolerant and more productive hybrid genotypes (F1307, F1314, F7902, F7928) and more tolerant genotypes (F1315, F1313, F7926, F7907) relative to the best parent in the soluble (S) and bound fractions (L) recorded a large amount of POX activity with small areas of necrotic lesion in contrast to less tolerant and productive genotypes (F1324, F1308, F7915 and F7919) which accumulated a small amount of POX activity in large areas of necrotic lesion. A negative and significant correlation ( $P < 0.01$ ) was observed between the development of necrosis and peroxidase activities. The profile of peroxidase isoforms (S) of the mesocarp of infected pods revealed the existence of a specific form (A2) after infection in tolerant genotypes. This isoform is linked to tolerance. The heritability values of POX activity obtained in fractions (S) in F13 family (♀SNK13 x ♂T79/467) and F79 family (♀T79/467 x ♂SNK13) are relatively high (0.69 and 0.64). The existing isoform (A2) in hybrid tolerant genotypes could lead in the creation of more productive and tolerant varieties making it available to farmers.

## PC1.35 THE ECONOMICS OF STOMATAL DEVELOPMENT

■ TUESDAY 4 JULY, 2017 POSTER SESSION

👤 RACHEL C DENLEY BOWERS (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), STUART A CASSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), NICHOLAS A M MONK (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

@ RCDENLEY-BOWERS1@SHEFFIELD.AC.UK

The number of stomata on a leaf can be measured in two ways: stomatal density (SD), which is the number of stomata in a given area; and stomatal index (SI), which is the number of stomata as a percentage of the total cell count.

Sometimes, two plants may have the same SD and different SIs. This suggests that the SD can be maintained by increasing either cell division or expansion.

Why would the plant choose one method over another? Which of these methods of maintaining stomatal density is most efficient?

## PC1.36 AN EMS-MUTAGENESIS SCREEN TO IDENTIFY MOLECULAR COMPONENTS OF BRASSINOSTEROID SIGNALING PATHWAY AT THE LEVEL OF GSK3 LIKE KINASES

■ TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ANAXI HOUBAERT (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), QING LU (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), RENÉ BENJAMINS (PLANT DEVELOPMENTAL BIOLOGY WAGENINGEN UNIVERSITY RESEARCH, NETHERLANDS), EUGENIA RUSSINOVA (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM)

@ ANHOU@PSB.UGENT.BE

Brassinosteroids (BRs) are a class of steroid hormones that are ubiquitously distributed throughout the plant kingdom. BR deficiency causes pleiotropic phenotypes including dwarfism, male sterility, delayed flowering, reduced apical dominance, and a light-grown morphology when grown in dark. BRs have a broad spectrum of activities that have a positive effect on the quantity and quality of crops and they increase plant resistance to stress and pathogens. The GSK3-type kinase BIN2 acts as a key negative regulator of BR signaling. Besides BR signaling, BIN2 also plays diverse roles in a lot of physiological and developmental processes such as stomatal development, tolerance for abiotic stress, ABA signaling, auxin signaling, root hair development and xylem differentiation. Considering that plants contain divergent GSK3 like kinases, it is important to identify more substrates to allow us to better understand how plants coordinate complicated physiological processes in response to diverse environmental stimuli. In a chemical genetics screen for compounds that cause constitutive BR responses in *Arabidopsis*, a small molecule that activates BR signaling by inhibiting GSK3 like kinases named bikinin was identified. The scientific aim of the proposed research is to identify downstream effectors of GSK3 like kinases through forward genetics approach.

Sofar, 5 putative mutants have been confirmed as suppressors of the bikinin-induced phenotypes. Those mutants were backcrossed to the nonmutagenized parental line and are currently in the pipeline for deep sequencing and target gene identification.

## PC1.37 SUB-CELLULAR RESPONSES OF THE WHEAT IMMUNE SYSTEM TO PATHOGENIC FUNGI

■ TUESDAY 4 JULY, 2017 POSTER SESSION

👤 FRANCESCO VALENTE (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF EXETER, UNITED KINGDOM), CECILIA RODRIGUES (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF EXETER, UNITED KINGDOM), EMMA WALLINGTON (NIAB CAMBRIDGE, UNITED KINGDOM), GRAHAM THOMAS (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF EXETER, UNITED KINGDOM), JEREMY METZ (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF EXETER, UNITED KINGDOM), ANDREW GREENLAND (NIAB CAMBRIDGE, UNITED KINGDOM), PATRICK J HUSSEY (SCHOOL OF BIOSCIENCES, DURHAM UNIVERSITY, UNITED KINGDOM), MICHAEL J DEEKS (DEPARTMENT OF BIOSCIENCES, UNIVERSITY OF EXETER, UNITED KINGDOM)

@ FV220@EXETER.AC.UK

The Ascomycete *Zymoseptoria tritici* is a hemibiotrophic fungus which is the main causal agent of wheat Septoria tritici blotch (STB). Capable of causing up to 40% yield loss, this is one of the most devastating foliar diseases of wheat worldwide. Detailed molecular understanding of the wheat immune response towards *Z. tritici* could lead to significant crop improvement for food production. Foliar infection begins with fungal penetration of the host tissues via host stomatal cavities within 12 hours post inoculation, followed by an endophytic phase that leads to chlorosis throughout the leaf surface and a necrotrophic phase during which symptom development occurs. Nonetheless, we are interested in analysing the early mechanism of hyphal recognition in wheat in response to stimuli caused by this pathogenic fungus. Hence, we are testing the hypothesis that the wheat immune response is playing a crucial role during the biotrophic phase in the early post inoculation stage both in compatible and incompatible host-microbe interactions. In order to test our scientific hypothesis, we are currently assessing the compatibility of three transformable *Zymoseptoria tritici* strains (IPO 323, K4979N2X1 and K6588N2X2) with four different wheat cultivars commonly utilised for wheat transformation (Cadenza, Fielder, Bobwhite and NB1). We are using live-cell imaging probes in these cultivars to quantify changes in plant subcellular architecture 3 to 5 days post fungal inoculation.

### PC1.38 ASCORBATE BIOSYNTHESIS AND ITS REGULATION BY *VTC2* IN THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● ANDRÉ VIDAL-MEIRELES (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY), JULIANE NEUPERT (MAX-PLANCK-INSTITUT FÜR MOLEKULARE PFLANZENPHYSIOLOGIE, GERMANY), LAURA ZSIGMOND (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY), LAISE ROSADO-SOUZA (MAX-PLANCK-INSTITUT FÜR MOLEKULARE PFLANZENPHYSIOLOGIE, GERMANY), LÁSZLÓ KOVÁCS (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY), VALÉRIA NAGY (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY), ANIKÓ GALAMBOS (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY), ALISDAIR R FERNIE (MAX-PLANCK-INSTITUT FÜR MOLEKULARE PFLANZENPHYSIOLOGIE, GERMANY), RALPH BOCK (MAX-PLANCK-INSTITUT FÜR MOLEKULARE PFLANZENPHYSIOLOGIE, GERMANY), SZILVIA Z TÓTH (BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY)

@ ANDREVMV@GMAIL.COM

Ascorbate (Asc) plays essential roles in stress resistance, development, signaling, hormone biosynthesis and regulation of gene expression. In plants, the main route for Asc biosynthesis is the L-galactose (Smirnoff-Wheeler) pathway, but in *Chlamydomonas reinhardtii* direct physiological and molecular evidence for the operation of the Smirnoff-Wheeler pathway was still lacking. To elucidate Asc biosynthesis in green algae, we decided to generate Asc-deficient *Chlamydomonas* transformants. As a target, we have chosen the *VTC2* gene, encoding GDP-L-galactose phosphorylase, since in higher plants it is known to be highly regulated and its activity has a strong influence on the Asc content; gene expression studies on *Chlamydomonas* suggested the same importance. For the gene silencing, we used the artificial microRNA (amiRNA) approach which is highly specific in *Chlamydomonas* and overcomes the problem of self-silencing observed with the siRNA approach.

The Asc concentrations on our *VTC2-amiRNA* lines were reduced to 10%, showing that GDP-L-galactose phosphorylase plays a pivotal role in Asc biosynthesis; the *VTC2-amiRNA* lines also grow more slowly, have lower chlorophyll content and smaller light-harvesting antenna, and are more susceptible to stress than the control strains. We also demonstrate that *VTC2* expression can be rapidly induced by reactive oxygen species even in the absence of photosynthesis and it lacks negative feedback regulation by Asc in the physiological concentration range. In contrast to plants, we also observe there is no circadian regulation of Asc biosynthesis.

In conclusion, our work demonstrates that Asc biosynthesis is highly regulated in *Chlamydomonas* via distinct mechanisms described previously from land plants.

### PC1.39 DEFINING THE EXPRESSION DOMAINS OF THE ARABIDOPSIS GLUTAREDOXIN GENES *AtGRXS5*, *AtGRXS6*, AND *AtGRXS8*

■ TUESDAY 4 JULY, 2017 POSTER SESSION

● MATTHEW A ESCOBAR (CALIFORNIA STATE UNIVERSITY SAN MARCOS, UNITED STATES), MIGUEL ROSAS (CALIFORNIA STATE UNIVERSITY SAN MARCOS, UNITED STATES), OSCAR DAVALOS (CALIFORNIA STATE UNIVERSITY SAN MARCOS, UNITED STATES)

@ MESCOBAR@CSUSM.EDU

Glutaredoxins are small redox enzymes that can reduce disulfide bonds in target proteins. Plants have far more glutaredoxin genes than other organisms, mostly due to a large group of class III glutaredoxins that is exclusive to Kingdom Plantae. Previously, we functionally analyzed a cluster of five class III glutaredoxin genes arranged in a tandem array on *Arabidopsis thaliana* chromosome 4, demonstrating that these genes are induced by nitrate and act as negative regulators of primary root growth. The purpose of this study was to characterize the specific cell- and tissue-level expression domains of three of these glutaredoxin genes: *AtGRXS5*, *AtGRXS6*, and *AtGRXS8*. The promoter regions of each gene were cloned upstream of the reporter gene *GUS* in a plant expression vector, which was used for plant transformation. Colorimetric *GUS* assays were then performed on the transgenic *Arabidopsis* lines expressing *AtGRXS5pro::GUS*, *AtGRXS6pro::GUS*, and *AtGRXS8pro::GUS* gene fusions. All three genes showed similar expression domains, with *GUS* activity localized in the phloem of root and shoot tissues throughout plant development. *GUS* gene expression was nitrate-dependent, demonstrating that nitrogen regulation of glutaredoxin gene expression is controlled at the transcriptional level. Preliminary studies of plants expressing N-terminal translational fusions between yellow fluorescent protein (YFP) and *AtGRXS5/6/8* suggest that these glutaredoxins are localized to the nucleus and cytosol. Collectively, both our findings and recent studies by others suggest that glutaredoxins may act as important phloem-mobile signals involved in plant nitrogen sensing and the regulation of root system architecture.



### PC1.40 CELL CULTURE OF *ACANTHOPHYLLUM GLANDULOSUM* L. AS AN ALTERNATIVE SOURCE OF SAPONIN

TUESDAY 4 JULY, 2017 POSTER SESSION

MITRA JAMSHIDI (SEHAT INDUSTRIAL AND COMMERCIAL CO, IRAN), FAEZEH GHANATI (TARBIAT MODARES UNIVERSITY, IRAN)

@ MIT.JAMSHIDI@GMAIL.COM

Saponins are structurally-related secondary compounds composed of steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties. Because of their surfactant and foaming properties, saponins are used in production of shampoos, liquid detergents, toothpastes. Saponins are also used as emulsifier and long-lasting foaming agent in beverages. *Acanthophyllum glandulosum* L. (Caryophyllaceae) is one of the endemic species in Iran. The plant has been traditionally used as a detergent due to the prevalence of saponin in its roots. Despite the wide use, the plant has never been cultivated, neither its various properties e.g., seasonal variations of growth have been studied. Plant tissue culture has potential to produce bioactive compounds with the great advantage of sustainability of culture. In the present study saponin contents of a rapid growing cell line of *Acanthophyllum glandulosum* L. were compared with the intact plant. Cultures were established from the seeds in a modified LS medium. Saponins were extracted with diethyl ether and n-butanol and were assessed spectrophotometrically using anisaldehyde-ethyl acetate and vanillin-sulfuric method for steroid and triterpenoid saponins, respectively. The content of saponins in calli was evaluated as  $3.25 \text{ mg/g DW}^{-1}$ , which was noticeable vs. of the intact roots,  $0.4 \text{ mg/g DW}^{-1}$ . The results clearly introduce the callus of *Acanthophyllum glandulosum* L. as an appropriate natural source for saponins. It is also noteworthy that saponins can be easily and efficiently extracted from this source.

### PC1.41 CRUCIAL ROLE OF FRUCTAN IN THE MAINTENANCE OF MEMBRANES OF WHEAT SEEDLINGS DURING SEVERE DROUGHT STRESS

TUESDAY 4 JULY, 2017 POSTER SESSION

FARNOOSH NEMATI (TARBIAT MODARES UNIVERSITY, IRAN), FAEZEH GHANATI (TARBIAT MODARES UNIVERSITY, IRAN)

@ FARNOOSH\_N62@YAHOO.COM

The plasma membrane is thought to be the major target for drought stress damage due to changes in composition and structures of water and phospholipids. Removal of water from the membrane disrupts the normal bilayer structure and results in the membrane becoming exceptionally porous when desiccated. Fructan has the capacity to stabilize membranes and prevent leakage during water cessation. In the present study the relationship between fructan content and membrane damages was evaluated in 4-day old seedlings of a drought-tolerant (Sirvan) and a drought-sensitive (Marvdasht) wheat cultivar exposed to 7 days water cessation and subsequent re-watering. In comparison with sensitive cultivar, the tolerant one accumulated more fructan ( $3.56 \pm 0.3 \mu\text{g/g FW}$ ) meanwhile decreased the level of membrane leakage and lipid peroxidation.

Freeze-thawing experiment showed that drought stress remarkably increased freeze-disrupt coefficient of sensitive cultivar (4.5 fold higher than of tolerant one) and severely damaged the epidermal and outer cortex cells of their leaves. The results suggest fructan as a flexible soluble carbohydrate with a direct protective effect on membrane integrity which plays a crucial role in the tolerance of wheat seedling against drought stress conditions.

### PC1.42 MERCURY-INDUCED BIOCHEMICAL AND ROS CHANGE IN TWO SPECIES RICE

TUESDAY 4 JULY, 2017 POSTER SESSION

YUNAN CHEN (NATIONAL SUN YET-SEN UNIVERSITY, TAIWAN)

@ YACHEN@MAIL.NYSU.EDU.TW

Mercury (Hg) is considered one of the most toxic metals in the worldwide with a long half-life. It is known to accumulate in the living organisms and cause serious damage on growth and metabolism of plants. Recent reports revealed that an oxidative stress could be involved in Hg toxicity, by either inducing free radical production or by decreasing enzymatic and non-enzymatic antioxidants. Reactive oxygen species (ROS) play an important role to against the stresses in the plant defense system and maintain cell growth and development. Many plants have been shown to accumulate a large quantity of proline in response to elevated toxic concentrations of heavy metals. Proline normally accumulates in cytoplasm to stabilize the structure of proteins and maintain cell redox status. Research has demonstrated proline accumulate in a large number of species in response to environmental stresses such as salinity, drought, cold, nutrient deficiency, heavy metals, pathogen infections and high acidity. Studies have shown proline accumulation may have a relationship with lipid peroxidation in plants under diverse kinds of stress. This study examines  $\text{H}_2\text{O}_2$  content, lipid peroxidation, and antioxidant enzyme activities in two cultivars of rice seedlings in response to Hg. Our preliminary observations indicated that rice seedlings of cultivar Tainung 67 (TNG67) are more tolerant to Hg than those of cultivar Taichung Native 1 (TN1). We demonstrated that the higher free proline level provided effective protection against Hg-induced stress, by reducing free radical damage in the cells.

### PC1.43 REGULATION OF GENE EXPRESSIONS IN THE REMOBILIZATION OF CARBON RESERVE IN STRAWS OF RICE AT GRAIN FILLING

TUESDAY 4 JULY, 2017 POSTER SESSION

JIANHUA ZHANG (CHINESE UNIVERSITY OF HONG KONG, HONG KONG), GUANQUN WANG (CHINESE UNIVERSITY OF HONG KONG, HONG KONG), NENGHUI YE (HUNAN AGRICULTURAL UNIVERSITY, CHINA)

@ JHZHANG@CUHK.EDU.HK

Carbon reserves in rice straws before flowering contribute to a significant portion of grain filling. However, the molecular mechanism

of carbon reserve remobilization from straws (stem and sheath) to grains remains unclear. In this study, we used super rice LYP9 and conventional rice 9311 showing different carbon remobilization behaviors and analyzed the transcriptomic profiles in their straws at three stages of grain filling. Among the differentially expressed genes (DGs), 5733 genes were uniquely up- or down-regulated at 30 days after anthesis (DAA) between LYP9 and 9311 in comparison to 681 at 10 DAA and 495 at 20 DAA, respectively, indicating the different regulations at late stage of grain filling. The KEGG pathway analysis and Gene Ontology (GO) classification of DGs showed that carbohydrate catabolic pathway, plant hormone signal transduction and photosynthesis pathway were the DGs-enriched, suggesting the difference of NSC content, photosynthesis and ABA content between the two cultivars during grain filling. Further comparison analysis and confirmation by qRT-PCR and enzyme assays suggest that genes involved in trehalose synthesis (TPP, TPS), starch degradation (beta-amylase) and sucrose synthesis (SPS, SS) were important for carbon reserves remobilization whereas ABA content was determined by the counteraction between NCED1 and ABA8ox1 gene. The higher expression level of all these genes and ABA content in 9311 resulted in better efficiency of carbon reserves remobilization in 9311 than cultivar of LYP9.

### PC1.44 EARLY EVENTS INDUCED BY COPPER INVOLVES THE ACTIVATION OF MOSAIC TRP CHANNELS, RELEASE OF AMINOACIDS, SEROTONINE AND ADRENALIN, AND ACTIVATION OF GLUTAMATE-, SEROTONIN- AND ADRENALIN-LIKE RECEPTORS IN THE MARINE ALGA *ULVA COMPRESSA*

TUESDAY 4 JULY, 2017 POSTER SESSION

ALEJANDRA MOENNE (UNIVERSITY OF SANTIAGO OF CHILE, CHILE), MELISSA GÓMEZ (UNIVERSITY OF SANTIAGO OF CHILE, CHILE), ALBERTO GONZÁLEZ (UNIVERSITY OF SANTIAGO OF CHILE, CHILE)

ALEJANDRA.MOENNE@USACH.CL

Copper ions induce the activation of TRP channels at 4, 8, 13, 80 and 86 min and at 5 and 9 h of exposure in *Ulva compressa*. In order to detect earlier responses, the alga was incubated with 10  $\mu$ M copper, and with inhibitors of human TRPs A1, C4/C5, C5/M8, M8 and V1 which these were incorporated just after copper addition (time 0), and after 1 and 2 min of copper exposure, and depolarization events at 4, 8 and 13 min were detected. Depolarizations involved the activation of TRP of A1/C5/M8 at time 0; TRPA1/C4/M8/V1 after 1 min; and TRPA1/C5/M8, after 2 min of copper exposure. In addition, inhibitors of glutamate-like receptor (GluR), serotonin and adrenalin receptors were used. Inhibitors of GluR of NMDA type, added at time 0, inhibitors GluR of AMPA/KA types, added after 1 and 2 min, and inhibitors of serotonin and adrenalin receptors added after 2 min inhibit depolarizations at 4, 8 and 12 min. Moreover, release of amino acids phenylalanine, tryptophan, methionine and arginine as well as serotonin and adrenalin to the extracellular medium was detected. In addition, protein kinases CaMK, PKA, PKC and PKG participate in depolarization events at 4, 8 and 12 min. Thus, copper excess induces the activation of mosaic TRP channels, the release of amino acids, serotonin, and adrenalin, and the activation of glutamate-, adrenalin- and serotonin-like receptors which are involved in depolarization events observed at 4, 8 and 12 min of copper exposure. Financed by Fondecyt 1160013.

### PC1.45 CALCIUM-INDUCED CALCIUM RELEASE INDUCED BY COPPER INVOLVES THE ACTIVATION OF FUNCTIONAL TRPS AND VDCC IN THE MARINE ALGA *ECTOCARPUS SILICULOSUS*

TUESDAY 4 JULY, 2017 POSTER SESSION

ALBERTO GONZÁLEZ (UNIVERSITY OF SANTIAGO OF CHILE, CHILE), ALEJANDRA MOENNE (UNIVERSITY OF SANTIAGO OF CHILE, CHILE)

ALBERTO.GONZALEZFI@USACH.CL

*Ectocarpus siliculosus* is a brown macroalgae distributed worldwide and the strain Ec524 from Chañaral, a copper polluted site in northern of Chile, exhibits tolerance to copper excess. The genome of *E. siliculosus* Ec524 encodes 15 potential mosaic Transient Receptor Potential (TRP) channels, mainly a mix of C and M types.

*E. siliculosus* was exposed to agonists of human TRP A1, C, M and V types for 60 min, or to 2.5  $\mu$ M copper for 12 h, and the increase of intracellular calcium was detected using a specific calcium fluorescent probe and confocal microscopy. In addition, to characterize the channels involved in the intracellular calcium increases, antagonists of human TRPs and VDCC were used.

Calcium increases were detected using agonists of human TRPs at 13, 29, 39 and 51 min of exposure, and exposure to copper also induced calcium increases at 13, 29, 39 and 51 min. These calcium increases were product of extracellular calcium entry. Copper exposure showed additional intracellular calcium increases at 3 and 9 h, that were product of calcium release from the endoplasmic reticulum after the activation of VDCC. Furthermore, protein kinases were found to be involved in the copper-induced activation of TRPs and VDCC. Thus, *Ectocarpus siliculosus* presented functional mosaic TRP channels activated by copper and VDCC that allows extracellular calcium entry leading to intracellular calcium release, in a calcium-dependent calcium release mechanism. Financed by Postdoctoral Fondecyt 3150440.

### PC1.46 CHANGES IN REDOX REGULATION AND ANTIOXIDANT SYSTEM DURING TRANSITION FROM C<sub>3</sub> TO SINGLE CELL C<sub>4</sub> PHOTOSYNTHESIS IN *BIENERTIA SINUSPERSICI*

TUESDAY 4 JULY, 2017 POSTER SESSION

BARIS UZILDAY (EGE UNIVERSITY, TURKEY), BARIS UZILDAY (EGE UNIVERSITY, TURKEY), RENGİN ÖZGÜR (EGE UNIVERSITY, TURKEY), TOLGA YALCINKAYA (EGE UNIVERSITY, TURKEY), ISMAIL TURKAN (EGE UNIVERSITY, TURKEY), ASKIM H SEKMEK (EGE UNIVERSITY, TURKEY)

BARIS.UZILDAY@EGE.EDU.TR

*Bienertia sinuspersici* (Chenopodiaceae), has a unique mechanism that perform C<sub>4</sub> photosynthesis in a single cell without need of Kranz anatomy. In this plant, two different compartments in a single chlorenchyma cell can be distinguished with two types of biochemically and structurally different chloroplasts that act as analogues of mesophyll cells bundle sheath cells of C<sub>4</sub> Kranz

plants. Development of this specialized mechanism is gradual during plant development. In cells of young leaves, different cellular compartments are not formed and  $C_4$  enzymes are not expressed. Therefore, young leaves of *Bienertia sinuspersici* perform  $C_3$  photosynthesis. Single-cell  $C_4$  photosynthesis can only be effectively performed when cell maturation is complete. Aim of this work was to investigate changes in redox regulation and antioxidant defence during transition from  $C_3$  to single cell  $C_4$  photosynthesis. First, gradual development of  $C_4$  photosynthesis was confirmed with protein blot and qRT-PCR analysis of  $C_4$  enzymes. After this activities and isoenzymes of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and glutathione pool and redox status (GSH/GSSG) were determined in young, developing and mature leaves. Activities of SOD, APX and POX decrease, while GR and DHAR was increased. Most striking results were the changes in isoenzyme patterns of SOD, CAT and GR which were gradual through transition to  $C_4$  photosynthesis. Our findings clearly show that it is not possible to rule out the need for an efficient redox regulation during the process of engineering single cell  $C_4$  photosynthesis into a  $C_3$  plant.

### PC1.47 COMPARATIVE PROTEOMICS ANALYSIS PROVIDES NEW CANDIDATES FOR ZINC HOMEOSTASIS REGULATION IN *ARABIDOPSIS*

TUESDAY 4 JULY, 2017 POSTER SESSION

SAHAND AMINI (UNIVERSITY OF LIEGE, BELGIUM), BORJANA ARSOVA (FORSCHUNGSZENTRUM JÜLICH, GERMANY), MAXIME SCHEEPERS (UNIVERSITY OF LIEGE, BELGIUM), DOMINIQUE BAIWIR (UNIVERSITY OF LIEGE, BELGIUM), GABRIEL MAZZUCHELLI (UNIVERSITY OF LIEGE, BELGIUM), EDWIN DE PAUW (UNIVERSITY OF LIEGE, BELGIUM), MICHELLE WATT (FORSCHUNGSZENTRUM JÜLICH, GERMANY), MARC HANIKENNE (UNIVERSITY OF LIEGE, BELGIUM)

@ SAHAND.AMINI@ULG.AC.BE

Zinc (Zn) is an essential micronutrient for plants and around two billion people are depending on grains and legumes as their main Zn source. On the other hand, this transition metal is toxic for plants at high concentrations in soils. This calls for a better unravelling of Zn homeostasis regulation mechanisms, including sensing and signaling in plants. In order to fulfill this aim, we are testing for novel proteins involved in Zn homeostasis in the model plant *Arabidopsis thaliana*. First, quantitative proteomics was performed on root and shoot samples obtained upon Zn starvation and re-supply in different spatio-temporal conditions. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was also performed for those treatments to measure the Zn concentration in tissues. It showed very rapid Zn uptake in root upon re-supply. Moreover, quantitative expression studies of known players of Zn homeostasis confirmed our large-scale proteomic results, although for a few genes lack of correlation between transcript and protein regulation was observed. Using clustering, statistical and gene ontology analyses, we selected candidate genes for further studies. Among more than 5000 detected proteins in roots by shotgun proteomics, 75 genes were selected for targeted analyses. In general, our results show that comparative proteomics study can be useful to reveal new players in the Zn regulatory network in plants, which can lead to new Zn biofortification and phyto remediation strategies.

### PC1.48 A NEW PLAYER IN PLANT REDOX HOMEOSTASIS

TUESDAY 4 JULY, 2017 POSTER SESSION

JULIA RACHOWKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), DOROTA KONOPKA-POSTUPOLSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND)

@ JULIA.R@IBB.WAW.PL

Annexins are a family of calcium and membrane binding proteins. They were discovered in all eukaryotic organisms and their expression occurs in all cells and tissues. So far the biological functions of these proteins in animal cells and organisms has been demonstrated but still very little is known about their plant homologues. Certain annexins are involved in the signal transduction and improve the plant tolerance and its adaptation to sub-optimal external conditions. In our recent work we showed that increased level of annexin ANXD36 in the potato has a significant impact on plant physiology and biochemistry. We noticed that during high light stress the level of ROS was reduced. Basic metabolic processes are a significant source of ROS. During evolution plant cells acquired the ability to understand these signals and to react to them to maintain metabolic balance. Since these processes are often a consequence of exposure to the environment it is currently believed that the ROS metabolism is a platform for interaction between metabolism and environmental factors. In our current project we examine the role of annexin 1 from *Arabidopsis thaliana* in the metabolism of ROS. We measured the level of low molecular weight antioxidants - glutathione and ascorbate, and examined the balance between their reduced and oxidized forms in the mutants with varied levels of annexin 1. Also, we analyzed the expression of genes involved in redox homeostasis. The observed correlation between antioxidant pool and annexin 1 level indicate important role of this protein in regulation of redox homeostasis.

## PC1.50 EVOLUTIONARY IMPLICATIONS OF PLASMODESMATA DENSITIES FOR C<sub>3</sub> VS. C<sub>4</sub> PHOTOSYNTHESIS

📅 TUESDAY 4 JULY, 2017      POSTER SESSION

👤 ROSEMARY G WHITE (CSIRO AGRICULTURE AND FOOD, AUSTRALIA), FLORENCE DANILA (AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA), W PAUL QUICK (THE INTERNATIONAL RICE RESEARCH INSTITUTE, PHILIPPINES), STEVEN KELLY (OXFORD UNIVERSITY, UNITED KINGDOM), SUSANNE VON CAEMMERER (AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA), ROBERT T FURBANK (AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA)

@ ROSEMARY.WHITE@CSIRO.AU

Proliferation of plasmodesmata (PD) connections between bundle sheath (BS) and mesophyll (M) cells has been proposed as a key step in evolution of the C<sub>4</sub> photosynthetic mechanism. Lack of quantitative data has hampered further exploration and validation of this hypothesis. Here, we quantify leaf anatomical traits thought to play a role in metabolite transport in 18 species of BEP and PACMAD grasses encompassing four origins of C<sub>4</sub> photosynthesis and all C<sub>4</sub> subtypes (NADP-ME, NAD-ME, and PCK). Anatomical trait values showed little phylogenetic signal within this cohort, however, there was evolutionary convergence in traits associated with metabolite transport within C<sub>4</sub> species. Specifically, C<sub>4</sub> species exhibited increased PD density at the M-BS cell interface when compared to C<sub>3</sub> species. Moreover NAD-ME species, irrespective of evolutionary origin, had higher PD density than other C<sub>4</sub> subtypes which correlates with an enhanced requirement for metabolite transport in these species. These data suggest that PD density is a key anatomical enabler of C<sub>4</sub> photosynthesis and that enhancement of PD connections between the mesophyll and bundle sheath cells is crucial for establishing a functional C<sub>4</sub> leaf.

# PC2 PLANT CELL CYCLE AND CYTOSKELETON

**ORGANISED BY:** JIM MURRAY (CARDIFF UNIVERSITY, UK), PATRICK HUSSEY (UNIVERSITY OF DURHAM, UK) AND WALTER DEWITTE (CARDIFF UNIVERSITY, UK)

## PC2.1 NUTRITIONAL CONTROL OF CELL SIZE BY THE GREATWALL-ENDOSULFINE-PP2A-B55 PATHWAY

📅 TUESDAY 4 JULY, 2017 ⌚ 13:40

👤 SERGIO MORENO (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA, SPAIN), LIVIA PÉREZ-HIDALGO (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN), NATHALIA CHICA (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN), ANA E ROZALÉN (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN), ANGELA RUBIO (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN), ALICIA VÁZQUEZ-BOLADO (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN), NATALIA GARCÍA-BLANCO (INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA CSIC UNIVERSITY OF SALAMANCA, SPAIN)

@ SMO@USAL.ES

Proliferating cells adjust their cell size depending on the nutritional environment. Cells are large in rich media and small in poor media. This physiological response has been demonstrated in both unicellular and multicellular organisms. We have recently shown that the greatwall-endosulfine (Ppk18-Igo1, in fission yeast) pathway couples the nutritional environment to the cell cycle machinery by regulating the activity of PP2A-B55 (Chica *et al.* 2016). In the presence of nutrients, greatwall (Ppk18) protein kinase is inhibited by TORC1 and PP2A-B55 is active. High levels of PP2A-B55 prevent the activation of mitotic Cdk1-Cyclin B and cells increase in size in G2 before they undergo mitosis. When nutrients are limiting, TORC1 activity falls off and the activation of greatwall (Ppk18) leads to the phosphorylation of endosulfine (Igo1) and inhibition of PP2A-B55, which in turn allows full activation of Cdk1-Cyclin B and entry into mitosis with a smaller cell size. Given the conservation of this pathway in plant and animal cells, it is reasonable to assume that this mechanism is also conserved.

Chica *et al.* 2016. *Current Biology* 26, 319-330.

## PC2.13 THE EVOLUTION OF PLANT CELL DIVISION

📅 TUESDAY 4 JULY, 2017 ⌚ 14:20

👤 HENRIK BUSCHMANN (OSNABRÜCK UNIVERSITY, GERMANY)

@ HENRIK.BUSCHMANN@BIOLOGIE.UNI-OSNABRUECK.DE

The mechanism of cell division has undergone significant alterations during the evolution from aquatic streptophyte algae to land plants. Two new structures evolved, the cytokinetic phragmoplast and the preprophase band (PPB) of microtubules, whereas the ancestral mechanism of cleavage and the centrosomes disappeared. Here we map cell biological data on the recently emerged phylogenetic tree of streptophytes. The tree suggests that after establishment of the phragmoplast mechanism several groups independently lost their centrosomes. Surprisingly, the phragmoplast shows reductions in the Zygnematophyceae (the sister to land plants), many of which returned to cleavage. The PPB on the other hand evolved stepwise and, most likely, originated in the algae. The phragmoplast-PPB mechanism established in this way served as a basis for the three-dimensional development of land plants.

### PC2.3 CELL-SIZE DEPENDENT PROGRESSION OF THE CELL CYCLE CREATES BOTH HOMEOSTASIS AND FLEXIBILITY OF PLANT CELL SIZE

TUESDAY 4 JULY, 2017 14:50

JAMES MURRAY (CARDIFF UNIVERSITY, UNITED KINGDOM), ANGHARAD JONES (CARDIFF UNIVERSITY, UNITED KINGDOM), WALTER DEWITTE (CARDIFF UNIVERSITY, UNITED KINGDOM), RICHARD SMITH (MAX PLANK INSTITUTE FOR PLANT RESEARCH, GERMANY), JAN TRAAS (ENS LYON, FRANCE)

MURRAYJA1@CARDIFF.AC.UK

Mean cell size at the point of division in plant cells is generally constant for specific conditions and cell types, but the mechanisms coupling cell growth and cell cycle control with cell size regulation are poorly understood in intact tissues. Here we show that the continuously dividing fields of cells within the shoot apical meristem of *Arabidopsis* show dynamic regulation of mean cell size dependent on developmental stage, genotype and environmental signals. We show cell size at division and cell cycle length can be effectively predicted using an iterative two-stage cell cycle model linking cell growth and two sequential cyclin dependent kinase (CDK) activities. A single phase model cannot predict the observed effects of alterations in G1/S and G2/M kinase activities as determined by using mutant and overexpression genotypes. Experimental results concur in showing that progression through both G1/S and G2/M is flexible in response to both genotype and environmental conditions and that both transitions are size dependent. We conclude that the cell-autonomous co-ordination of cell growth and cell division previously observed in unicellular organisms also exists in intact plant tissues, and that observed cell size in growing tissues may be an emergent rather than directly determined property of cells.

### PC2.2 GROWTH AND CELL CYCLE - NEW INSIGHTS ON MECHANISMS OF MUTUAL COORDINATION AS REVEALED BY DIFFERENT TEMPERATURE TREATMENT IN GREEN ALGAE DIVIDING BY MULTIPLE FISSION

TUESDAY 4 JULY, 2017 16:00

KATERINA BISOVA (INSTITUTE OF MICROBIOLOGY AS CR, CZECH REPUBLIC), IVAN IVANOV (INSTITUTE OF MICROBIOLOGY AS CR, CZECH REPUBLIC), VILEM ZACHLEDER (INSTITUTE OF MICROBIOLOGY AS CR, CZECH REPUBLIC)

BISOVA@ALGA.CZ

Some green algae can divide into more than two daughter cells by a mechanism called multiple fission. Growth and cell cycle progression at different growth rates are coordinated so that the daughter cell size remains stable at different light intensities whilst it changes with temperature. This was proposed to be achieved by 'critical cell size' attained at commitment point (equivalent of G1/S transition). To gain more insight into coordination between growth and cell cycle progression, we performed a series of experiments that affect critical

cell size, e. g. experiments at different temperatures and shifts between them, in the green alga *Scenedesmus quadricauda*. The *S. quadricauda* divided into 8 daughter cells at 20°C and 30°C but the cell cycle lengths differed by a factor of two. When the cultures grown at 20°C were transferred to 30°C into dark to avoid growth interference they divided into more cells than committed at 20°C, the division occurred sooner and faster than in control conditions. In contrast, the cells transferred from 30°C to 20°C mostly divided only nuclei and not cells and even then to significantly lower percentage than the control cells. This suggested that the same 'critical cell size' was perceived differently at different temperatures. Since peak CDK activity correlated with mitosis/es and protoplast division/s it suggests the differences in daughter cell size in different temperatures are not dependent on critical cell size but rather on the level of CDK activity. This work was supported by the GA CR (grant no. 15-09231S).

### PC2.4 THE INTERPLAY BETWEEN GIBBERELLIN SIGNALING AND CELL CYCLE CONTROL

TUESDAY 4 JULY, 2017 16:30

CAMILLE M BLAKEBROUGH-FAIRBAIRN (CARDIFF UNIVERSITY, UNITED KINGDOM), WALTER DEWITTE (CARDIFF UNIVERSITY, UNITED KINGDOM), FRANCESCO MASIA (CARDIFF UNIVERSITY, UNITED KINGDOM), WOLFGANG W LANGBEIN (CARDIFF UNIVERSITY, UNITED KINGDOM), WOLFGANG BUSCH (GREGOR MENDEL INSTITUTE, AUSTRIA), KRISTINE HILL (UNIVERSITY OF TUEBINGEN, GERMANY), MALCOLM BENNETT (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JIM AH MURRAY (CARDIFF UNIVERSITY, UNITED KINGDOM)

BLAKEBROUGH-FAIRBAIRN CM@CARDIFF.AC.UK

Due to the sessile nature of plants, cell expansion and proliferation are the key developmental processes that drive plant growth. Regulation of these mechanisms enables plants to alter their growth rates in response to environmental and developmental stimuli. This is integrated and co-ordinated by diverse hormonal pathways. Gibberellins (GAs) are plant-specific hormones that promote growth and regulate various developmental processes by signalling the destruction of a class of nuclear-localised growth repressors known as DELLA proteins. Little is known about the link between GA and cell proliferation. In *Arabidopsis thaliana*, there are five DELLA proteins that act as co-transcriptional regulators with overlapping but distinct functions. Two DELLA proteins associated with negative regulation of the GA signalling pathway are GAI (gibberellin insensitive) and RGA (repressor of *ga1-3*). Our evidence suggests that GAI and RGA regulate the G<sub>1</sub> to S phase of the plant cell cycle and are functionally different. GAI contains the LxCxE amino acid motif that putatively mediates binding to the RETINOBLASTOMA RELATED (RBR) protein, which prevents the G<sub>1</sub>-S transition. FRET-FLIM has revealed an in situ interaction between GAI and RBR, but not with RGA that contains a mutated motif. Furthermore, loss of RGA function reduces the effect of the cell cycle and CYCLIN DEPENDENT KINASE (CDK) inhibitor, KIP-RELATED PROTEIN 2 on root growth whilst loss of GAI does not. Hence we suggest that the GAI-RBR complex represses cell proliferation and the GAI-RBR association might be under the control of CDK activity.

## PC2.5 THE ARABIDOPSIS HOMEBOX GENE SHOOT MERISTEMLESS HAS CELLULAR AND MERISTEM-ORGANISATIONAL ROLES WITH DIFFERENTIAL REQUIREMENTS FOR CYTOKININ AND CYCD3 ACTIVITY

📅 TUESDAY 4 JULY, 2017 ⌚ 16:45

👤 SIMON SCOFIELD (CARDIFF UNIVERSITY, UNITED KINGDOM)

@ SCOFIELDS@CARDIFF.AC.UK

The Arabidopsis class-1 KNOX gene SHOOT MERISTEMLESS (STM) encodes a homeodomain transcription factor essential for shoot apical meristem (SAM) formation and sustained activity. STM activates cytokinin biosynthesis in the SAM, but it is unclear the extent to which STM function is mediated through cytokinin. Here we show that STM inhibits cellular differentiation and endoreduplication, acting through cytokinin and the cytokinin-inducible CYCD3 cell cycle regulators, establishing a mechanistic link to cell cycle control which provides sustained mitotic activity to maintain a pool of undifferentiated cells in the SAM. Equivalent functions are revealed for the related KNOX genes KNAT1/BP and KNAT2 through ectopic expression. STM is also required for proper meristem organisation and can induce de novo meristem formation when expressed ectopically, even when cytokinin levels are reduced or cytokinin signaling is impaired. This function in meristem establishment and organisation can be replaced by KNAT1/BP, but not KNAT2, despite its activation of CK responses, suggesting promotion of CK responses alone is insufficient for SAM organisation. We propose that STM has dual cellular and meristem-organisational functions that are differentially represented in the class-1 KNOX gene family and have differing requirements for CK and CYCD3.

## PC2.6 CYCD ENHANCED CYTOKININ SENSITIVITY: A LINK BETWEEN MORPHOGENESIS AND CELL DIVISION IN HIGHER PLANTS?

📅 TUESDAY 4 JULY, 2017 ⌚ 17:00

👤 WALTER DEWITTE (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), EMILY SORNAY (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), ANGHARAD JONES (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), LISA DE MULDER (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), ENGELMANN JENNY (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), DAN GRIFFITHS (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), LIRON SHENHAV (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM), ONDREJ NOVAK (LABORATORY OF GROWTH REGULATORS, CZECH REPUBLIC), MIREK STRNAD (LABORATORY OF GROWTH REGULATORS, CZECH REPUBLIC), BEATRIX HORVATH (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), BEN SCHERES (UNIVERSITY OF WAGENINGEN, NETHERLANDS), JIM MURRAY (CARDIFF SCHOOL OF BIOSCIENCES, UNITED KINGDOM)

@ DEWITTEW@CARDIFF.AC.UK

Entry into the mitotic cell cycle is triggered by cyclin-cyclin dependant kinases complexes that inactivate the Retinoblastoma protein, promoting G1 to S phase transition. CYCD cyclins/CDK complexes inactivate RBR by hyperphosphorylation, and as such they are rate limiting for cell division in patterning and growth in Arabidopsis thaliana. Since CYCD genes are induced by mitogenic signals such as cytokinins and sugars, they have been described downstream targets of mitogenic signalling, acting at the interface of the cell cycle and development and physiology. Now several lines of evidence indicate that the CYCD3 genes mediate the response of tissues towards cytokinins. Varying CYCD levels affects regeneration in tissue culture, greening of calli in auxin homeostasis without influencing endogenous cytokinin levels. Induction of CYCD3 influences the transcript levels encoding for ARR cytokinin response regulators, and modulates the expression of the TCS::GFP cytokinin signalling reporter. This suggests that cytokinin induced CYCD3 genes act in a positive feed-forward loop with cytokinins, linking cell division with cytokinin mediated morphogenesis.

## PC2.7 CONTROL OVER DIVISIONS AND TRANSITIONS IN THE STOMATAL LINEAGE

WEDNESDAY 5 JULY, 2017 09:00

DOMINIQUE BERGMANN (STANFORD UNIVERSITY, UNITED STATES), ABIGAIL SIMMONS (STANFORD UNIVERSITY, UNITED STATES), KELLI DAVIES (STANFORD UNIVERSITY, UNITED STATES)

DBERGMANN@STANFORD.EDU

Developmental cell fate transitions require the regulation of division and differentiation processes in order to correctly pattern and organize tissues. In Arabidopsis stomatal development, the potential for multiple asymmetric divisions among stomatal lineage precursors allows flexibility and expansion of the population used to build the tissue, while a single symmetric division in late stomatal precursors is required to create the guard cell pair. How the transitions into and out of intermediate states are regulated, however, is not well understood. In animals, the conserved DREAM/MMB complex regulates gene expression during proliferation and differentiation. We identified homologues of LIN54/MIP120, a core DNA-binding component of the DREAM/MMB complex, as targets of the master regulator transcription factor SPEECHLESS and demonstrate their role in enforcing efficient transitions between intermediate cell states in the stomatal lineage.

## PC2.8 THE CDKG1 PROTEIN KINASE IS ESSENTIAL FOR MALE MEIOSIS AT HIGH AMBIENT TEMPERATURE

WEDNESDAY 5 JULY, 2017 09:40

JOHN DOONAN (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), JOHN NIBAU (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), DESPIONA DADAROU (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), FOTIENI TSILIMIGKA (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), DYLAN PHILIPS (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), GLYN JENKINS (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), NICOLA CAVALLARI (MAX F. PERUTZ LABORATORIES MEDICAL UNIVERSITY OF VIENNA, AUSTRIA), ANDREA BARTA (MAX F. PERUTZ LABORATORIES MEDICAL UNIVERSITY OF VIENNA, AUSTRIA), TAO ZHENG (INSTITUTE OF VIROLOGY AND BIOTECHNOLOGY ZHEJIANG ACADEMY OF AGRICULTURAL SCIENCE, CHINA)

JOHN.DOONAN@ABER.AC.UK

The Arabidopsis CDKG gene defines a clade of cyclin-dependent protein kinases, structurally and functionally related to kinases found in the Ph1 locus that suppress homeologous recombination in wheat. We recently demonstrated that CDKG1/CYCLINL1 is essential for synapsis and recombination during male meiosis at high ambient temperature. In order to determine where in the meiotic recombination pathway the CDKG1 is acting, we crossed

cdkg1-/- mutants with several well characterized meiotic mutants. Our results suggest that CDKG1 acts upstream of the decision to go through a class I or class II crossover pathway. In addition, we found that CDKG1 is alternatively spliced in a temperature-dependent manner. The two splice variants have distinct sub-cellular localizations suggesting a subspecialisation of isoform function as was observed with the CDKG1 mammalian counterpart CDK11.

## PC2.9 MOLECULAR CONTROL OF FORMATIVE CELL DIVISIONS IN THE ARABIDOPSIS ROOT

WEDNESDAY 5 JULY, 2017 09:55

IVE DE SMET (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM)

IVSME@PSB.VIB-UGENT.BE

Plants rely on coordinated formative cell division for the formation of new cell types and tissues. For example, in the Arabidopsis primary root tip, columella stem cells - upon formative cell division - give rise to new stem cells and daughter cells that will differentiate. In addition to controlling primary root growth, developing lateral roots is another strategy which allows the plant to maximize the area over which nutrients are absorbed, and further allows the plant to anchor itself more firmly in the soil. The development of lateral root primordia goes through several well-described stages, with the first stages being essential for proper lateral root primordium development. Typically, stage 1 comprises two rounds of asymmetric cell divisions of a small set of pericycle founder cells, forming smaller daughter cells with distinct cell fates. At stage 2, a rotation in the plane of division occurs; the cells divide periclinally toward the outer tissues forming an outer layer and an inner layer. Here, I will discuss our latest results on the role of small signalling peptides (RALFL34 and CEP5), the receptor kinase ACR4, the phosphatase PP2A, and the cell wall remodelling enzyme EXPA1 in regulating the above-described processes.



## PC2.10 RETINOBLASTOMA – A CENTRAL REGULATOR OF DNA DAMAGE RESPONSE

WEDNESDAY 5 JULY, 2017 11:00

ARP SCHNITTGER (UNIVERSITY OF HAMBURG, GERMANY)

@ ARP.SCHNITTGER@UNI-HAMBURG.DE

The retinoblastoma protein (Rb), which typically functions as a transcriptional repressor of E2F-regulated genes, represents a major control hub of the cell cycle. In addition, Rb has been implicated in many aspects of eukaryotic life including cell differentiation. Here, we show that loss of the Arabidopsis Rb homolog RETINOBLASTOMA-RELATED 1 (RBR1) leads to cell death, especially upon exposure to genotoxic drugs. Slowing down the cell cycle by concomitantly reducing cyclin-dependent kinase (CDK) activity largely prevented cell death. However, even the *rbr1 cdk* double mutant had elevated levels of DNA damage after exposure to genotoxic drugs indicating a direct role of RBR1 in the DNA-damage response (DDR). Consistent with its role as a transcriptional repressor, we find that RBR1 directly binds to and represses key DDR genes such as *RADIATION SENSITIVE 51 (RAD51)*, leaving it unclear why *rbr1* mutants are hypersensitive to DNA damage. However, we find that RBR1 is also required for RAD51 localization to DNA lesions. We further show that RBR1 is itself targeted to DNA break sites, dependent on the activity of a plant specific class of CDKs, i.e. CDKB1s, which we have recently identified as key regulators of DDR in plants. Thus, there is a regulatory cascade emerging that is important for the assembly of DNA-bound repair complexes. Based on its central role in DDR, we have now obtained the genome-wide binding profile of RBR1 in Arabidopsis. This has led to the identification of several novel genes involved in DDR that are presented here.

## PC2.11 SPATIAL CONTROL OF CYTOKINESIS IN *ARABIDOPSIS THALIANA*

WEDNESDAY 5 JULY, 2017 13:50

SABINE MULLER (UNIVERSITY OF TUEBINGEN, GERMANY), ARVID HERRMANN (UNIVERSITY OF TUEBINGEN, GERMANY), PANTELEIMON LIVANOS (UNIVERSITY OF TUEBINGEN, GERMANY), ELISABETH LIPKA (UNIVERSITY OF TUEBINGEN, GERMANY)

@ SABINE.MUELLER@ZMBP.UNI-TUEBINGEN.DE

The timing and orientation of cell divisions in plants significantly contributes to cellular morphogenesis. During cytokinesis, cell plate formation initiates in the division plane and its expansion progresses laterally towards the parental wall, aided by the cytoskeletal phragmoplast. Intriguingly, the site of cell plate attachment, designated the cortical division site is already selected at the G2/M transition and delineated at the cell cortex by the preprophase band. However, the molecular pathways that enable maintenance of the cortical division site and its recognition by the approaching phragmoplast are poorly defined.

Simultaneous mutation of the functionally redundant pair of *PHRAGMOPLAST ORIENTING KINESIN (POK)1* and *POK2* lead to oblique cell wall insertions due to the mismatch of preprophase

band and phragmoplast position in *pok1 pok2* double mutants. POK1 is recruited to the preprophase band and then retained at the cortical division site throughout mitosis. Cortical division site markers TANGLED and RanGAP1, which both interact with the carboxy (C)-terminal of POK1 are lost from the cortical division site in *pok1 pok2* mutants suggesting that POKs act as a molecular framework to preserve cortical division site identity.

To gain further insight on how POK proteins serve as scaffolds for cortical division site resident proteins and how they contribute to phragmoplast guidance, we pursue cell biological characterization of POK dependent pathways using the *pok1 pok2* double mutant as a genetic tool and we dissect POK functions by characterization of their protein domains.

## PC2.12 KINESIN-4-MEDIATED SHORTENING OF MICROTUBULE OVERLAP REGIONS AS A MECHANISM TO CONTROL POLARITY IN MICROTUBULE ARRAYS

WEDNESDAY 5 JULY, 2017 14:30

TIJS KETELAAR (WAGENINGEN UNIVERSITY, NETHERLANDS), JEROEN DE KEIJZER (WAGENINGEN UNIVERSITY, NETHERLANDS), RUBEN VAN SPOORDONK (WAGENINGEN UNIVERSITY, NETHERLANDS), JOANNE VAN DER MEER - VERWEIJ (WAGENINGEN UNIVERSITY, NETHERLANDS), MARCEL JANSON (WAGENINGEN UNIVERSITY, NETHERLANDS)

@ TIJS.KETELAAR@WUR.NL

During plant cytokinesis, cells initiate a new cell wall segment that separates the two daughter cells. For this, the cell plate, a disk-shaped cell wall precursor is deposited. Cell plate deposition starts in the centre of the cell that expands radially towards the parental cell walls. The position of the cell plate is templated by the bipolar phragmoplast microtubule network that is thought to deliver vesicles containing cell wall precursors to the division plane. Recently we have shown that in the moss, *Physcomitrella patens* initial accumulation of materials for cell plate formation occurs along regions of microtubule overlap in the centre of the phragmoplast. Kinesin-4 mediated shortening of these microtubule overlaps is required to spatially confine cell plate deposition. In the absence of shortening, wider cell plate deposition resulted into cell walls that were thick and irregularly spaced. Thus, the regularly spaced short microtubule overlaps in the phragmoplast midzone provide a lattice on which a new cell wall segment can be scaffolded.

Next, we focused on the interphase microtubule array of tip-growing protonema cells and show that Kinesin-4 performs a similar function in shortening of microtubule overlaps in this array. Loss of Kinesin-4 leads to bipolarisation and hyperparallelization of the microtubule array, which in turn reduces the ability of caulonema tip cells to alter their growth direction. These results identify Kinesin-4 mediated control of microtubule overlap lengths as a novel mechanism to maintain a uniform polarity and limit the amount of cross-linking in the plant interphase microtubule array.

## PC2.14 INTERACTIONS OF THE PLANT CYTOSKELETON WITH MEMBRANES

WEDNESDAY 5 JULY, 2017 15:45

PATRICK J HUSSEY (DURHAM UNIVERSITY, UNITED KINGDOM)

P.J.HUSSEY@DURHAM.AC.UK

Plants do not have the wide variety of adaptor proteins to produce the specialized sites of interaction between actin and membranes that are present in animal cells. However, actin-membrane interactions within plant cells are critical for the positioning of subcellular compartments, for coordinating intercellular communication, and for membrane deformation. Novel factors are therefore likely to provide interfaces at actin-membrane contacts in plants, for example, the plant-specific Networked (NET) superfamily of actin-binding proteins. The coordinated action of other actin binding/regulating proteins may also be involved in the cytoskeleton's interaction with membranes, for example, the actin nucleating complex Arp2/3 and its regulatory network involving the SCAR/WAVE complex. Here, recent developments on the molecular mechanism of the actin cytoskeleton - membrane interaction will be discussed.

## PC2.15 GENOME-WIDE CHROMATIN MAPPING WITH SIZE RESOLUTION REVEALS A DYNAMIC SUB-NUCLEOSOMAL LANDSCAPE IN ARABIDOPSIS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

EMILY SORNAY (CARDIFF UNIVERSITY, UNITED KINGDOM), DANIEL A PASS (CARDIFF UNIVERSITY, UNITED KINGDOM), NICHOLAS A KENT (CARDIFF UNIVERSITY, UNITED KINGDOM), ANGELA MARCHBANK (CARDIFF UNIVERSITY, UNITED KINGDOM), JIM A H MURRAY (CARDIFF UNIVERSITY, UNITED KINGDOM)

SORNAYE@CARDIFF.AC.UK

All eukaryotic genomes are entwined as chromatin, where DNA is interlaced with both regularly patterned nucleosomes and other mobile/labile sub-nucleosomal-sized protein structures such as transcription factors (TF) and initiation complexes. The nucleosomal landscape depicting chromatin structure maps are generated using the differential micrococcal nuclease (MNase) digestion combined with paired-end sequencing (Kent et al. 2011, *Nucleic Acids Research*). Additionally, incorporation of RNAseq expression data provides insight into the transcriptional activity of the identified DNA-bound with varying particle size.

Combining differential intensities of MNase-seq and total RNAseq on *Arabidopsis thaliana* Col-0 wild-type in vitro cell culture, has enabled the mapping of small complexes surrounding genomic features and their differing strengths of correlation to gene expression. We previously showed that the presence of sensitive positions and a labile -1 nucleosome upstream of the TSS of active genes.

Cell cycle is a dynamic process. The regulation of its progression and chromatin structure are intertwined and essential to the transfer of the proper genetic and epigenetic information to the daughter cells. E2F<sub>c</sub> and CYCD3 are core regulators involved in the transmission G1-to-S-phase of the cell cycle. Applying differential

MNase-seq and RNA-seq to knock-out/over-expressors of E2F<sub>c</sub> and CYCD3 respectively, we show significant chromatin reorganisation effects occurring during cell cycle.

## PC2.16 MITOCHONDRIAL-DEPENDENT NO HOMEOSTASIS DRIVES ROOT DEVELOPMENT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

LUIS SANZ (SPANISH-PORTUGUESE AGRICULTURAL RESEARCH INSTITUTE (CIALE), SPAIN), TAMARA LECHÓN (SPANISH-PORTUGUESE AGRICULTURAL RESEARCH INSTITUTE (CIALE), SPAIN), NOEL BLANCO-TOURINÁN (INSTITUTE FOR PLANT MOLECULAR AND CELL BIOLOGY (IBMCP), SPAIN), VIRGINIA PALOMARES (SPANISH-PORTUGUESE AGRICULTURAL RESEARCH INSTITUTE (CIALE), SPAIN), MIGUEL A BLÁZQUEZ (INSTITUTE FOR PLANT MOLECULAR AND CELL BIOLOGY (IBMCP), SPAIN), IVETT BÁRÁNY (CENTRE FOR BIOLOGICAL RESEARCH (CIB), SPAIN), MIGUEL A MORENO-RISUEÑO (CENTRE FOR PLANT BIOTECHNOLOGY AND GENOMICS (CBGP), SPAIN), MARI C RISUEÑO (CENTRE FOR BIOLOGICAL RESEARCH (CIB), SPAIN), PILAR S TESTILLANO (CENTRE FOR BIOLOGICAL RESEARCH (CIB), SPAIN), WALTER DEWITTE (CARDIFF UNIVERSITY, UNITED KINGDOM), ÓSCAR LORENZO (SPANISH-PORTUGUESE AGRICULTURAL RESEARCH INSTITUTE (CIALE), SPAIN)

LUSAN@USAL.ES

Root development has been greatly studied through the use of the model organism *Arabidopsis thaliana*. Development of the *Arabidopsis* root is a dynamic process that involves the integration of different signals to regulate complex gene networks. Our recent research has uncovered a role for nitric oxide (NO) on primary root growth. NO accumulates in cortex/endodermis stem cells and their immediate progeny, generating endodermal and cortical tissues, suggesting that NO could play an important role in regulating stem cell decisions, as has been reported in animals.

Mitochondria play an essential role during root growth to supply energy and required substrates. Current studies propose that mitochondria are one of the major producers of NO in plants. In particular, the PROHIBITIN3 (PHB3) and NO-ASSOCIATED1 (NOA1) genes are expressed in root mitochondria and have been reported to influence NO levels in different tissues.

We performed a transcriptomic meta-analysis between NOA1 and PHB3 targets, and we found a putative link between both proteins during root development. Loss-of-function mutant at *phb3* provokes defects in root development, including decreased meristematic cell production, while it strikingly increases expression of mitotic marker CYCB1;1. In addition, PHB3 and NOA1 jointly affect mitochondrial biogenesis and stem cell activity by regulating WUSCHEL-related homeobox5 (WOX5) expression, which is specifically expressed in the quiescent center (QC).

# PC3 MEMBRANE DYNAMICS: SIGNALLING AND POLARITY

**ORGANISED BY:** NICK MONK (UNIVERSITY OF SHEFFIELD, UK)  
AND KATIE FISHER (UNIVERSITY OF SHEFFIELD, UK)

## PC3.1 TUNING NOTCH SIGNALLING THROUGH AND AN ENDOCYTIC REGULATORY NETWORK: REVISITING OLD GENETIC PROBLEMS WITH NEW INSIGHTS

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 MARTIN BARON (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), MARIAN B WILKIN (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), HIDEYUKI SHIMIZU (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), SIMON WILKIN (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), BARBORA TRUBENOVA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), NICK MONK (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), GIULIA MONTECONE (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), ZHI HUANG (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ MARTIN.BARON@MANCHESTER.AC.UK

The Notch gene encodes a fundamentally important, highly conserved cell signalling receptor and there are few biological processes that are not impacted on by its activity, from organogenesis to stem cell regulation, from brain development to memory formation, with widespread implications for human health. There have been 100 years of study of the Notch gene following pioneering work on mechanisms of inheritance by Thomas Hunt Morgan. Mutational analysis of Notch subsequently revealed its complex genetics with many different context-dependent alleles exhibiting tissue specific, often temperature sensitive phenotypes whose mechanistic bases are poorly understood. Through computational simulation, cell biology and whole genome RNAi studies, we showed that the setting of Notch signalling levels is deeply embedded in the physiology of the cell through the operation of an endocytic trafficking network that tunes signal activity by regulating Notch flux towards inhibitory or signal activation outcomes. Our new model can explain long standing observations of perplexing temperature and genetic background dependent switching between loss and gain of function Notch signalling phenotypes in *Drosophila* and is providing a new overview to understand the relationship between structure and function of Notch and its mutant phenotypes.

## PC3.2 CRUMBS PREVENTS ECTOPIC NOTCH ACTIVATION IN *DROSOPHILA* BY INHIBITING LIGAND-INDEPENDENT ENDOCYTOSIS

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 LINDA NEMETSCHKE (MPI-CBG, GERMANY), ELISABETH KNUST (MPI-CBG, GERMANY)

@ NEMETSCH@MPI-CBG.DE

Crumbs and Notch are two evolutionarily conserved, apically localized transmembrane proteins with big extracellular domains rich in EGF-like repeats. Crumbs is one of the key determinants of apical-basal polarity. Notch is a ubiquitously expressed receptor, which performs a plethora of functions; therefore, its activity has to be tightly regulated. Here we show that *Drosophila* Crumbs prevents Notch endocytosis in developing wings through direct interaction between the two proteins. Notch endocytosis in the absence of Crumbs does not require the ligands Delta and Serrate or  $\gamma$ -secretase activity. Yet, it results in activation of the ligand-independent, Deltex-dependent Notch signalling pathway. This function of Crumbs is not due to general defects in apico-basal polarity, as localisation of other apical proteins is unaffected. Our data reveal a new mechanism to control localisation and activity of the potent Notch receptor, and uncover a novel role of Crumbs, which is independent of its role in apico-basal polarity.

## PC3.3 ROLE OF THE Rsr1 GTPase IN *CANDIDA ALBICANS* HYPHAL GUIDANCE – AND BEYOND

📅 MONDAY 3 JULY, 2017 ⌚ 10:10

👤 TINA BEDEKOVIC (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), ALEXANDRA C BRAND (UNIVERSITY OF ABERDEEN, UNITED KINGDOM)

@ TINA.BEDEKOVIC@ABDN.AC.UK

*Candida albicans* is an opportunistic fungal pathogen that can cause systemic infections in immunocompromised patients, where the formation of invasive hyphal filaments contributes to fatal levels of sepsis and organ failure. Hypha-mediated tissue damage depends on directional growth responses that are regulated by the small N-Ras-like GTPase, Rsr1. In *S. cerevisiae*, Rsr1 is a K-Ras and specifies

the yeast bud site where it is activated by its guanine-nucleotide exchange factor (GEF), Bud5, and de-activated by its GTPase activating protein (GAP), Bud2. However, its role in *C. albicans* hyphae is less well understood. The site of Rsr1 activity is thought to be regulated by the localization of Bud5 and Bud2 at the tip and subapical region of hyphae, respectively. We generated mutant strains expressing a single copy of Rsr1 GDP-locked (Rsr1<sup>K16N</sup>) or GTP-locked (Rsr1<sup>G12V</sup>) and fused them with YFP to further elucidate the regulation of Rsr1 in hyphae. Here we show that YFP-Rsr1K16N and YFP-Rsr1G12V are differentially localised. Affinity purification of heterologously-expressed GST-tagged mutant versions of Rsr1 against lysates of yeast and hyphae suggests that activity state and cell morphology differentially determine the binding partners of Rsr1. These data, together with phenotypic analyses, indicate that Rsr1 may not only have a role in growth-site selection, but also in nuclear ploidy and cell stress responses. These findings suggest that the role of N-Ras Rsr1 GTPase in *C. albicans* extends beyond directional growth.

### PC3.4 COORDINATION IS KEY - RHO GTPASE RECRUITMENT AND PROTEIN COMPLEX ASSEMBLY AT THE ROOT HAIR INITIATION DOMAIN

MONDAY 3 JULY, 2017 10:25

PHILIPP DENNINGER (COS HEIDELBERG, GERMANY), GUIDO GROSSMANN (COS-HEIDELBERG, GERMANY)

PHILIPP.DENNINGER@COS.UNI-HEIDELBERG.DE

We study the establishment of polar plasma membrane domains using root hair initiation in *Arabidopsis* as a model system. This process involves recruitment and activation of small GTPases, a common mechanism in all eukaryotes. Despite the conserved nature of polar growth, our understanding of the mechanisms how the plasma membrane is specified locally and how protein recruitment is coordinated is limited. To resolve the stepwise assembly of the tip growth machinery we followed the timed association of 34 proteins involved in root hair initiation and present a time-line of membrane domain formation. Rho-like GTPases from plants (ROPs) are among the first proteins to accumulate at the root hair initiation site, followed by multiple factors of different functions. ROPs are known key players for polar growth, but it remains unclear whether they mark the site of hair emergence or follow upstream determinants. To identify genes with a role in ROP recruitment, we analyzed known and putative ROP interactors. We identified candidates that associate with the root hair initiation domain concurrently with ROP2. In loss-of-function mutants, ROP2 accumulation and, consequently, root hair initiation was impaired. Upon overexpression of selected markers, ectopic polar domains were formed at the plasma membrane that were able to recruit ROP2. Our results suggest a mechanism that involves recruitment factors that mark the root hair initiation domain and are necessary and sufficient for the polar accumulation of Rho-GTPases to the plasma membrane - a key step during the initiation of tip growth.

### PC3.5 THE COMPLEX RELATIONSHIP BETWEEN POLARIZED GROWTH AND CELL POLARITY

MONDAY 3 JULY, 2017 14:00

NATASHA S SAVAGE (UNIVERSITY OF LIVERPOOL, UNITED KINGDOM)

NSAVAGE@LIVERPOOL.AC.UK

Cell morphology and cell function are inextricably linked. One factor of morphology control is localized (polarized) growth. Exocytic vesicles provide the plasma membrane needed for cell growth. Localizing the delivery, and insertion, of exocytic vesicles to specific growth areas is the job of the Rho GTPases Cdc42 and Rac1.

In this presentation we will talk about the interplay between polarized patches of Cdc42/Rac1, which mark the sites of vesicle insertion (polarized growth), and the act of vesicle insertion itself. We will use work undertaken in fungal species and in silico to support the discussions but the basic principles addressed have implications on all higher eukaryotes.

### PC3.6 MOLECULAR MECHANISMS OF COORDINATED CELL POLARISATION IN THE *DROSOPHILA* WING

MONDAY 3 JULY, 2017 14:40

KATHERINE H FISHER (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), NICHOLAS A MONK (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), DAVID STRUTT (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

K.H.FISHER@SHEFFIELD.AC.UK

The beautiful and diverse patterns observed throughout nature originate from a common process of symmetry breaking. Animal stripes, the branches of a tree and even the characteristic shape of our own hands are examples of spatial patterns, where growth or deformation occur at varying rates over tissues and preferentially in particular directions. This patterned growth is self-organised, coordinated by internal rules at a cellular level. A major challenge is to decipher these rules with the ultimate aim of preventing or treating developmental defects that arise from errors in this process.

The specification and coordination of orientations within growing tissues remain poorly understood. My research employs powerful genetics and image analysis of the developing *Drosophila* wing to determine how cells become polarised and how polarity is coordinated at a tissue scale. I have developed a computational model to determine whether particular hypothesised molecular mechanisms can account for the observed patterns of polarity. We have experimentally tested model predictions to uncover how the Fat/Dachsous molecular system can extract vectorial information from a chemical gradient to polarise cells evenly across a tissue. This work demonstrates the power of an integrative approach in uncovering the molecular mechanisms by which such long-range gradients are interpreted.

### **PC3.7 MODELLING BASIC MECHANISMS OF PLANAR CELL POLARITY GENERATION AND COORDINATION IN EPITHELIA**

📅 **MONDAY 3 JULY, 2017**      ⌚ **15:10**

👤 **NICK MONK (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM),  
LU XIAO (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)**

@ **N.MONK@SHEFFIELD.AC.UK**

Cells in epithelial sheets often exhibit planar cell polarity (PCP) – overt polarisation within the plane of the epithelium. The establishment and coordination of PCP is known to depend on transmembrane protein complexes, which adopt non-uniform distributions within each cell during cell polarisation. I will present mathematical models of transmembrane complex formation, with the aim of understanding the extent to which juxtacrine signalling events can account for observed features of PCP, and the requirements for externally imposed global polarity information.

# PC4 LIFE AT THE INTERFACE: PLANT MEMBRANE-PROTEIN DYNAMICS/INTERACTIONS DURING ENVIRONMENTAL CHANGE

**ORGANISED BY:** ANGUS MURPHY (UNIVERSITY OF MARYLAND, UNITED STATES), PIERS HEMSLEY (UNIVERSITY OF DUNDEE, UK) AND WENDY PEER (UNIVERSITY OF MARYLAND, UNITED STATES)

**SESSION SPONSORED BY:** FRONTIERS

## PC4.1 ABA RECEPTORS TRANSIENTLY INTERACT WITH MEMBRANES THROUGH C2-DOMAIN CAR PROTEINS

WEDNESDAY 5 JULY, 2017 09:00

PE德罗 L RODRIGUEZ (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), ALBERTO COEGO (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), BORJA BELDA-PALAZON (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), JORGE LOZANO-JUSTE (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), JOSE JULIAN (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), MARIA A. FERNANDEZ (INSTITUTO DE BIOLOGIA MOLECULAR Y CELULAR DE PLANTAS, SPAIN)

@ PRODRIGUEZ@IBMCP.UPV.ES

Regulation of ion transport in plants is essential for cell homeostasis and stress response. Abiotic stress unbalances cell ion homeostasis and plants tend to re-adjust it regulating membrane transporters and channels. The plant hormone abscisic acid (ABA) and the second messenger  $Ca^{2+}$  are central in such processes since they are involved in the regulation of protein kinases and phosphatases that control ion transport activity in response to environmental stimuli. Membrane-delimited ABA signal transduction plays a critical role in this process, but little information is available on the molecular mechanisms linking core signaling components to the plasma membrane. We have shown that transient calcium-dependent interactions of ABA receptors with plasma membrane is mediated through a 10-member family of C2-domain ABA-related (CAR) proteins, CAR1 to CAR10, in *Arabidopsis thaliana*. CAR1 and CAR4 interact with several ABA receptors in plasma membrane as well as in nucleus of plant cells. The crystal structure of CAR1 and CAR4 was solved, which revealed that, in addition to a classical calcium-dependent lipid-binding C2-domain, a specific CAR signature is responsible for the interaction with ABA receptors and their recruitment to phospholipid vesicles. This interaction is relevant for receptor function and ABA signaling since different car triple mutants affected in *CAR1*, *CAR4*, *CAR5* and *CAR9* genes showed reduced sensitivity to ABA in seedling establishment and root growth assays. In summary, we identified ABA receptor-interacting partners that mediate a transient  $Ca^{2+}$ -dependent interaction with phospholipid vesicles, which affects receptor sub-cellular localization and positively regulates ABA signaling.

## PC4.2 BULK INTERNALIZATION, SORTING, AND TURNOVER OF TRANSPORTERS ASSOCIATED WITH ORDERED LIPID DOMAINS IN RESPONSE TO SALT STRESS IN ARABIDOPSIS IS DISTINCT FROM ABA DEPENDENT PROCESSES

WEDNESDAY 5 JULY, 2017 09:40

ANGUS MURPHY (UNIVERSITY OF MARYLAND, UNITED STATES), CHANGXU PANG (UNIVERSITY OF MARYLAND, UNITED STATES), DORON SHKOLNIK (UNIVERSITY OF MARYLAND, UNITED STATES), WENDY A PEER (UNIVERSITY OF MARYLAND, UNITED STATES), WIEBKE TAPKEN (UNIVERSITY OF MARYLAND, UNITED STATES), MARK JENNESS (UNIVERSITY OF MARYLAND, UNITED STATES), JOSHUA BLAKESLEE (OHIO STATE UNIVERSITY, UNITED STATES)

@ ASMURPHY@UMD.EDU

Salt-induced internalization of plasma membrane (PM) ATP Binding Cassette (ABC) and Intrinsic Proteins (PIPs) has been reported in *Arabidopsis* and other plant species. Internalization of these transporters has been variously described as selective and ABA-dependent. For instance, transcript abundance associated with the ABCB4 root auxin transporter gene is regulated via the ABI4 transcription factor and microRNA844 under nitrogen limitation that induces ABA signalling processes. In shorter timeframes, ABA also impacts trafficking of ABCB4 at the *trans* Golgi Network with the result of diversion of the protein to the prevacuolar compartment. Presumably, regulation of ABCB4 in the root epidermis regulates auxin-dependent growth, but may also limit secondary nitrate channel activity associated with ABCB4. However, 75-100 mM NaCl or KCl treatment provokes an almost instantaneous internalization of ABCB4, PIP isoforms, and ABCG36/PEN3/PDR9 in larger endosomal structures. This internalization is not ABA dependent, co-occurs with rapid decreases in root turgor, membrane depolarization, and cytosolic pH, as well as appearance of vacuolar "bulbs". The rapidity of the process and impact on a set of proteins previously shown to be associated with ordered membrane domains suggests the presence of a process similar to salt-induced, "bulk flow" membrane internalization described in *Saccharomyces cerevisiae*. Roots restored to control conditions recover, with differential rates of recovery observed with various membrane markers. It is proposed

that these processes are primarily a consequence of turgor-induced membrane turnover, but have a secondary protective effect in reducing permissive ion uptake when the lipid environment at the PM is perturbed.

#### PC4.6 THE ARABIDOPSIS ASPARTYL PROTEASE 2 FUNCTIONS IN THE TRANS-GOLGI NETWORK

WEDNESDAY 5 JULY, 2017 11:00

WENDY PEER (UNIVERSITY OF MARYLAND, UNITED STATES), CHANGXU PANG (UNIVERSITY OF MARYLAND, UNITED STATES), DORON SHKOLNIK (UNIVERSITY OF MARYLAND, UNITED STATES), ANGUS S MURPHY (UNIVERSITY OF MARYLAND, UNITED STATES)

WAPEER@UMD.EDU

Plant aspartyl proteases localized to the apoplast function in germination and defence. A separate clade of aspartyl proteases, represented here by ASPARTYL PROTEASE 2 (APA2) contain a saposin-like lipid interaction domain and function within the lumen of the endomembrane system. APA2 is synthesized as a propeptide and is auto-activated at acidic pH (pH ~5.5 and below). An APA2-CFP functional fusion partially co-localized with the pre-vacuolar compartment (PVC) marker BP80-GFP and the *trans*-Golgi Network (TGN) marker SYP61-YFP. The SYP61 compartment was shown to be pH 5.6, and saposin domains mediate membrane fusion at acidic pH. The ATP-Binding Cassette subfamily B transporter ABCB4, a plasma membrane, fasciclin-like arabinogalactan-anchored protein which was previously found to traffic to the plasma membrane via the SYP61 compartment, was identified as a substrate for APA2. APA2 cleaves an oligopeptide corresponding to an extracellular (luminal) loop of ABCB4, and *in vitro* assays of plant extracts showed ABCB4 degradation in the presence of APA2 at acidic pH. The saposin-like domain may facilitate proteolysis *in vivo*. Stress treatments increased APA2 co-localization with SYP61 and BP80. APA2 appears to have a role in ABCB4 redirection to the PVC via VTI11/12 following stress treatment.

#### PC4.7 ENDOCYTIC MECHANISMS OF MEMBRANE PROTEINS IN PLANTS - A SINGLE-MOLECULE PERSPECTIVE

WEDNESDAY 5 JULY, 2017 11:30

XIAOJUAN LI (BEIJING FORESTRY UNIVERSITY, CHINA), JINXING LIN (BEIJING FORESTRY UNIVERSITY, CHINA), LUSHENG FAN (INSTITUTE OF BOTANY CHINESE ACADEMY OF SCIENCES, CHINA), RUILI LI (BEIJING FORESTRY UNIVERSITY, CHINA)

LIXJ@BJFU.EDU.CN

The plant plasma membrane (PM) is highly dynamic. Thus, measuring the mobility of PM proteins is key to understanding cellular signaling mechanisms. In the past few years, we have developed VA-TIRFM to image PM proteins in intact plant cells, provided new information on the spatiotemporal dynamics of specific molecules.

Clathrin-mediated endocytosis plays an essential role in many cellular and developmental processes. Based on single-molecule and genetic approaches, we demonstrated that Arabidopsis AP2 $\sigma$  is closely associated and physically interacts with the clathrin light chain (CLC). In addition, the density and turnover rate of the CLC spots are significantly reduced in the *ap2 $\sigma$*  mutant. These findings led us to conclude that AP2 is involved in the CCV initiation, assembly and maturation stages, which is required for clathrin-mediated endocytosis.

In recent years, the possible functions of plant membrane raft-like domains have been described. We found that Arabidopsis flotillin1 (AtFlot1) was associated with the membrane microdomains. We further demonstrated that the dynamic behavior and localization of Flot1 puncta were different from that of CLC puncta. Moreover, reduction in meristem size and retardation in seedling growth were detected in amiRNA AtFlot1 transgenic lines. These findings suggested that AtFlot1 is involved in a clathrin-independent endocytic pathway and functions in seedling development.

Further studies based on single-molecule approaches revealed that clathrin and AtFlot1-associated membrane microdomains cooperatively regulate PM protein dynamics and endocytosis, such as aquaporin (PIP2;1), and the membrane microdomain alters PM proteins activities by positively or negatively affecting their clustering and signal transduction.

## PC4.8 MOLECULAR MECHANISM OF TOUCH SENSING AND SIGNALLING

WEDNESDAY 5 JULY, 2017 13:50

RAINER HEDRICH (UNIVERSITY OF WÜRZBURG, GERMANY)

HEDRICH@BOTANIK.UNI-WUERZBURG.DE

The response of the carnivorous plant *Dionaea muscipula* to mechanical stimulation has captured the imagination of scientist early on. Insects attracted by the odour of the fly trap and exploring the inner trap lobe accidentally displace sensory trigger hairs causing firing of individual action potentials (APs). Trigger hair stimulation is only recognized as potential food when a moving object elicits two APs: Excitation-contraction of the capture organ by the second AP trigger results in local turgor changes and immediate buckling of the trap's two lobes into a cage. Excitation-contraction coupling during fast trap closure relies on molecular mechanisms operated by the trigger hair.

To address the nature and electro-biology of *Dionaea's* mechanosensory organ, we have isolated multicellular trigger hairs from the fly trap and identified their transcriptomic profile focusing on genes with pronounced differential expression in the multicellular mechanosensor. In addition to gene clusters required for housekeeping functions, we identified transcripts associated with mechanosensation and ion transport. Bioinformatics analyses identified trigger hair expressed channel types likely associated with peculiar features of the action potential originating in the mechanosensor.

## PC4.9 THE ANION CHANNEL SLAH3 AND ITS MULTIPLE MODES OF REGULATION

WEDNESDAY 5 JULY, 2017 14:30

INES KREUZER (UNIVERSITY OF WÜRZBURG MOLECULAR PLANT PHYSIOLOGY AND BIOPHYSICS, GERMANY), GEIGER DIETMAR (UNIVERSITY OF WÜRZBURG MOLECULAR PLANT PHYSIOLOGY AND BIOPHYSICS, GERMANY)

IFUCHS@BOTANIK.UNI-WUERZBURG.DE

SLAC/SLAH anion channels are master switches of plant stress responses. While SLAC1 expression is restricted to guard cells, its homolog SLAH3 is expressed in various tissues including guard and mesophyll cells and roots. Interestingly, SLAH3 activity is modulated in a tissue/cell specific way. When expressed in aerial tissues, SLAH3 anion channels are not active per se but require extracellular nitrate and phosphorylation by calcium-dependent kinases (CPKs). In roots, however, SLAH3 does not need phosphorylation for activity: there, SLAH3 co-localises with SLAH1 channel subunits in the pericycle cells adjacent to the xylem vessels. When co-expressed in *Xenopus* oocytes, the electrically silent SLAH1 subunit gates SLAH3 open even in the absence of nitrate and CPKs. Apparently, the heteromerization of SLAH1/SLAH3 facilitates SLAH3-mediated chloride efflux from pericycle cells into the root xylem. Under salt stress or ABA treatment, the transcription of SLAH1 and SLAH3 is massively reduced, which represents an elegant way of circumventing excessive chloride uptake under saline conditions. In *Arabidopsis* mesophyll cells, SLAH3 is regulated differently: here, SLAH3 is phosphorylated and activated by CPK21, which is itself controlled by the protein phosphatase ABI1. All ABA signalling components regulating membrane-delimited

steps downstream of ABI1 are at least transitionally associated with stable lipid nanodomains. Particularly, CPK21 and SLAH3 predominantly interact in such membrane domains, which results in channel activation and anion efflux. In the presence of ABI1 (i.e. absence of ABA), SLAH3 and CPK21 do not enter common lipid nanodomains, which inhibits their interaction and thus prevents SLAH3-mediated anion currents.

## PC4.10 HOW DO HIGHER PLANTS SENSE TEMPERATURE?

WEDNESDAY 5 JULY, 2017 15:00

IOANNA KOSTAKI (UNIVERSITY OF BRISTOL, UNITED KINGDOM), VERITY C BONNELL (UNIVERSITY OF BRISTOL, UNITED KINGDOM), ALISTAIR M HETHERINGTON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), ANTONY N DODD (UNIVERSITY OF BRISTOL, UNITED KINGDOM), KEARA A FRANKLIN (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

BZKIK@BRISTOL.AC.UK

Understanding how higher plant cells perceive temperature is a major challenge in biology. We report a novel approach to address this question using stomatal guard cells as a model cell system. Guard cells sense environmental signals such as light, temperature, drought and CO<sub>2</sub> and adjust the aperture of the stomatal pore to regulate gas exchange. Increases in temperature promote stomatal opening in order to facilitate evaporative leaf cooling. We have exploited this response in isolated epidermal tissue to identify putative temperature sensing mechanisms. Our data demonstrate that loss of functional alleles of known components in temperature signalling and stomatal opening (*ARP6*, *FT1*, *PIF4*, *PHOT1* and *PHOT2*) do not affect high temperature-mediated guard cell movement. Furthermore, our data suggest that disrupting the interaction of 14-3-3 proteins with the plasma membrane H<sup>+</sup>-ATPase inhibits high temperature-mediated stomatal opening but does not affect the transcriptional induction of heat shock proteins. Transcriptional responses to elevated temperature may therefore be uncoupled from events that take place in the plasma membrane. Finally, we have found that most members of the non-epsilon group of the 14-3-3 family contribute to high temperature-mediated stomatal opening, while it appears that the H<sup>+</sup>-ATPases AHA1 and AHA2 differentially regulate light and temperature responses, respectively. Although it is likely that there are multiple temperature sensing mechanisms operating in higher plants, guard cells provide a unique opportunity to analyse gene expression and a physiological output at the single cell level.



## PC4.11 STAYING-TIGHT: SHAPING PLASMODESMATA MEMBRANE CONTACT SITES

📅 WEDNESDAY 5 JULY, 2017 ⌚ 15:45

👤 EMMANUELLE BAYER (CNRS UNIVERSITY OF BORDEAUX LABORATORY OF MEMBRANE BIOGENESIS, FRANCE), WILLIAM NICOLAS (CNRS UNIVERSITY OF BORDEAUX LABORATORY OF MEMBRANE BIOGENESIS, FRANCE), MAGALI GRISON (CNRS UNIVERSITY OF BORDEAUX LABORATORY OF MEMBRANE BIOGENESIS, FRANCE), SYLVAIN TRÉPOT (INSTITUT CURIE, FRANCE), KARL OPARKA (INSTITUTE OF MOLECULAR PLANT SCIENCES UNIVERSITY OF EDINBURGH, UNITED KINGDOM), JENS TILSNER (BIOMEDICAL SCIENCES RESEARCH COMPLEX UNIVERSITY OF ST ANDREWS, UNITED KINGDOM), LYSIANE BROCARD (BORDEAUX IMAGING CENTRE PLANT IMAGING PLATFORM UMS 3420 INRA-CNRS-INSERM-UNIVERSITY OF BORDEAUX, FRANCE)

@ EMMANUELLE.BAYER@U-BORDEAUX.FR

Remarkably and unlike other eukaryotic cell junctions, plasmodesmata (PD) pores establish both membrane and cytoplasmic continuity throughout the plant. Within the PD pores, the plasma membrane (PM) and the endoplasmic reticulum (ER) come to remarkably close proximity and are connected by 'spokes'<sup>1</sup> whose function and identity remain unknown. Both the ER and PM membrane domains lining PD are highly specialised and contain a specific set of proteins and lipids that are critical for proper function<sup>2,3</sup>. Inherent to their structure, PD constitute a specialised type of membrane contact site (MCS), a general term describing areas of close (10-30 nm) apposition between two membranes<sup>4,5</sup>. In yeast and human cells MCS are well-established sites for inter-organelle signalling<sup>6</sup>. In PD, the function of ER-PM tethering remains an enigma. According to current models however, modulation in the gap between the ER and the PM defines the space available for molecular trafficking, governing the size exclusion limit (SEL) of the pores. However, how PD channels are built and organised within the narrow space between the ER and the PM, and how ER-PM spacing affects cell-to-cell connectivity remains little understood. Here we used electron tomography to gain access to the 3D ultrastructure of PD and shed light on the structural plasticity of their ER-PM junctions.

## PC4.12 CALCIUM RELEASE FROM THE PLANT ENDOPLASMIC RETICULUM OCCURS DURING BLUE-LIGHT RETROGRADE SIGNALING FROM THE ER-CHLOROPLAST JUNCTION

📅 WEDNESDAY 5 JULY, 2017 ⌚ 16:15

👤 LAWRENCE R GRIFFING (TEXAS AM UNIVERSITY, UNITED STATES), SARA MAYNARD (TEXAS AM UNIVERSITY, UNITED STATES), MEGHAN CONNOLLY (TEXAS AM UNIVERSITY, UNITED STATES)

@ GRIFFING@TAMU.EDU

In animal cells and yeasts, but not in plants, calcium released from the endoplasmic reticulum (ER) occurs through calcium channels that are either IP<sub>3</sub> receptors or ryanodine receptors and modulates a vast array of signaling responses. The genes for these calcium release channels are not found in plants. Here, however, direct evidence for the ER acting as a store for calcium release, upon the receipt of an abiotic signal in plants is presented. The signal is high intensity 405 nm blue light focused directly on the ER-chloroplast junction. Immediately upon illumination, calcium ions are released into the cytosol, generating a unique calcium signature as detected with cytoplasmically-expressed YCnano65. Photostimulation of other regions of the ER produces a calcium signature that differs in both the timing and magnitude of the response. The evidence that the increased cytosolic calcium ion level comes from the ER includes the following three tests. 1) The response is not inhibited by exogenous lanthanum, indicating that plasma membrane channels are not involved. 2) There is a specific change in the response upon treatment with the ECA (ER-calcium ATPase) inhibitor, cyclopiazonic acid. 3) When calcium concentration is monitored with the ER-targeted calcium sensor, D1ER, the ER calcium ion concentration in the lumen of the ER decreases. The increased cytosolic calcium and changes in the ER ion status may play a role in retrograde signaling to the nucleus which produces a response that overcomes the ER stress which ensues upon photostimulation.

### PC4.13 NEIGHBOR DETECTION AT THE LEAF TIP ADAPTIVELY REGULATES UPWARD LEAF MOVEMENT THROUGH SPATIAL AUXIN DYNAMICS

WEDNESDAY 5 JULY, 2017 16:45

CHRYSOULA K PANTAZOPOULOU (UTRECHT UNIVERSITY, NETHERLANDS), FRANCA J BONGERS (UTRECHT UNIVERSITY-WAGENINGEN UNIVERSITY, NETHERLANDS), JESSE J KÜPERS (UTRECHT UNIVERSITY, NETHERLANDS), EMILIE REINEN (UTRECHT UNIVERSITY, NETHERLANDS), DEBATOSH DAS (UNIVERSITY OF MUNICH, GERMANY), JOCHEM B EVERS (WAGENINGEN UNIVERSITY, NETHERLANDS), NIELS P R ANTEN (WAGENINGEN UNIVERSITY, NETHERLANDS), RONALD PIERIK (UTRECHT UNIVERSITY, NETHERLANDS)

C.PANTAZOPOULOU@UU.NL

Vegetation stands have a heterogeneous light quality distribution, including the red:far-red light ratio (R:FR) that informs plants about proximity of neighbors. Adequate responses to changes in R:FR are important for competitive success. These responses include upward leaf movement (hyponasty) and elongation of stems and petioles. How the detection and response to R:FR are spatially linked and how this spatial coordination between the two affects plant performance remains unresolved. We show in *Arabidopsis thaliana* and *Brassica nigra* that localized FR-enrichment at distinct positions in the leaf induces either hyponasty or petiole elongation. Using a combination of organ-level transcriptome analysis, molecular reporters and physiological measurements we show that auxin dynamics are key to remote response to localized FR-enrichment. Finally, using a computational 3D plant model, we demonstrate that R:FR sensing in a specific leaf position can be advantageous and stimulates the performance of plants at high plant densities.

### PC4.14 THE ROLE OF EXTRACELLULAR VESICLES IN PLANT-MICROBE INTERACTIONS

THURSDAY 6 JULY, 2017 09:00

ROGER W INNES (INDIANA UNIVERSITY, UNITED STATES), BRIAN D RUTTER (INDIANA UNIVERSITY, UNITED STATES)

RINNES@INDIANA.EDU

Exosomes are extracellular vesicles (EVs) that play a central role in intercellular signaling in mammals by transporting proteins and small RNAs. Plants are also known to produce EVs, particularly in response to pathogen infection. The functions of plant EVs are unknown, however. EV production is enhanced in response to infection with *Pseudomonas syringae* or treatment with salicylic acid, suggesting that EVs play a role in defense. Consistent with this hypothesis, proteomic analyses of purified EVs revealed that they are highly enriched in proteins involved in biotic and abiotic stress responses, while sRNA-seq analyses revealed that EVs carry numerous miRNAs. We hypothesize that EVs may mediate host-induced gene silencing during infection by fungal pathogens. In this talk I will present our ongoing work to test this hypothesis, along with the results of reverse genetic screens in which we are identifying *Arabidopsis* genes required for protein loading into EVs.

### PC4.15 S-ACYLATION IN PLANTS – GREASING MEMBRANE PROTEIN FUNCTION?

THURSDAY 6 JULY, 2017 09:40

PIERS A HEMSLEY (UNIVERSITY OF DUNDEE, UNITED KINGDOM), CHARLOTTE H HURST (UNIVERSITY OF DUNDEE, UNITED KINGDOM), DIONNE TURNBULL (UNIVERSITY OF DUNDEE, UNITED KINGDOM), MAIJU A LAURILA (UNIVERSITY OF DUNDEE, UNITED KINGDOM)

P.A.HEMSLEY@DUNDEE.AC.UK

S-acylation is a lipid based post-translational modification of proteins thought to alter a proteins physical properties leading to alterations in how a protein interacts with the membrane environment. It is unique amongst lipid modifications as it is reversible; this lends it the ability to act as a regulatory mechanism. We have shown that ~40% of the plant membrane proteome is S-acylated and that S-acylation affects both integral and peripheral membrane proteins. The study of S-acylation is still in its infancy but we have found that S-acylation is key for the function of two plant-defining groups of proteins; the cellulose synthase complex (CSC) and receptor-like kinases (RLKs). We recently demonstrated that the CSC, a hetero-18-mer, contains ~100 S-acyl groups and is the most heavily S-acylated protein complex ever described. We will discuss the roles and implications of S-acylation in CSC function. Receptor-like kinases are responsible for perception of almost all extracellular physical stimuli in plants. The S-acylation state of RLKs changes upon ligand binding and we will discuss our findings in the context of RLK signalling regulation. We will also detail our work on defining the dynamic S-acyl proteome to increase understanding of how this enigmatic modification regulates membrane protein function.

### PC4.16 SUBCELLULAR REGULATION OF PIP2;5 PLASMA MEMBRANE AQUAPORIN BY LIPID ENVIRONMENTS AND INTERACTING PROTEINS

THURSDAY 6 JULY, 2017 10:10

ANA R FOX (INSTITUT DES SCIENCES DE LA VIE UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM), FRANÇOIS CHAUMONT (INSTITUT DES SCIENCES DE LA VIE UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM)

ANA.FOX@UCLouvain.be

Plants continuously sense and respond to a fluctuating environment by an accurate regulation of channels activity in their membranes. Among these channels are the plasma membrane intrinsic proteins (PIPs), a subfamily of aquaporins that facilitates the passive diffusion of water and/or small solutes across biological membranes. Maize PIP2;5 is highly expressed in root tissues including in the endo- and exodermis and has been suggested to be involved in the radial movement of water. We are currently characterizing the subcellular behaviour of this aquaporin, specifically in relation to changes in the lipid environment and to its interaction with other proteins. To this aim, we generated Maize Black Mexican Sweet (BMS) suspension cultured cells expressing PIP2;5 tagged with the monomeric Yellow Fluorescent Protein. We then modified the lipid environment of

YFP:PIP2;5 using inhibitors of sterols and sphingolipids biosynthesis and observed changes in the diffusion pattern of YFP:PIP2;5 in the plasma membrane by fluorescence recovery after photobleaching. We are also implementing the BioID assay (proximity dependent biotin identification) to screen for PIP interacting proteins in a cellular environment. We generated BMS cells expressing PIP2;5 tagged with a promiscuous biotin protein ligase. Thus, in the presence of biotin, the proteins that are interacting with the fusion protein will be biotinylated, easily purified and identified by mass spectrometry. The first results will be described.

### PC4.17 DYNAMICS OF MEMBRANE-RESIDENT CELL SURFACE RECEPTORS & PARTNERS

THURSDAY 6 JULY, 2017 10:55

THOMAS OTT (UNIVERSITY OF FREIBURG, GERMANY)

THOMAS.OTT@BIOLOGIE.UNI-FREIBURG.DE

Considering the dense packing of proteins at the plasma membrane (PM) and the plethora of environmental stimuli that tissues need to perceive and to integrate simultaneously, cells evolved membrane-based substructures called membrane micro-domains (MDs). These compartments may serve as central hubs for the specific assembly of signalling complexes including receptors. Even though many individual proteins have been described to laterally segregate into MDs at the cell surface, it has been a long-standing question whether pathway-specific components indeed localize to the same MD. I will show evidence for this intriguing phenomenon, unravel essential molecular building blocks and propose a sequence of events that are required and sufficient to maintain an infection-related MD in vivo.

### PC4.18 STOMATAL IMMUNITY REQUIRES SUSTAINING OF FLG22 RESPONSES THROUGH RabG3b-MEDIATED TRAFFICKING

THURSDAY 6 JULY, 2017 11:25

MICHAELA KOPI SCHKE (THE SAINSBURY LABORATORY NORWICH, UNITED KINGDOM), GILDAS BOURDAIS (THE SAINSBURY LABORATORY, UNITED KINGDOM), MARY TETLOW (THE SAINSBURY LABORATORY, UNITED KINGDOM), SILKE ROBATZEK (THE SAINSBURY LABORATORY, UNITED KINGDOM)

MICHAELA.KOPI SCHKE@SAINSBURY-LABORATORY.AC.UK

The FLAGELLIN SENSING 2 (FLS2) receptor kinase mediates immunity against bacterial infections through the perception of flagellin (flg22). A critical defence response is the closure of stomata to prevent bacteria from entering the plant interior. Yet, the molecular events underlying sustained flg22-induced stomatal closure remain unclear. Here, we report the identification of the Rab7-like small GTPase RabG3B specifically regulating stomatal closure induced by flg22 but not abiotic stresses. Consistently, *rabg3b* mutants exhibited enhanced susceptibility to surface infected bacteria. Time course analysis showed that *rabg3b* mutants are competent for closing stomata induced by flg22 but fail to sustain stomatal closure. Given its function in late endosome to vacuole

trafficking, we hypothesize that RabG3B is involved in the turnover of activated FLS2 receptors. By Y2H screening we identified interactors of a constitutively active version of RABG3B which could be potential effectors of this Rab7 GTPase. Interestingly, one of them is a described tethering factor between the vacuole and actin cytoskeleton, suggesting a potential role of this tether in endosome-to-vacuole trafficking. We will present data on this tether and provide evidences of the role of RabG3B in FLS2 turnover required to sustain flg22-induced stomatal closure.

### PC4.19 MOLECULAR INTERACTIONS AT THE PLANT CELL SURFACE CONTINUUM

THURSDAY 6 JULY, 2017 13:50

JOHN RUNIONS (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), JOSEPH MCKENNA (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM)

JRUNIONS@BROOKES.AC.UK

Plants sense and respond to many biotic and abiotic factors in their environment using receptor-mediated signalling mechanisms at the cell surface. For many years, cytoskeleton, plasma membrane and cell wall have been studied in isolation but they act concertedly to coordinate development and stress response. We use advanced imaging experimental techniques such as photobleaching recovery and total internal reflection in an attempt to understand the role played by cytoskeleton and cell wall in governing plasma membrane protein dynamics and interaction. Formin1, an actin interacting protein spans the plasma membrane and is anchored within the cell wall. This provides stability of actin microfilament structure and is probably important in maintenance of plasma membrane microdomain structure. The central question of ongoing research is, 'Does the cell wall play a role in plasma membrane structuring?' Cell wall mutant analysis combined with pharmacological treatments are beginning to provide evidence of this interaction. Total internal reflection allows us to image single molecules such as FLAGELLIN SENSITIVE2 (FLS2). Diffusion rates and domains alter dramatically in cell wall mutant lines, or when plants are treated with pathogen effectors such as flg22.

### PC4.20 GLUTAMATE RECEPTOR-LIKE (GLR) CHANNELS IN PLANTS: EVOLUTION AND FUNCTION ON Ca<sup>2+</sup> HOMEOSTASIS IN SPERM AND MALE REPRODUCTION

THURSDAY 6 JULY, 2017 14:30

JOSÉ A FEIJÓ (UNIVERSITY OF MARYLAND, UNITED STATES)

JFEIJO@UMD.EDU

I will present data suggesting an evolutionary conservation of GLR channels related to a male reproductive function. In *Physcomitrella patens* (Pp) double GLR KO plants show no visible defects on somatic development, but are sterile. We have determined that sterility is carried by the male gamete lineage. We thus designed a sperm navigation assay and determined that chemotaxis is affected in the KO sperm. In accordance to a role in Ca<sup>2+</sup> homeostasis, KO sperm has

lower levels of cytoplasmic  $Ca^{2+}$ . Characterization by patch-clamp of GLR1 on protoplasts and heterologous mammalian expression showed that, despite chloride and strong cation non-selective conductances, PpGLRs have a  $Ca^{2+}$  conductivity. Surprisingly, on the rare events that fertilization occurs on the KO plants, the sporophyte does not develop normally, on what seems like an extra checkpoint for fertilization dependent on GLRs. We have queried our transcriptomic databases, and found that the BELL2 transcription factor, which is fundamental for the haploid to diploid transition in *Chlamydomonas*, is almost totally repressed on the double KO. We hypothesized that the sporophyte development is dependent on BELL2, and generated complementation lines of the double GLR KO with BELL2 under a the GLR2 promoter, which is specifically expressed during gamete formation. The complemented line rescued the phenotype thus confirming the dependency of BELL2 for transition to diploid status. Our work revealed an unexpected dependency of GLR for male reproduction, leading us to suggest this role as the major selective pressure to conserve this family of genes throughout terrestrial plant evolution.

#### PC4.21 ABCB TRANSPORTERS AND THEIR FUNCTION ON THE PLASMA MEMBRANE: EXCLUDERS, EFFLUXERS AND CHANNELS?

THURSDAY 6 JULY, 2017 15:00

MARK K JENNESS (UNIVERSITY OF MARYLAND, UNITED STATES), ANGUS S MURPHY (UNIVERSITY OF MARYLAND, UNITED STATES)

MJENNESS@UMD.EDU

A subset of plant ATP binding cassette subfamily B (ABCB) transporters mediate the cellular export of the phytohormone auxin. Loss of these transporters results in reduced polar auxin transport and altered plant architecture but no significant defects in embryogenesis or organogenesis. A number of lines of evidence suggest that isotropically-oriented ABCBs exhibit channel-like activity for small anions and prevent re-uptake of auxin at the plasma membrane (PM) after auxin is directionally-exported by polarized PIN transporters. On a macroscopic scale, cellular exclusion by ABCBs also defines boundaries of polar auxin transport streams. Biochemical and modeling studies predict that a majority of cellular auxin partitions into membrane-aqueous interfaces and that auxin binding sites in ABCB proteins are situated within membrane leaflets. When standard cold loading of the artificial auxin 2,4-D is replaced with room temperature loading in *S. pombe*, net uptake of 2,4-D is reduced in cells heterologously expressing Arabidopsis ABCB19, consistent with a primary function of membrane exclusion. Re-examination of auxin transport and auxin-dependent phenotypes in Arabidopsis shows that loss of ABCB function results in pooling of auxin in regions of high auxin biosynthesis and that PIN activity alone is not sufficient to maintain proper transport streams. ABCB1 and ABCB19 are required to mobilize auxin from small cells in meristematic regions and ABCB21 makes an additional contribution to restriction of auxin to root vasculature.

#### PC4.3 EFFECT OF GA, SA AND JA ON PIP AQUAPORIN EXPRESSION IN FRAGARIA X ANANASSA LEAVES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

BRITT M E MERLAEN (UNIVERSITEIT GENT, BELGIUM), ELLEN DE KEYSER (ILVO - PLANT, BELGIUM), MARIE-CHRISTINE VAN LABEKE (UNIVERSITEIT GENT, BELGIUM)

BRITT.MERLAEN@UGENT.BE

Aquaporins are water channels that facilitate passive water transport across inter- and intracellular membranes. They regulate water transport at cellular level. There are several types of aquaporins, but because of their abundance and subcellular location, the plasma membrane intrinsic proteins (PIPs) are thought to contribute substantially to regulating plant water relations and to participate in abiotic stress responses. The question rises which hormones are involved in signal transduction.

*Fragaria x ananassa* cv. Figaro plants were transferred to a watery solution containing either no hormones (control), 0.5 mM salicylic acid (SA), 0.05 mM gibberellic acid (GA) or 0.1 mM jasmonic acid (JA). During these treatments, stomata opened transiently under influence of SA and JA (less than 6 and 11 hours respectively). GA caused opening of the stomata during the first 12 hours. After 24 hours, stomata were closed in all treatments, possibly due to a lack of aeration.

Genes similar to FaPIP1;1 and FaPIP1;2 are downregulated by GA after 6h. Genes similar to FaPIP1;3 are upregulated by JA after 12h. Genes similar to FaPIP2;1 are upregulated by JA and downregulated by SA after 6h. Expression of genes similar to FaPIP2;2 does not seem to be influenced by any of the tested hormones. The responses of the different PIP aquaporins are diverse, but seem to be limited in time to 6 to 12 hours after the start of the treatment. In other species, the effect on the expression seems to vary with aquaporin, hormone concentration, tissue and duration of the treatment.

#### PC4.4 DISCOVERING DE-S-ACYLATING ENZYMES IN ARABIDOPSIS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MAIJU A LAURILA (UNIVERSITY OF DUNDEE, UNITED KINGDOM), PIERS A HEMSLEY (UNIVERSITY OF DUNDEE, UNITED KINGDOM)

M.A.LAURILA@DUNDEE.AC.UK

S-acylation is the only reversible lipid-based post-translational modification of proteins. Even though 24 protein acyl transferases -enzymes that add S-acyl groups onto proteins- have been identified and verified in Arabidopsis, the identities and numbers of any plant de-S-acylating enzymes remain unknown. More than 500 proteins have been suggested to be S-acylated in plants and a still unexplored number of these are likely to be de-S-acylated as means of regulating localisation and function. It is therefore very probable that one or more families of plant APTs await discovery. APT enzymes from mammals and Toxoplasma have previously been identified by inhibitor-based approaches using competitive activity-based protein profiling (cABPP). Known inhibitors of APTs from mammals prevent de-S-acylation of the plant small GTPase ROP6 in vivo

indicating that these inhibitors are effective in plants. We have also shown that these inhibitors can directly inhibit plant serine hydrolase activities *in vivo*. By combining cABPP with quantitative SILAC proteomics we are identifying serine hydrolases in plants that are sensitive to known inhibitors of de-S-acylation and may therefore constitute novel plant de-S-acylating activities.

## PC4.5 THE CHARACTERIZATION OF THE PLASTIDIC HOMOLOG OF THE VACUOLAR GLUCOSE TRANSPORTER1 (VGT1)

📅 WEDNESDAY 5 JULY, 2017    POSTER SESSION

👤 PRATIWI PRANANINGRUM (TU KAISERSLAUTERN, GERMANY), KATHRIN PATZKE (TU KAISERSLAUTERN, GERMANY), BETTINA BÖLTER (LUDWIG-MAXIMILIANS UNIVERSITY, GERMANY), PATRICK KLEMENS (TU KAISERSLAUTERN, GERMANY), ILKA HAFERKAMP (TU KAISERSLAUTERN, GERMANY), H E NEUHAUS (TU KAISERSLAUTERN, GERMANY)

@ PRATIWI@RHRK.UNI-KL.DE

The transport of sugars across membrane barriers is essential for higher plants, since sugar represent transport and storage units of cellular energy generation and thus play a fundamental role during developmental processes and stress responses. In addition to transport across the plasma membrane, carrier-mediated sugar transport has also been demonstrated across organellar membranes, such as the inner plastid envelope or the vacuolar membrane, named tonoplast. The monosaccharide transporter family is diverse and contains seven distinct clades. In this study, we will focus on VGT-like protein family of monosaccharide transporter in which comprised of three genes, At3g03090, At5g17010 and At5g59250. Recently, two genes from this family, AtVGT1 (At3g03090) and AtVGT2 (At5g17010), have been shown to localize to the vacuolar membrane of *Arabidopsis thaliana* (*A. thaliana*) and it has been shown that VGT1 transports glucose and a proton-coupled antiport. In contrast, here we show that the protein encoded by At5g59250, the third member of this sub-group, locates to the chloroplast membrane. In the present study, we want to further characterize the function of this protein in the sugar transport mechanism in plants. We will introduce the current status of the investigation progress.

# PC6 MOLECULAR CONTROL OF PLANT GROWTH DURING ABIOTIC STRESS

**ORGANISED BY:** ULRIKE BECHTOLD (UNIVERSITY OF ESSEX, UK) AND BEN FIELD (CNRS MARSEILLE, FRANCE)

**SESSION SPONSORED BY:** CLF PLANTCLIMATICS GmbH AND JOURNAL OF EXPERIMENTAL BOTANY

## PC6.1 PROTEIN IMPORT INTO CHLOROPLASTS AND ITS REGULATION BY THE UBIQUITIN-PROTEASOME SYSTEM

📅 MONDAY 3 JULY, 2017 ⌚ 09:00

👤 PAUL JARVIS (UNIVERSITY OF OXFORD, UNITED KINGDOM)

@ PAUL.JARVIS@PLANTS.OX.AC.UK

Most proteins in chloroplasts and other plastids are encoded in the nucleus and synthesized on cytosolic ribosomes. They are made in precursor form, each one with an amino-terminal targeting signal, or transit peptide. The transit peptide directs the protein through a post-translational targeting pathway, and is cleaved upon arrival inside the plastid. This targeting or import process is mediated by the coordinate action of two proteinaceous import machines, one in each of the envelope membranes. The import machinery of the outer envelope membrane is called the TOC complex, and that in the inner membrane is called the TIC complex. Components of the TOC and TIC complexes have been identified through biochemical analyses. Interestingly, genome sequence information has revealed that many of these components (particularly receptor components of the TOC complex) exist in multiple homologous forms. We and others have used genetic approaches to dissect the functional significance of these different TOC protein isoforms in *Arabidopsis*. Results indicate that the different isoforms operate in different import pathways with distinct precursor recognition specificities; i.e., different import pathways exist for different precursor protein classes. Operation of these client-specific import pathways controls the organellar proteome, plays a role in the differentiation of different plastid types, and is regulated by direct action of the ubiquitin-proteasome system (UPS). Regulation of the TOC apparatus by the UPS is mediated by the SP1E3 ligase of the plastid outer envelope membrane. In addition to its well-established role in plastid developmental processes, SP1 also acts in abiotic stress tolerance.

## PC6.2 INVESTIGATING THE ROLE OF THE BACTERIAL ALARMONE (p)ppGpp IN THE CHLOROPLAST OF PLANTS

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 SEDDIK HARCHOUNI (AIX-MARSEILLE UNIVERSITY, FRANCE), SAMANTHA ENGLAND (CEA, FRANCE), JULIEN VIEU (AIX-MARSEILLE UNIVERSITY, FRANCE), BENOIT MENAND (CNRS, FRANCE), BEN FIELD (CNRS, FRANCE)

@ SEDDIK.HARCHOUNI@ETU.UNIV-AMU.FR

One of the main stress responses in bacteria is the stringent response which is mediated by the alarmone guanosine 5'-(tri)diphosphate 3'-diphosphate ((p)ppGpp). (p)ppGpp is a key regulatory nucleotide that controls gene expression to promote survival. In plants, elements of the bacterial stringent response were inherited from the cyanobacterial ancestor of the chloroplast 1.5 billion years ago. In plants (p)ppGpp is found in the chloroplast and, like in bacteria, levels of ppGpp increase markedly when plants are subjected to abiotic stresses such as wounding and heat shock. Recently, we showed that (p)ppGpp is a potent regulator of chloroplast gene expression *in vivo*, and regulates the growth and development of the model plant *Arabidopsis thaliana*. I am investigating the role of (p)ppGpp in the moss *Physcomitrella patens*, which is a model organism that occupies an interesting evolutionary position between algae and vascular plants. To understand the role of (p)ppGpp in moss we have created transgenic lines that inducibly over produce (p)ppGpp by expression of a (p)ppGpp synthase (SYN). We will discuss how (p)ppGpp affects moss growth and stress responses, and how (p)ppGpp has both conserved and specific functions with respect to land plants. Indeed, our findings suggest that (p)ppGpp signalling is a flexible process that may fulfill diverse roles in the photosynthetic eukaryotes.

### PC6.3 THE ROLE OF RETROGRADE SIGNALS DURING PLANT STRESS RESPONSE

📅 MONDAY 3 JULY, 2017 ⌚ 09:40

👤 ÅSA STRAND (UMEÅ UNIVERSITY, SWEDEN)

@ ASA.STRAND@UMU.SE

Cells can sense changes in the environment by external cues that affect different receptors on the cell surface. Exposure to stress also inhibits metabolic activities and causes severe constraints on cellular energy homeostasis. Recovery of energy homeostasis by restoring respiration and photosynthesis is therefore essential for stress acclimation and plant productivity during stress. Organelles play crucial roles as stress sensors in the cell and communicate their status through so-called retrograde signals to regulate nuclear gene expression. Thus, the stress signalling response is not linear but rather a complex integration of signalling networks originating in different cellular compartments. The Mediator kinase module subunit CDKE1/CDK8 was identified through a screen for mutants that did not respond correctly to retrograde signals triggered by oxidative stress. The *cdke1/cdk8* mutant demonstrated a genome-uncoupled phenotype in response to retrograde signals originating in both mitochondria and plastids. As a consequence the mutant showed severely impaired ability to recover energy metabolism following exposure to stress. CDKE1 is potentially a central nuclear component integrating mitochondrial and plastid retrograde signals playing a role in regulating energy metabolism during the response to stress.

### PC6.4 ADAPTATION OF PLANTS TO COLD TEMPERATURES BY A CHLOROPLAST-BASED SIGNALLING CIRCUIT

📅 MONDAY 3 JULY, 2017 ⌚ 10:25

👤 DORA L CANO RAMIREZ (UNIVERSITY OF BRISTOL, UNITED KINGDOM), BETHAN F MANLEY (UNIVERSITY OF BRISTOL, UNITED KINGDOM), KEARA A FRANKLIN (UNIVERSITY OF BRISTOL, UNITED KINGDOM), ANTONY N DODD (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

@ DORA.CANORAMIREZ@BRISTOL.AC.UK

Survival of photosynthetic organisms requires the co-ordination of biological processes with daily and seasonal changes in the environment. To achieve this, environmental signals must be perceived and integrated through signalling pathways to achieve correct co-ordination of gene expression. We are investigating mechanisms that communicate this environmental information to chloroplasts (e.g. Noordally et al. Science 2013). Chloroplasts contain a small circular genome that encodes essential components of the photosynthetic apparatus. Nuclear-encoded sigma factors are evolutionarily-conserved RNA polymerase subunits that communicate circadian and environmental information from the nucleus to chloroplast-encoded genes. We have identified a novel low-temperature signalling pathway that involves sigma factor-mediated signalling to chloroplasts and underpins optimum plant performance under both low and freezing temperatures. We have demonstrated that this pathway increases freezing tolerance and

photosynthetic efficiency at low temperatures, identified upstream and downstream regulators of the pathway, and demonstrated close integration with the circadian oscillator. Overall, we have identified a novel low-temperature signalling network that involves both anterograde signalling to chloroplasts, and retrograde signalling from chloroplasts to the nuclear genome.

### PC6.5 MITOCHONDRIAL AND CHLOROPLAST STRESS RESPONSES ARE REGULATED BY DISTINCT TOUCH- AND ORGANELLE DYSFUNCTION-DEPENDENT PATHWAYS

📅 MONDAY 3 JULY, 2017 ⌚ 10:40

👤 OLIVIER VAN AKEN (MOLECULAR CELL BIOLOGY UNIT DEPARTMENT OF BIOLOGY LUND UNIVERSITY, SWEDEN), INGE DE CLERCQ (VIB - DEPARTMENT OF PLANT SYSTEMS GHENT UNIVERSITY, BELGIUM), SHAOBAI HUANG (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), ANETA IVANOVA (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), ETHAN FORD (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), RYAN LISTER (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), SIMON R LAW (DEPARTMENT OF PLANT PHYSIOLOGY UMEÅ PLANT SCIENCE CENTRE UMEÅ UNIVERSITY, SWEDEN), MARTYNA BRODA (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), BARRY J POGSON (ARC COE PLANT ENERGY BIOLOGY AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA), FRANK VAN BREUSEGEM (VIB - DEPARTMENT OF PLANT SYSTEMS BIOLOGY GHENT UNIVERSITY, BELGIUM), JAMES WHELAN (ARC COE PLANT ENERGY BIOLOGY LATROBE UNIVERSITY, AUSTRALIA), A HARVEY MILLAR (ARC COE PLANT ENERGY BIOLOGY UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA)

@ OLIVIER.VAN\_AKEN@BIOL.LU.SE

Previous studies have identified various transcription factors that modulate retrograde regulation of mitochondrial and chloroplast functions in *Arabidopsis thaliana*. However, the relative contribution of these regulators and whether they act downstream of separate or overlapping stress signalling cascades is still unknown. We demonstrate that multiple stress-related signalling pathways, with distinct kinetic signatures, converge on overlapping gene sets involved in energy organelle function. The transcription factor ANAC017 is almost solely responsible for transcript induction of retrograde marker genes due to chemical or genetic inhibition of organelle function. ANAC017 is a key regulator of mitochondrial and specific types of chloroplast retrograde signalling, with repercussions for protein activity and metabolism. However, an independent and highly transient gene expression pathway, which is induced within 10-30 minutes after spray treatments, also targets energy organelle functions, and is related to touch and wounding responses. Metabolite analysis demonstrated that the early response is concurrent with rapid changes in tricarboxylic acid cycle intermediates and large changes in transcript abundance of genes encoding mitochondrial dicarboxylate carrier proteins. Furthermore, we showed that the transcription factors AtWRKY15 and AtWRKY40 have repressive regulatory roles in this touch-responsive gene expression. In conclusion, this study shows that

several regulatory systems can independently affect energy organelle function in response to stress, providing alternative ways to exert operational control.

### PC6.6 HIGH-THROUGHPUT PHENOTYPING TO DECODE THE COMPLEXITY OF NATURAL VARIATION FOR RESPONSE TO THE ENVIRONMENT IN ARABIDOPSIS

📅 MONDAY 3 JULY, 2017 ⌚ 14:00

👤 OLIVIER LOUDET (INRA VERSAILLES, FRANCE)

@ OLIVIER.LOUDET@VERSAILLES.INRA.FR

Following a long history of quantitative genetics in crop plants, it is now quite popular as well to use naturally-occurring variation contained in *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map QTLs and try and resolve them at the gene level. Apart from being able to exploit - in multiple genetic backgrounds - allelic variation that cannot be easily retrieved from classical mutagenesis, the success of the QTL studies has often been because of the use of quantitative phenotyping. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the abiotic environment (especially drought stress, nitrogen limitation and their combinations). We are using a combination of our unique high-throughput phenotyping robot (the Phenoscope), RNA-seq, fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale thanks to the Phenoscope should allow to resolve enough quantitative loci to start drawing a more general picture as to how and where in the pathways adaptation is shaping natural variation. I will present recent results obtained when trying to decipher the genetic architecture of dynamic growth response to the environment, to illustrate our strategies and research.

### PC6.7 GENETIC ADAPTATION OF PREHISTORIC BARLEY TO OSMOTIC STRESS

📅 MONDAY 3 JULY, 2017 ⌚ 14:40

👤 CHIOMA U OKPARA (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), PETER CIVAN (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), TERRY BROWN (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ CHIOMA.OKPARA@MANCHESTER.AC.UK

Barley (*Hordeum vulgare L.*) is one of the early crops that was domesticated in the Near East, and accompanied farmers during migration. Barley, which grows naturally in southwest Asia, was carried to southeast Europe and eventually spread to other parts of the continent. Considering that the northern part of Europe is colder and

wetter than their Asian origin, barley must have faced unfavourable climatic/ecological conditions thereby influencing their growth and development in this new environment. Some transcription regulatory genes (TRG) found in plants, including barley, have been linked to adaptation to different environmental conditions, where they either activate transcription of certain genes or inactivate them. DREB1 is an example of a stress related TRG involved in cold/salt tolerance. We tested the hypothesis that domesticated barley evolved adaptive changes in the DREB1 gene and its promoter, which were presumably beneficial in cold and wet environments. Hence, wild and landraces of georeferenced barley seeds were sampled. Genomic DNA was extracted followed by long-range PCR amplification (using information from the Morex genome assembly as a reference to design primers), library preparation and subsequently next generation sequencing (NGS) using the MiSeq platform. NGS results show synonymous substitutions in the DREB1 coding region for all the landraces while a few wild accessions carry nonsynonymous substitutions. Interestingly, multiple SNPs found in the promoter region grouped the samples into two distinct haplogroups each containing wild and cultivated barley. These genetic data are being analysed using genealogy, network, and correlation with bioclimatic variables.

### PC6.8 COMPARATIVE INTERACTION ANALYSIS BETWEEN ARBUSCULAR MYCORRHIZA FUNGI (AMF) (RHIZOPHAGUS IRREGULARIS), NPK AND DROUGHT TOLERANCE TO GROWTH AND YIELD OF NERICA

📅 MONDAY 3 JULY, 2017 ⌚ 14:55

👤 ZAINAB A ABUBAKAR (GOMBE STATE UNIVERSITY, NIGERIA), AHMAD ABDULHAMEED (ABUBAKAR TAFAWA BALEWA UNIVERSITY, NIGERIA), HUSSAINI ABDU (ABUBAKAR TAFAWA BALEWA UNIVERSITY, NIGERIA), ADANZE C NZEAKO (ABUBAKAR TAFAWA BALEWA UNIVERSITY, NIGERIA), MUHAMMED N YAKUBU (ABUBAKAR TAFAWA BALEWA UNIVERSITY, NIGERIA), MUHAMMED MUHAMMED (ABUBAKAR TAFAWA BALEWA UNIVERSITY, NIGERIA)

@ ZEEPHA22@YAHOO.COM

Abiotic stresses such as drought, salinity, submergence, and nutrient deficiencies limit rice production. These stresses have been identified as among the major factors negatively affecting crop yield and productivity worldwide. The present study was conducted to evaluate the response of New Rice For Africa (NERICA) cultivars exposed to drought at 0%, 5% and 10% doses of *Rhizophagus irregularis* (Arbuscular Mycorrhiza Fungi (AMF)) and at 0 kg/h, 90 kg/h and 180 kg/h NPK doses respectively. Root and shoot mass and yield (100 grain) were measured. The findings of this research showed that NERICA has high NPK use efficiency with 90 kg/h (half recommended dose), and 5% AMF (*Rhizophagus irregularis*) under droughted condition with relatively higher yield than 180 kg/h (recommended dose) and 10% AMF under both droughted and the irrigation treatments. A relatively half recommended dose of the NPK significantly increased yield in the irrigated treatment but not root and shoot mass. NERICA, thus has tolerance to low NPK and water deficit. The results of this study calls for further studies to determine the appropriate levels of NPK that requires 5% *R. irregularis* to enhance yield in NERICA. It further recommends that this methodology be applied in other rice cultivars. Key: New rice for Africa (Nerica), Arbuscular Mycorrhiza Fungi (AMF), NPK, drought.



## PC6.9 PHOSPHATE STATUS-DEPENDENT CONTROL OF INTERACTIONS WITH ROOT-INFECTING FUNGI IN PLANTS

📅 MONDAY 3 JULY, 2017 ⌚ 15:10

👤 YUSUKE SAIJO (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), TAE-HONG LEE (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), KENTARO OKADA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), MIDORI TANAKA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), KEI HIRUMA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN)

@ SAIJO@BS.NAIST.JP

When key nutrients are limited, plants exploit root-associated beneficial microbes for nutrient acquisition, while retaining effective resistance against pathogens. It remains poorly understood how plants modulate defense responses to microbial challenge, according to the nature of their encountered microbes and the availability of key nutrients, such as inorganic phosphate (Pi). Here, we report that transcriptional responses to damage-associated molecular pattern (DAMP) peptides via their receptors PEPRs, but not to fungal chitin, are enhanced in *Arabidopsis thaliana*. Sensitized PEPR signaling leads to a substantial increase in transcriptional activation of genes engaged in Trp-derived metabolic pathways, involving production of the antimicrobials camalexin and indole glucosinolates. We pursue the biological significance and molecular mechanisms for PEPR signaling sensitization under low Pi conditions, by employing a beneficial endophyte, *Colletotrichum tofieldiae* (Ct) (Hiruma et al, Cell 2016), and its pathogenic relative *C. incanum* (Ci). As previously described for a leaf-infecting pathogenic relative *C. higginsianum* (Yamada et al, EMBOJ 2016), Ci and Ct infection both leads to depletion of the co-receptor kinase BAK1, which seems to suppress BAK1-dependent fungal resistance. However, our genetic studies point to the existence of separate requirements for the PEPR pathway and Pi starvation response (PSR) pathways in plant interactions between the two closely related pathogenic and endophytic fungi.

## PC6.16 SUMO MEDIATED CELL SIGNALLING PATHWAYS REVEAL HORMONE BYPASS MECHANISMS IN PLANTS THAT AFFECT GROWTH AND DEFENCE

📅 MONDAY 3 JULY, 2017 ⌚ 16:10

👤 ARI SADANANDOM (UNIVERSITY OF DURHAM, UNITED KINGDOM)

@ ARI.SADANANDOM@DURHAM.AC.UK

Plants survive adverse conditions by modulating their growth in response to changing environmental signals. Gibberellins (GA) play a key role in these adaptive responses by stimulating the degradation of growth repressing DELLA proteins. GA binding to its receptor GID1 enables association of GID1 with DELLAs. This leads to the ubiquitin-mediated proteasomal degradation of DELLAs and consequently growth promotion. We report that DELLA-dependent growth control can also be regulated independently of GA. We demonstrate that a proportion of DELLAs are conjugated to the Small Ubiquitin-like

Modifier (SUMO) protein which increases during stress. We identify a SUMO interacting motif (SIM) in GID1 and demonstrate that SUMO-conjugated DELLA binds to this motif in a GA-independent manner. The consequent sequestration of GID1 by SUMO-conjugated DELLAs leads to an accumulation of non-SUMOylated DELLAs and subsequent beneficial growth restraint during stress. We conclude that plants have developed a non-GA mechanism to control growth during stress.

## PC6.17 GLUTATHIONE HOMEOSTASIS AND CONTROL OF ROOT GROWTH IN ARABIDOPSIS

📅 MONDAY 3 JULY, 2017 ⌚ 16:40

👤 ANDREAS J MEYER (UNIVERSITY OF BONN, GERMANY), SAJID A K BANGASH (UNIVERSITY OF BONN, GERMANY)

@ ANDREAS.MEYER@UNI-BONN.DE

The tripeptide glutathione (reduced form: GSH; oxidized form: GSSG) is a key player in maintaining cellular thiol-redox homeostasis and as such also considered to be involved in a multitude of abiotic stress reactions. Beyond this, GSH is required as a co-factor of several biochemical reactions including coordination of Fe-S-cluster during their transfer from the assembly machinery to the respective apoproteins as well as detoxification of toxic metabolites and xenobiotics. Based on the growth phenotypes of partially GSH-deficient mutants, GSH and the thiol redox status have been linked to developmental processes but direct evidence for a functional link is still lacking. To investigate the role of GSH in root growth, we took advantage of an allelic series of GSH-deficient mutants that display a severe root phenotype correlated with GSH content. When exposed to severe water stress in a fully automated phenotyping experiment we unexpectedly found no significant differences between mutants and wild-type plants. This observation is further corroborated by the lack of any pronounced changes in the glutathione redox potential ( $E^{GSH}$ ) monitored with redox-sensitive GFP. To further test for the role of GSH in control of root growth we generated several double mutants affected in glutathione redox homeostasis in different forms. Characterization of these mutants at phenotypic and physiological level reveals a distinct role of glutathione in growth control.

## PC6.18 LOW PHOSPHATE ACTIVATES STOP1-ALMT1 TO RAPIDLY INHIBIT ROOT CELL ELONGATION

📅 MONDAY 3 JULY, 2017 ⌚ 16:55

👤 THIERRY DESNOS (CEA, FRANCE), COLINE BALZERGUE (CEA, FRANCE), THIBAUT DARTEVELLE (CEA, FRANCE), CHRISTIAN GODON (CEA, FRANCE), EDITH LAUGIER (CEA, FRANCE), CLAUDIA MEISRIMLER (CEA, FRANCE), JEAN-MARIE TEULON (CEA, FRANCE), AUDREY CREFF (CEA, FRANCE), MARIE BISSLER (CEA, FRANCE), CORINNE BROUCHOUD (AIX-MARSEILLE UNIVERSITÉ, FRANCE), AGNÈS HAGÈGE (CEA, FRANCE), JENS MÜLLER (LIPB, GERMANY), SERGE CHIARENZA (CEA, FRANCE), HÉLÈNE JAVOT (CEA, FRANCE), NOËLLE BECUWE-LINKA (CEA, FRANCE), PASCALE DAVID (CEA, FRANCE), BENJAMIN PÉRET (CNRS, FRANCE), ETIENNE DELANNOY (CEA, FRANCE), MARIE-CHRISTINE THIBAUD (CEA, FRANCE), JEAN ARMANGAUD (CEA, FRANCE), STEFFEN ABEL (LIPB, GERMANY), JEAN-LUC PELLEQUER (CEA, FRANCE), LAURENT NUSSAUME (CEA, FRANCE)

@ THIERRY.DESNOS@CEA.FR

Environmental cues profoundly modulate cell proliferation and cell elongation to inform and direct plant growth and development. External phosphate limitation (-Pi) inhibits primary root growth in many plant species. However, the underlying Pi sensory mechanisms are unknown.

In a large scale EMS mutagenesis, we identified numerous mutant alleles of two new Arabidopsis genes whose WT activity inhibits the primary root growth in -Pi: the transcription factor STOP1 and its direct target *ALMT1*, encoding a malate exudation channel. We observed that the -Pi stress stimulates transcription of *ALMT1* and that the long primary root of *stop1* mutants is due to the lack of *ALMT1* expression.

We showed that STOP1, ALMT1 and the cell wall ferroxidase LPR1 rapidly inhibit cell elongation. Using a combination of atomic force microscopy (AFM) micro-indentation, pharmacology and mutants, we demonstrated that STOP1, ALMT1 and LPR1 mediate Fe and peroxidase-dependent cell wall stiffening of root epidermal cells in the transition zone.

During the subsequent slow inhibition of cell proliferation in the primary root meristem, which is mediated by LPR1, but largely STOP1-ALMT1-independent, we showed that Fe and callose accumulate in the stem cell niche, leading to meristem reduction.

Our work uncovers STOP1 and ALMT1 as a new signalling pathway of low Pi availability and exuded malate as an unexpected apoplastic inhibitor of root cell wall expansion (*Nature Communications*, in press).

## PC6.19 REDUCTION OF IAA METHYLTRANSFERASE ACTIVITY COMPENSATES FOR HIGH-TEMPERATURE MALE STERILITY IN ARABIDOPSIS

📅 MONDAY 3 JULY, 2017 ⌚ 17:10

👤 MOHAMAD ABBAS (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JORGE HERNÁNDEZ-GARCÍA (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), NOEL BLANCO-TOURINÁN (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), NORMA ALIAGA (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), EUGENIO G. MINGUET (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), DAVID ALABADI (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN), MIGUEL A. BLAZQUEZ (INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE PLANTAS, SPAIN)

@ MOHAMAD.ABBAS@NOTTINGHAM.AC.UK

High temperature is a general stress factor that causes a decrease in crop yield. It has been shown that auxin application reduces the male sterility caused by exposure to higher temperatures. However, the fact that auxin is a key regulator of plant development poses the risk of undesired side effects caused by widespread applications, and the generation of new plant varieties with local accumulation of auxin in reproductive organs may represent an alternative. We have explored the possibility of increasing indoleacetic acid (IAA) in ovaries by knocking out the IAA METHYLTRANSFERASE1 (*IAMT1*) gene in Arabidopsis thaliana. The *iamt1* mutant showed increased auxin signaling in funiculi, which correlated with a higher growth rate of wild-type pollen in contact with mutant ovaries and premature ovule fertilization. While the production of seeds per fruit was similar in the wild type and the mutant at 20°C, exposure to 29°C caused a more severe decrease in fertility in the wild type than in the mutant. Loss of *IAMT1* activity was also associated to the production of more nodes after flowering and higher tolerance of the shoot apical meristem to higher temperatures. As a consequence, the productivity of the *iamt1* mutant under higher temperatures was more than double of that of the wild type, with almost no apparent trade-off.

## PC6.20 HOW DO PLANTS MANAGE THEIR ENERGY?

📅 TUESDAY 4 JULY, 2017 ⌚ 10:30

👤 ELENA BAENA-GONZÁLEZ (INSTITUTO GULBENKIAN DE CIÊNCIA, PORTUGAL)

@ EBAENA@IGC.GULBENKIAN.PT

Management of energy resources is important for the survival of all living organisms. Energy management occurs at the cellular level to support processes that are most relevant for a particular environment or developmental stage but also at the whole organism level where an efficient coordination of activities in different tissues and organs is required for optimal use of available resources and proper growth and development. In plants energy management is a major determinant of crop productivity, as it affects processes such as stress tolerance, flowering, branching, senescence, or seed

filling. However, how the energy management network operates is poorly understood. One major component of this network is the SnRK1 protein kinase complex. SnRK1 undergoes activation under conditions of energy deficiency, shifting the cell's metabolism from anabolic towards a catabolic mode and consequently playing pivotal roles in carbon allocation and remobilization during stress but also in normal conditions. In this talk I will present our strategies and progress in the dissection of the poorly understood SnRK1 signaling pathway, as a first step towards understanding how plants manage their resources and how plant growth is modified in response to the environment.

### PC6.21 INCREASING WHEAT YIELD AND RESILIENCE USING A NOVEL TREHALOSE-6-PHOSPHATE (T6P) PRECURSOR

📅 TUESDAY 4 JULY, 2017 ⌚ 11:10

👤 CARA A GRIFFITHS (ROTHAMSTED RESEARCH, UNITED KINGDOM), RAM SAGAR (OXFORD UNIVERSITY, UNITED KINGDOM), YIQUN GENG (OXFORD UNIVERSITY, UNITED KINGDOM), LUCIA F PRIMAVESI (ROTHAMSTED RESEARCH, UNITED KINGDOM), MITUL K PATEL (OXFORD UNIVERSITY, UNITED KINGDOM), MATTHEW J PAUL (ROTHAMSTED RESEARCH, UNITED KINGDOM), BENJAMIN G DAVIS (OXFORD UNIVERSITY, UNITED KINGDOM)

@ CARA.GRIFFITHS@ROTHAMSTED.AC.UK

Diminishing arable land, population growth and variable global climates are increasing the pressure on agricultural plant species to become higher-yielding and have greater resilience. Crop improvement can be facilitated by traditional selective breeding and genetic modification approaches, however may not induce the changes needed in the time the world requires it. Trehalose-6-phosphate (T6P) is a central sugar signal in plants that regulates sucrose allocation and use through regulating the activity of Sucrose non-fermenting kinase 1 (SnRK1). In this research, a chemical approach was utilised to increase crop yield and resilience that relies on increasing the concentration of T6P within the plant using a caged-T6P precursor. Excitedly, when a solution of caged-T6P is applied to drought-stressed wheat at jointing stage, recovery of the plants and the capacity to grow new tissue is increased after rewatering. Furthermore, and perhaps the most striking result, is that seed yield of non-stressed plants sprayed with a caged-T6P solution is increased by up to 20%. This research demonstrates for the first time that a chemical approach can be used to alter carbohydrate biology in plants to increase drought resilience, in addition to increasing yields by up to 20%, exceeding that shown in recent work in GM and breeding technologies.

### PC6.22 TEMPERATURE COMPENSATION OF STARCH DEGRADATION IN *ARABIDOPSIS THALIANA*: ARE TETRATRICOPEPTIDE REPEAT (TPR)-LIKE SUPERFAMILY PROTEINS INVOLVED?

📅 TUESDAY 4 JULY, 2017 ⌚ 11:25

👤 EVA-THERESA PYL (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), TOMASZ CZERNIAK (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), CHRISTIN ABEL (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), SARAH M PILKINGTON (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), DOREEN FEIKE (JOHN INNES CENTRE, UNITED KINGDOM), ALISON M SMITH (JOHN INNES CENTRE, UNITED KINGDOM), RONAN SULPICE (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MARK STITT (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

@ PYL@MPIMP-GOLM.MPG.DE

To cope with the daily cycle of light and dark, plants accumulate carbon (C) reserves in the light period, often as starch, and then remobilize them at night to support metabolism, maintenance and growth. The rate of starch degradation is affected by the night temperature and depends on the growth conditions. When C supply is limited, the rate of starch degradation is completely temperature-compensated, responding quickly even to sudden changes in night temperature, thereby preventing exhaustion of C reserves before dawn. However, when the plant has more than enough starch to survive through the night, the rate of starch degradation is temperature sensitive and depends on the night temperature. An *Arabidopsis thaliana* C-starvation reporter line was mutagenized with EMS and the M<sub>1</sub> plants were selfed. The M<sub>2</sub> population was grown with a night temperature of 10°C, and screened for mutants that experience C-starvation following an unexpected warm night temperature of 19°C. One of the identified mutants was confirmed to be unable to properly adjust its rate of starch degradation, so its starch reserves were already depleted 2h before the end of the warm night. Candidate genes were identified by SHORE mapping and tested by phenotyping T-DNA insertion lines. This confirmed that the causal mutation resides in a gene encoding a tetratricopeptide repeat-like superfamily protein (*TPR1*). This has a close homologue, *TPR2*, but *tpr2* null mutants have no obvious starch phenotype. A double *tpr1 tpr2* mutant has been made to test its response to unexpected early warm nights.

## PC6.23 THE ROLE OF THE TOR KINASE IN THE REGULATION OF PLANT NUTRIENT AND STRESS SIGNALLING

TUESDAY 4 JULY, 2017 11:40

CHRISTIAN MEYER (IJPB INRA VERSAILLES, FRANCE)

@ CHRISTIAN.MEYER@INRA.FR

Plants cannot escape from environmental stresses and thus have to adapt their growth to external conditions by regulating cellular processes such as metabolism and hormonal balance to preserve energy resources. The Target of Rapamycin (TOR) kinase is an evolutionarily conserved and central regulatory element that integrates nutrient, energy and hormone signals to regulate growth in yeast, animals and plants. Indeed, the TOR pathway controls essential biological outputs like mRNA translation, autophagy, metabolic adaptations and hormone responses, collectively contributing to growth and stress survival.

We will show that a decrease in TOR activity reduces the accumulation of stress-related hormone (abscisic acid) and metabolites (raffinose and proline). Furthermore, reducing TOR activity by genetic or pharmaceutical means has a strong effect on the global transcriptome and proteome as well as on primary metabolism. For example, the expression of a vast majority of nuclear genes coding for plastidic ribosomal proteins, which are regulated by many stresses in a coordinated way, is globally controlled by the TOR activity. We will also present the identification of novel components of the plant TOR signalling pathway.

In conclusion it seems that the TOR kinase is an important sensor and regulatory component in plants that is essential for growth and metabolic adaptations to stresses.

## PC6.24 LIGHT-INDEPENDENT SUGAR SIGNALLING IN ARABIDOPSIS

TUESDAY 4 JULY, 2017 12:10

ANGELA ROMAN-FERNANDEZ (DEPARTMENT OF BIOLOGY UNIVERSITY OF YORK, UNITED KINGDOM), HEATHER EASTMOND (DEPARTMENT OF BIOLOGY UNIVERSITY OF YORK, UNITED KINGDOM), WAHEED ARSHAD (DEPARTMENT OF BIOLOGY UNIVERSITY OF YORK, UNITED KINGDOM), IAN A GRAHAM (DEPARTMENT OF BIOLOGY UNIVERSITY OF YORK, UNITED KINGDOM), MICHAEL J HAYDON (THE UNIVERSITY OF MELBOURNE, AUSTRALIA)

@ ANGELA.ROMAN-FERNANDEZ@YORK.AC.UK

Sugars are the prime carbon and energy sources in most cells but also play pivotal roles as signalling molecules. In plants, the model of sugar sensing and signalling pathways has been inferred by homology to animal or yeast systems and by the study of loss-of-function mutants in Arabidopsis. The major components identified include HEXOKINASE 1 (HXK1), Snf1-RELATED KINASE 1 (SnRK1), trehalose-6-phosphate (T6P), G-protein signalling and TARGET OF RAPAMYCIN (TOR) kinase, but a great deal remains to be elucidated about the precise molecular mechanism of sugar sensing and signalling in plant cells. Previous investigation of plant sugar signalling has mostly been performed in the light when signalling pathways are already activated by photosynthetically-derived

endogenous sugars. Experimental approaches to distinguish sugar and light have recently uncovered novel roles for sugars in circadian entrainment. Using similar approaches, we have performed a forward genetic screen in Arabidopsis using a transcriptional luciferase reporter for *COLD*, *CIRCADIAN RHYTHM AND RNA BINDING 2* (*CCR2*) in a light-independent assay to identify *sor* mutants of Arabidopsis, which are hypo-responsive to sucrose. The transcriptional response of *sor* mutants to various metabolic sugars and sugar uptake assays suggest these mutants are not impaired in transport or catabolism. Furthermore, we have detected altered expression of transcriptional markers for SnRK1 and TOR kinase pathways in *sor* mutants, which is consistent with altered sugar signalling. The full extent of sugar-responsive transcriptional phenotypes in *sor* mutants are being defined by RNA-Seq analyses to reveal the affected transcriptional networks in these mutants.

## PC6.25 INFLUENCE OF LIGHT ON SHOOT STEM CELL REGULATION AND DEVELOPMENT

TUESDAY 4 JULY, 2017 12:25

ANNE PFEIFFER (COS UNIVERSITY HEIDELBERG, GERMANY), DENIS JANOCHA (COS UNIVERSITY HEIDELBERG, GERMANY), YIHAN DONG (COS UNIVERSITY HEIDELBERG, GERMANY), JAN U LOHMANN (COS HEIDELBERG, GERMANY)

@ ANNE.PFEIFFER@COS.UNI-HEIDELBERG.DE

The development of the first leaves is actively suppressed in etiolated Arabidopsis seedlings. Unlike other photomorphogenic growth patterns, the activation of the shoot apical meristem (SAM) depends not solely on the perception of light, but additionally on nutrient availability. We dissect how light as well as light derived metabolic signals are integrated at the SAM to overcome this developmental arrest. Light is hereby perceived through the plant's photoreceptors, which activate the stem cell regulator WUSCHEL in the SAM independently of photosynthesis by inhibiting the potent repressor of photomorphogenesis COP1. Metabolic signals, on the other hand, are transduced to the meristem through activation of the TARGET OF RAPAMYCIN (TOR) kinase. We show that TOR is furthermore inhibited by COP1 and that the regulation of TOR activity is involved in transducing the light signal to the SAM. The TOR kinase acts therefore as central integrator of light derived signals and a key regulator of meristematic activity at the shoot apex. Light and nutrient signalling are additionally integrated at the level of the plant hormone cytokinin (CK). While light prevents CK breakdown at the SAM via repression of the CYTOKININ OXIDASES 5 and 6, CK downstream signalling depends strictly on the presence of photosynthetic products. Ensuing that both, light and nutrients are available at the same time allows the plant to resourcefully time the developmental transition towards new organ development.

## PC6.26 MASTER REGULATOR HSFS IN ARABIDOPSIS: ARE THEY MOLECULAR SWITCHES BETWEEN GROWTH AND DEFENCE?

TUESDAY 4 JULY, 2017 13:40

PHILIP M MULLINEAUX (UNIVERSITY OF ESSEX, UNITED KINGDOM), WALEED S ALBIHLAL (UNIVERSITY OF SURREY, UNITED KINGDOM), RAMONA PERSAD (UNIVERSITY OF ESSEX, UNITED KINGDOM), IRABONOSI OBOMIGHE (UNIVERSITY OF ESSEX, UNITED KINGDOM), THOMAS BLEIN (INSTITUTE OF PLANT SCIENCES PARIS-SACLAY, FRANCE), MARINO EXPOSITO-RODRIGUEZ (UNIVERSITY OF ESSEX, UNITED KINGDOM), MARTIN CRESPI (INSTITUTE OF PLANT SCIENCES PARIS-SACLAY, FRANCE), ULRIKE BECHTOLD (UNIVERSITY OF ESSEX, UNITED KINGDOM)

@ MULLIN@ESSEX.AC.UK

There are 21 Heat Shock Transcription Factor genes (*HSFs*) in *Arabidopsis thaliana* and evidence is accumulating that these not only control stress responses but also aspects of plant growth and development. The clade A1 class of *HSFs* are 4 paralogs that appear to influence the expression of many, if not all, other *HSFs* and are termed master regulators of the *HSF* family. We identified developmental and stress-responsive genes controlled by *HSFA1b* by analysing genome-wide binding of *HSFA1b*-eYFP, combined with transcriptomics, in control, heat stressed and *HSFA1b* over-expressing plants. A wide array of genes associated not only with stress responses but also growth and development functions were shown to be both directly and indirectly regulated by *HSFA1b*. *HSFA1b*-regulated genes include those coding natural antisense transcripts (NATs) which potentially regulate the expression of >400 target loci. Further, we intersected binding targets of *HSFA1b* with those of 7 other transcription factors (TFs), which suggests that *HSFA1b* is one of a number of TFs that transduce environmental and endogenous cues into a core common network of stress-responsive and developmental genes. We conclude that in wild type plants, under both benign and stress conditions, that *HSFA1b* provides a conduit for environmental cues to subtly and constantly influence growth and development. Further, how *HSFs* engage in priming the expression of stress-responsive genes, which poise the plant to either activate defences to survive the onset of an adverse condition or conversely, initiate acclimation leading to enhanced growth, will be considered.

## PC6.27 ROOT HYDROTROPISM IS CONTROLLED VIA A CORTEX-SPECIFIC GROWTH MECHANISM

TUESDAY 4 JULY, 2017 14:20

DANIELA DIETRICH (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), LEI PANG (TOHOKU UNIVERSITY, JAPAN), AKIE KOBAYASHI (TOHOKU UNIVERSITY, JAPAN), JOHN A FOZARD (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), VERONIQUE BOUDOLF (GHENT UNIVERSITY, BELGIUM), RAHUL BHOSALE (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), REGINA ANTONI (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), TUAN NGUYEN (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), SOTARO HIRATSUKA (TOHOKU UNIVERSITY, JAPAN), NOBUHARU FUJII (TOHOKU UNIVERSITY, JAPAN), YUTAKA MIYAZAWA (YAMAGATA UNIVERSITY, JAPAN), TAE-WOONG BAE (TOHOKU UNIVERSITY, JAPAN), DARREN M WELLS (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), MARKUS R OWEN (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), LEAH R BAND (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), ROSEMARY J DYSON (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), OLIVER E JENSEN (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JOHN R KING (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), SAOIRSE R TRACY (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), CRAIG J STURROCK (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), SACHA J MOONEY (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JEREMY A ROBERTS (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), RISHIKESH P BHALERAO (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), JOSE R DINNENY (CARNEGIE INSTITUTION FOR SCIENCE, UNITED STATES), PEDRO L RODRIGUEZ (UNIVERSIDAD POLITECNICA DE VALENCIA, SPAIN), AKIRA NAGATANI (KYOTO UNIVERSITY, JAPAN), YOICHIROH HOSOKAWA (NARA INSTITUTE OF SCIENCE TECHNOLOGY, JAPAN), TOBIAS I BASKIN (UNIVERSITY OF MASSACHUSETTS, UNITED STATES), TONY P PRIDMORE (UNIVERSITY OF NOTTINGHAM, UNITED STATES), LIEVEN DE VEYLDER (GHENT UNIVERSITY, BELGIUM), HIDEYUKI TAKAHASHI (TOHOKU UNIVERSITY, JAPAN), MALCOLM J BENNETT (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM)

@ DANIELA.DIETRICH@NOTTINGHAM.AC.UK

Plants use tropisms to acclimate to changes in environmental conditions and link their direction of growth to cues provided by light, gravity and water. Hydrotropism allows roots to forage for water, a process known to depend on abscisic acid (ABA) but whose molecular and cellular basis remains unclear. We show that hydrotropism still occurs in roots after laser and manual ablation removed the meristem and root cap. Additionally, targeted expression studies reveal that hydrotropism depends on the ABA signalling kinase, SnRK2.2, and the hydrotropism-specific MIZ1, both acting specifically in elongation zone cortical cells. Conversely, hydrotropism, but not gravitropism, is inhibited by preventing differential cell-length increases in the cortex, but not in other cell types. Using multiscale mechanical modelling, we show that a differential growth response in the cortex cell file is sufficient to achieve the root tip angles observed in the hydrotropism assays. We conclude that root tropic responses to gravity and water are driven by distinct tissue-based mechanisms. In addition, unlike its role in root gravitropism, the elongation zone performs a dual function during a hydrotropic response, both sensing a water potential gradient and subsequently undergoing differential growth.

## PC6.28 AN ORGAN-ON-A-CHIP APPROACH FOR INVESTIGATING ROOT-ENVIRONMENT INTERACTIONS IN HETEROGENEOUS CONDITIONS

📅 TUESDAY 4 JULY, 2017 ⌚ 14:35

👤 GUIDO GROSSMANN (CENTRE FOR ORGANISMAL STUDIES HEIDELBERG UNIVERSITY, GERMANY), CLAIRE E STANLEY (INSTITUT FÜR NACHHALTIGKEITSWISSENSCHAFTEN AGROSCOPE, SWITZERLAND), JAGRITI SHRIVASTAVA (CENTRE FOR ORGANISMAL STUDIES HEIDELBERG UNIVERSITY, GERMANY), RIK BRUGMAN (CENTRE FOR ORGANISMAL STUDIES HEIDELBERG UNIVERSITY, GERMANY), DIRK VAN SWAAY (INSTITUTE FOR CHEMICAL AND BIOENGINEERING ETH ZÜRICH, SWITZERLAND)

@ GUIDO.GROSSMANN@COS.UNI-HEIDELBERG.DE

Plant roots grow in highly heterogeneous environments where they need to locally adapt their architecture to optimally absorb nutrients and water, interact with pathogenic and symbiotic microbes, and cope with diverse abiotic stress conditions. Our lab is developing RootChips, integrated microfluidic perfusion and imaging platforms that facilitate microscopic studies of root-environment interactions under precisely controlled conditions.

Here we present a technique that allows investigating how roots perceive and respond to local stimuli and develop in asymmetric environments, where one side of the root is exposed to beneficial and the other side to adverse conditions. The dual-flow-RootChip (dfRootChip) employs a micropillar array for guided root growth and utilizes laminar flow to treat both sides of a root separately. We present applications that range from monitoring of physiology and development under asymmetric conditions, tracing molecular uptake and selective drug treatments, to local inoculation with microbes. We measured calcium responses in roots treated with biotic and abiotic elicitors and observed elicitor-specific signal propagation across the root from treated to untreated cells. We further demonstrate cell-autonomous regulation of root hair growth under local availability of phosphate, a nutrient that often exhibits patchy distribution in soil. Our approach sheds light on cell autonomy and lateral coordination of morphological adaptation and facilitates studies on root physiology, signalling and development in heterogeneous environments at the organ level.

## PC6.29 PLANT SYSTEMS BIOLOGY: APPLICATION TO RICE FOR UNDERSTANDING METABOLIC AND REGULATORY CHARACTERISTICS UNDER DIFFERENT LIGHT CONDITIONS FOR CROP IMPROVEMENT

📅 TUESDAY 4 JULY, 2017 ⌚ 14:50

👤 BIJAYALAXMI MOHANTY (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE), MEIYAPPAN LAKSHMANAN (BIOPROCESSING TECHNOLOGY INSTITUTE, SINGAPORE), SUN-HYUNG LIM (NATIONAL ACADEMY OF AGRICULTURAL SCIENCE RURAL DEVELOPMENT ADMINISTRATION, KOREA (SOUTH)), JAE KWANG KIM (INCHEON NATIONAL UNIVERSITY, KOREA (SOUTH)), SUN-HWA HA (KYUNG HEE UNIVERSITY, KOREA (SOUTH)), DONG-YUP LEE (NATIONAL UNIVERSITY OF SINGAPORE BIOPROCESSING TECHNOLOGY INSTITUTE, SINGAPORE)

@ CHEBIJAY@NUS.EDU.SG

Light is the primary energy source and important signaling component for growth and development of plants. A comprehensive understanding on how different light sources affect the growth, development and production of secondary metabolites both at metabolic and gene regulation levels will provide a significant insight towards improved rice varieties. To do so, we employed systems biology approach to study such light-driven metabolic and regulatory mechanism in rice. In this study, we first developed a constraint-based genome scale metabolic model of rice and subsequently integrated transcriptome and metabolomic data with the model to find out different light-specific transcriptional signatures of rice metabolism. Specifically, effects of blue- and red-light signals on the primary and secondary metabolism identified the involvement of different phytohormones such as abscisic acid (ABA), ethylene, gibberellin and jasmonate. The subsequent analysis on the promoter region of relevant genes further identified several light-specific putative cis-elements and their associated transcription factors. Moreover, the analysis on the light-specific transcriptional regulation of the biosynthesis of higher accumulation carotenoids and phenolic compounds revealed a crosstalk between different phytohormones and the possible involvement of key transcription factors such as bHLH, bZIP, MYB, WRKY, ZnF and ERF upon exposure to blue light. Overall, our analysis provides a possible transcriptional regulatory mechanism underlying involvement of different secondary metabolic pathways in response to blue light and provide several potential applications for crop improvement.

### PC6.36 REDOX CYCLING DURING THE CELL CYCLE IN THE EMBRYONIC ROOT MERISTEM AND ITS DISRUPTION BY MILD OXIDATION

TUESDAY 4 JULY, 2017 16:00

CHRISTINE H FOYER (UNIVERSITY OF LEEDS, UNITED KINGDOM)

@ C.FOYER@LEEDS.AC.UK

Redox regulation of the plant cell cycle has long been postulated but not been clearly demonstrated. Using their *in vivo* reduction-oxidation (redox) reporter (roGFP2), we explored the possibility of redox cycling within the cell cycle in the proliferating cells of the embryonic root meristem of germinating *Arabidopsis* seeds. A transient oxidation of the cytosol and then the nuclei was observed at G1 followed by re-reduction at G2 and mitosis. However, this redox cycling at G1/G2 was perturbed in mutants with low redox buffering capacity resulting from impaired synthesis and accumulation of ascorbate. The nuclei in the dividing cells of these mutants showed a significant increase in the degree of oxidation, together with an impaired capacity to increase in size during the early stages of the cell cycle. In addition, data will be presented showing that the seed transcriptome is altered by oxidation in the mother plant resulting in larger embryos after imbibition, without consistently affecting dormancy and longevity.

### PC6.37 PHOTORECEPTORS ARE INVOLVED IN *ARABIDOPSIS* GROWTH UNDER SALT STRESS CONDITIONS *IN VITRO*

TUESDAY 4 JULY, 2017 16:30

IRINA STRIZH (FACULTY OF BIOLOGY M.V. LOMONOSOV MOSCOW STATE UNIVERSITY, RUSSIA)

@ IRINA.STRIZH@MAIL.RU

Growing *Arabidopsis* in Petri dishes under full illumination of the root and shoot is the common and well accepted experimental method in plant biology. It is also well known that root of the most plants should grow in darkness so there is an opinion that light is a stress factor for the root growing. Abiotic stresses could cause an acceleration of the growing to escape the problem or an inhibition of root growing in particular. Light promote root growing, but salt stress inhibits it. The idea of the present work was to compare the involvement of these two stress abiotic factors for *Arabidopsis* growing *in vitro*. We have measured primary root growth and also compared germination rate and survival time of plants growing continuously on 0, 50, 100, 150 mM NaCl. As object of research *phyA*, *phyB*, *phot1*, *phot2*, *cry1*, *cry2* mutants (background Col-0) were used. The more prominent differences between wild type and mutants have been found under 150 mM NaCl in media. Mutants on photoreceptors have shown definitely longer survival time than wild type. We can conclude that photoreceptors not only involved in primary root growth and germination under salt and light stresses, but can influence salt tolerance of the adult plants as well.

### PC6.38 IMPROVING POST-SUBMERGENCE RECOVERY IN *ARABIDOPSIS* THROUGH ROS MEDIATION

TUESDAY 4 JULY, 2017 16:45

ELAINE YEUNG (UTRECHT UNIVERSITY, NETHERLANDS), HANS VAN VEEN (UTRECHT UNIVERSITY, NETHERLANDS), DIVYA VASHISHT (GREGOR MENDEL INSTITUTE, AUSTRIA), JULIA BAILEY-SERRES (UNIVERSITY OF CALIFORNIA RIVERSIDE, UNITED STATES), LAURENTIUS ACJ VOESENEK (UTRECHT UNIVERSITY, NETHERLANDS), RASHMI SASIDHARAN (UTRECHT UNIVERSITY, NETHERLANDS)

@ E.Y.YEUNG@UU.NL

Recovery from flooding stress is dependent not only on plants' viability under water, but their post-submergence tolerance as well. Some plants die after the flood water recedes, thus investigating the recovery phase may provide insight into plants' overall flooding tolerance. This study compared a submergence-tolerant and -intolerant *Arabidopsis* accession. Both showed similar submergence-mediated damage, but they were studied for their different recovery rates upon de-submergence. Contrasting physiological and molecular responses under post-submergence recovery was investigated using a ribosome-sequencing approach. Differences between the accessions in the submergence and the recovery phase were identified. Gene Ontology identified categories related to oxidative stress, photosynthetic processes, desiccation stress, and hormone responses. Formation of damaging reactive oxygen species (ROS) molecules occurs when plants are exposed to a sudden burst of oxygen during the transition to the air environment after a long submergence period. Oxidative stress leads to breakdown of important organelles, thus damaging the photosynthetic apparatus and induction of downstream desiccation responses. When cell death and senescence occur in the leaves, plant regrowth is inhibited. Thus, the ability to restrict or recover from ROS production during de-submergence is important for recovery. Studying the post-flooding responses with ribo-seq revealed the molecular processes inducing protective mechanisms during recovery.

### PC6.10 CIA2 AND CIL TRANSCRIPTION FACTORS ARE REQUIRED FOR OPTIMAL PHOTOSYNTHESIS IN *ARABIDOPSIS THALIANA*

TUESDAY 4 JULY, 2017 POSTER SESSION

PIOTR GAWRONSKI (WULS, POLAND), PAWEŁ BURDIĄK (WULS, POLAND), JAKUB MIELECKI (WULS, POLAND), ANNA KOZŁOWSKA-MAKULSKA (WULS, POLAND), STANISŁAW KARPINSKI (WULS, POLAND)

@ PETER.GAWRONSKI@GMAIL.COM

Because of their nature, plants are unable to avoid naturally fluctuating environment conditions such as high light, UV radiation or pathogen attack. Plant response to stress conditions is determined by highly complex signalling network between organelles such as chloroplast and nucleus. Apart from hosting the process of photosynthesis chloroplasts serve as sensors of visible light and

other environmental cues. In a series of events termed as retrograde signalling they release a plethora of retrograde signalling and factors that lead to changes in nuclear gene expression and trigger plant acclimation. Great majority of chloroplast proteins are nuclear encoded and are imported into the organelle from cytoplasm. Among them, there is a surprisingly high number of putative transcription factors (TFs) with yet unknown function. Using reverse-genetics approach, we screened over 200 knockout lines representing putative TFs predicted for chloroplast targeting. We focused on a *cia2* mutant (mutation in *CHLOROPLAST IMPORT APPARATUS 2*, *CIA2* gene), which showed increased sensitivity to high light and UV-AB radiation. Since homology searches identified *CIA2-like* (*CIL*) gene encoding protein highly similar to CIA2, we introduced *cil* mutation into *cia2* background. When compared to wild type plants, *cia2/cil* had decreased non-photochemical quenching, CO<sub>2</sub> assimilation and chlorophyll content. We also observed that processing of chloroplast 23S rRNA and expression of nuclear-encoded, chloroplast-targeted ribosomal proteins are decreased in *cia2/cil*. Our data suggest that CIA2 and CIL, by controlling an expression of nuclear genes, influence translation in chloroplasts and are required for optimal photosynthesis in *Arabidopsis*.

### PC6.11 LIGHT REGULATION OF STOMATAL DEVELOPMENT IN *ARABIDOPSIS THALIANA*, INTEGRATION OF HORMONAL AND TRANSCRIPTIONAL CONTROL

TUESDAY 4 JULY, 2017 POSTER SESSION

- MARIE HRONKOVÁ (UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE AND BIOLOGY CENTRE CAS, CZECH REPUBLIC), DANA WIESNEROVÁ (BIOLOGY CENTRE CAS ČESKÉ BUDĚJOVICE, CZECH REPUBLIC), MARIE ŠIMKOVÁ (BIOLOGY CENTRE CAS ČESKÉ BUDĚJOVICE, CZECH REPUBLIC), PETRE DOBREV (INSTITUTE OF EXPERIMENTAL BOTANY AS CR PRAGUE, CZECH REPUBLIC), JIŘÍ ŠANTRŮČEK (UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE AND BIOLOGY CENTRE CAS ČESKÉ BUDĚJOVICE, CZECH REPUBLIC)

@ HRONKOVA@UMBR.CAS.CZ

Stomata are epidermal pores, crucial for plant survival, as they balance gas exchange and transpiration, affecting photosynthetic rate. Hence, not only stomatal opening but also stomatal development is tightly regulated through internal and external factors that are integrated by a complex signalling network. Light represents an important external factor that strongly promotes stomata formation. Stomatal development is under the control of an intrinsic program mediated by a secretory peptide gene family, *EPIDERMAL PATTERNING FACTORS* (*EPFs*), including positively acting *STOMAGEN* (*EPFL9*). In our recently published work we linked this phenomenon with higher expression level of this and some other related genes.

According literature, auxin is a negative regulator of stomatal development in dark-grown seedlings (through MONOPTEROS repression of *STOMAGEN* expression in mesophyll cells). Abscisic acid (ABA), known to induce stomatal closure, acts to promote maximal leaf growth but the suboptimal water supply alters the role of ABA to a direct growth inhibitor.

Endogenous auxin and ABA levels in the leaves of different age grown under low and high light intensity were matched with expression levels of selected genes. How these signals are integrated to optimize stomatal development and plant growth under certain

light conditions? This will be discussed together with stomatal insensitivity to ABA as closure signal in young leaves, exposed to higher relative humidity in the leaf rosette. It might be favourable to the diffusion of atmospheric CO<sub>2</sub>, especially in highly illuminated leaves with high demand for photosynthetic substrate, but could also critically impair the tolerance to the water stress.

### PC6.12 DEVELOPMENT OF THE ROOT VASCULATURE DEPENDS ON ABA AND IS AFFECTED BY ABIOTIC STRESS

TUESDAY 4 JULY, 2017 POSTER SESSION

- PRASHANTH RAMACHANDRAN (PHYSIOLOGICAL BOTANY DEPARTMENT OF ORGANISMAL BIOLOGY UPPSALA UNIVERSITY, SWEDEN), FRAUKE AUGSTEIN (PHYSIOLOGICAL BOTANY DEPARTMENT OF ORGANISMAL BIOLOGY UPPSALA UNIVERSITY, SWEDEN), JAN DE VRIES (DEPARTMENT OF BIOCHEMISTRY MOLECULAR BIOLOGY DALHOUSIE UNIVERSITY, CANADA), GUODONG WANG (COLLEGE OF LIFE SCIENCES SHAANXI NORMAL UNIVERSITY, CHINA), ANNELIE CARLSBECKER (PHYSIOLOGICAL BOTANY DEPARTMENT OF ORGANISMAL BIOLOGY UPPSALA UNIVERSITY, SWEDEN)

@ PRASHANTH.RAMACHANDRAN@EBC.UU.SE

As the initiation point for water uptake, a well-developed xylem in the root serves an important role in plant survival. The root is constantly exposed to changing environment, and this would enforce a need to adapt not only root growth but also its anatomy e.g. for more efficient water uptake. In a previous study we have uncovered a bidirectional communication between the vascular stele and the surrounding endodermis via microRNA 165/166, determining the identity of the xylem cells (Carlsbecker & Lee et al., 2010, *Nature* 465: 316-321). This patterning mechanism intersects with hormonal signaling to maintain stable xylem patterning (Müller et al., 2016, *Plant Physiology* 170: 956-970). However, the effect of abiotic stress on root vascular development has not been extensively studied. To approach the potential influence of abiotic stress on vascular development we have chosen to address the role of abscisic acid (ABA) and ABA-mediated abiotic stress on xylem development in *Arabidopsis thaliana*. The stereotypical vascular organization of the *Arabidopsis* root facilitates the analysis of cell identity and differentiation related events. We find that ABA biosynthesis and core ABA signaling components participate to control both these processes. We demonstrate that the effect of ABA on xylem development is non-cell autonomous and explore the relation to the vascular patterning process mediated by endodermally derived microRNAs, miR165/166. We discuss the possibility that ABA's influence on vascular development may be a means to adapt to abiotic stresses such as drought, and present our attempts to test this hypothesis.



### PC6.13 ZEITLUPE INTERACTS WITH OPEN STOMATA 1 AND REVEALS A CLOCK-REGULATED STOMATAL APERTURE CONTROL

TUESDAY 4 JULY, 2017 POSTER SESSION

MANUELA JURCA (UMEÅ UNIVERSITY, SWEDEN), ANASTASIA MATROSOVA (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), MIKAEL JOHANSSON (BIELEFELD UNIVERSITY, GERMANY), CRISTIAN IBÁÑEZ (LA SERENA UNIVERSITY, CHILE), IWANKA KOZAREWA (UMEÅ UNIVERSITY, SWEDEN), JOHAN SJÖLANDER (UMEÅ UNIVERSITY, SWEDEN), LASZLO BAKÓ (UMEÅ UNIVERSITY, SWEDEN), ALEX A R WEBB (CAMBRIDGE UNIVERSITY, UNITED KINGDOM), MARIA ISRAELSSON-NORDSTRÖM (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), MARIA E ERIKSSON (UMEÅ UNIVERSITY, SWEDEN)

@ MANUELA.JURCA@UMU.SE

OPEN STOMATA 1 (OST1) is a nexus in stomatal aperture control, standing as a merging point in ABA, carbon dioxide and stress signaling. We show that the photoreceptor and clock component ZEITLUPE (ZTL) regulates stomatal movements since *ztl* mutant plants show strong ABA insensitivity, with more opened stomata than wild-type, similar to mutant *ost1-3* plants. On a biochemical level we confirm a physical interaction between ZTL and OST1 proteins, thus establishing a link between light perception, ABA signaling and stress responses. This work expands on current knowledge of ABA signaling pathways and suggests an important point of regulation of diurnal circadian-regulated stomatal movements while allowing adjustments to a stressful environment. Also, the positive regulation by ZTL on stomatal closing responses are conserved between *Arabidopsis* and *Populus*. Thus, the OST1 interaction with ZTL may offer a molecular model where the clock regulates water use and stress responses and important for biomass production of trees.

### PC6.14 NOVEL REGULATORS OF LIGHT-DRIVEN SHOOT ARCHITECTURE: A COMPARATIVE APPROACH

TUESDAY 4 JULY, 2017 POSTER SESSION

SARA BUTI (UTRECHT UNIVERSITY, NETHERLANDS), CHARLOTTE M M GOMMERS (CRAG CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), LAURENTIUS A C J VOESENEK (UTRECHT UNIVERSITY, NETHERLANDS), RONALD PIERIK (UTRECHT UNIVERSITY, NETHERLANDS)

@ S.BUTI@UU.NL

Plants growing at high densities can adopt two different strategies to deal with shade caused by neighbours: escape growth (shade avoidance response) or being quiescent (shade tolerance response). The Shade Avoidance Syndrome (SAS) is initiated in response to far-red light reflected by neighbours, and allows plants to escape from the shade through an upward orientation of the leaves and accelerated elongation of hypocotyls, internodes and petioles. SAS occurs in most crop species that are grown at high density in monocultures. However, it is a wasteful investment because the

allocation of resources towards the stem goes at the expense of yield. Shade tolerant plants that grow in forest understories cannot outgrow the trees around them and consequently suppress shade avoidance. The aim of this work is to understand which molecular and physiological mechanisms shade tolerant plants have evolved to suppress shade avoidance. A comparative study system of two *Geranium* species with opposite strategies is used: *G. pyrenaicum* (shade avoider) and *G. robertianum* (shade tolerant forest understory species). This work is focused on studying the function of candidate genes identified through an RNAseq transcriptome study on these two contrasting *Geranium* species in order to find regulators of their antithetical light response strategies.

### PC6.15 INVESTIGATING THE ROLE OF CHLOROPLAST GAPDH IN DETERMINING REGULATION CALVIN CYCLE IN DROUGHT AND NON-DROUGHT CONDITIONS

TUESDAY 4 JULY, 2017 POSTER SESSION

MOHAMMED ALQURASHI (UNIVERSITY OF ESSEX, UNITED KINGDOM), CHRISTINE A RAINES (UNIVERSITY OF ESSEX, UNITED KINGDOM), ULRIKE BECHTOLD (UNIVERSITY OF ESSEX, UNITED KINGDOM)

@ MKHALQ@ESSEX.AC.UK

Manipulation of photosynthetic processes offers an opportunity to increase food production. Current and previous studies have shown manipulation of photosynthesis has the potential to increase crop yield. Gene regulatory network inference from time series gene expression data identified key genes involved in regulation of photosynthetic carbon assimilation under drought stress. Three highly connected genes were identified as being important in influencing drought-induced transcriptional responses of the genes that encode for enzymes of the Calvin cycle. These genes are: the drought inducible transcription factor RAP2.12 (a member of the ethylene response factor family), the Calvin cycle gene GAPA-2, and a putative transcription factor. The model output suggests that increase in GAPA-2 gene expression and a decrease in the levels of RAP2.12 may have a positive effect in the expression of Calvin cycle genes under drought conditions. The main goal of this project is to produce plants with significantly improved biomass production and photosynthetic performance under drought stress, by controlling the expression of multiple genes that are critical in the regulation of the Calvin cycle. We produced lines, which over express GAPA-2 in a RAP2.12 knockout (*rap2.12*), to find out whether these two proteins are playing an important role in the regulation of Calvin cycle enzymes under drought and non-drought condition. Up to date, molecular analyses were conducted and identified double homozygous mutants in two independent *rap2.12* mutants containing a GAPA-2 over-expression construct. Growth analysis, and drought experiment underway. Gene expression levels for drought and non-drought plants will be evaluated by qPCR.

### PC6.30 TEMPERATURE SENSING AND SIGNALLING IN *ARABIDOPSIS* METABOLISM

TUESDAY 4 JULY, 2017 POSTER SESSION

HELENA A HERRMANN (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GILES JOHNSON (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JEAN-MARC SCHWARTZ (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

HELENA.HERRMANN@POSTGRAD.MANCHESTER.AC.UK

The allocation of transient carbon assimilates such as sugars, starch, malate and fumarate shifts in *Arabidopsis thaliana* as the plant is subjected to different stresses. Using both modelling and wet lab techniques we explore how the tightly regulated interplay of carbon assimilates provides the conditions necessary for photosynthetic acclimation. We aim to understand both the metabolic changes which signal an acclimation response as well as the different metabolic states required for acclimation. We have identified cytosolic fumarate accumulation as a necessary requirement for acclimation to both cold and warm temperatures.

### PC6.31 ARABIDOPSIS GROWTH AND DEVELOPMENT: THE ROLE OF HEAT SHOCK FACTOR A1b (HSFA1b)

TUESDAY 4 JULY, 2017 POSTER SESSION

IRABONOSI OBOMIGHIE (UNIVERSITY OF ESSEX, UNITED KINGDOM)

OSYRINA2@YAHOO.COM

Heat Shock Factors (HSFs) are central to the transcriptional regulation of the heat stress response in *Arabidopsis*. Of the 21 HSFs found in *Arabidopsis*, the clade A1 family of HSF (HSFA1a/A1b/A1d/A1e) are implicated in the control of HSP gene expression and regulation during stress defence but they have an additional role in the regulation of development-associated gene expression, which is much less understood. In order to determine the role of one of its members (HSFA1b) in growth and development, transcriptomic changes of HSFA1b overexpressing plants (35S:HSFA1bOx) compared to wild type plants identified a number of genes with developmental roles. In agreement with the gene expression data, 35S:HSFA1bOx plants flower early with an increase in seed yield despite a reduced rosette area. Further analysis of the transcriptomics data revealed 2 ARGONAUTE genes (AGO1 and AGO2) involved in both plant development and resistance to pathogens. AGO1 and 2 is directly and indirectly regulated by HSFA1b respectively under heat stress with significant altered expression. We decided to investigate how much of the 35S:HSFA1bOx phenotype is as a result of AGO1&2 altered expression. Preliminary results suggest that AGO1&2 may not be epistatic to HSFA1b as the developmental phenotypes associated with 35S:HSFA1bOx plants was observed in the 35S:HSFA1bOx-ago1/2 double knockout mutants. However, 35S:HSFA1bOx plants have also been shown to positively influence basal resistance to plant pathogens *Hyaloperonospora arabidopsidis* (HPA) and *Pseudomonas syringae pv tomato*. Experiments are being conducted to ascertain the involvement of AGO1&2 as both have been implicated in the resistance of plant pathogens.

### PC6.32 HORMONAL INTERACTIONS IN ROOT RESPONSES TO MECHANICAL IMPEDANCE

TUESDAY 4 JULY, 2017 POSTER SESSION

AMY G R JACOBSEN (DURHAM UNIVERSITY, UNITED KINGDOM), JEN F TOPPING (DURHAM UNIVERSITY, UNITED KINGDOM), KEITH LINDSEY (DURHAM UNIVERSITY, UNITED KINGDOM)

A.G.R.JACOBSEN@DURHAM.AC.UK

The plant root encounters a number of diverse abiotic and biotic environmental stresses in the soil, such as mechanical impedance, drought, temperature, nutrients and pathogens. Plant roots must be able to respond to such stress appropriately and do so through changes to growth and development. Such changes involve effects on the behaviour of the stem cell population in the meristem, the activity of the meristem, the extent of cell expansion in the elongation zone, and the frequency of initiation and extent of elongation of lateral roots. These developmental changes are mediated by interactions between several classes of hormones that form a complex network with key regulatory genes.

Plants often encounter barriers to their growth in soils. For example, in drying soil strength increases with decreasing water content. Mechanical impedance has previously been shown to reduce root elongation and may have a negative impact on crop yields. It is therefore important to understand how root growth and development is regulated in response to encountering a barrier.

The work described here aims to investigate how plant roots sense and respond to barriers to their growth and the molecular signalling pathways involved in controlling this response.

### PC6.33 MOLECULAR BREEDING OF SALT TOLERANT WHEAT

TUESDAY 4 JULY, 2017 POSTER SESSION

JOHANNA V LETHIN (GÖTEBORG UNIVERSITY BIOLOGICAL AND ENVIRONMENTAL SCIENCE, SWEDEN), SHARHIAR SHAKIL (DEPARTMENT OF PURE AND APPLIED BIOCHEMISTRY, LUND UNIVERSITY, SWEDEN), OLOF OLSSON (DEPARTMENT OF PURE AND APPLIED BIOCHEMISTRY, LUND UNIVERSITY, SWEDEN), HENRIK ARONSSON (GÖTEBORG UNIVERSITY DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCE, SWEDEN)

JOHANNA.LETHIN@BIOENV.GU.SE

Over the last few decades, there has been a significant increase in irrigated land. This has led to that around 2000 ha of irrigated agricultural land is lost every day due to high salinity levels. The size of high salinity areas has been growing from 45 million ha in the early 1990s to its current volume of 62 million ha[1]. Globally, 3.1% of the total land mass is affected by salinity, with over half of the world's countries afflicted[2]. Presently, about one-fifth of the world's irrigated lands are too salty for agriculture. Clearly, this negative trend has to be broken.

Our aim is to develop wheat (*Triticum aestivum* L.) varieties with an increased and sufficient salinity tolerance for cultivation on areas that are so salt contaminated that they can no longer be used. We identified a successful local wheat variety commonly grown in Bangladesh denoted Gom-25, it has a high root biomass and low

transpiration rate and is therefore already fairly well adapted to salt stress [3]. However, much better varieties are needed in order to reuse some parts of the 1 million ha area of salt contaminated land in Bangladesh (Figure 1). Starting from Gom-25, we therefore increased variation in this cultivar introducing EMS (Ethyl Methane Sulphonate) based mutations into the Gom-25 genome. Mutated wheat lines with an increased salinity tolerance is now selected from this population.

### PC6.34 $Ca^{2+}$ -DEPENDENT EXTERNAL NADPH OXIDATION IS AN ANCIENT PROCESS COMPARE TO EXTERNAL NADH OXIDATION IN PLANTS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 MENGSHU HAO (LUND UNIVERSITY, SWEDEN), ALLAN G RASMUSSEN (LUND UNIVERSITY, SWEDEN)

@ MENGSHU.HAO@BIOL.LU.SE

In plants, NADH and NADPH are key reductant carriers that maintain redox and antioxidant status, and that closely links to biosynthesis, catabolism, signalling, and biotic and abiotic stress. EF-hand-containing type II NAD(P)H dehydrogenases are located at outside of the inner mitochondrial membrane in plants. As an example, potato and *Arabidopsis thaliana* NDB1 are external type II NADPH dehydrogenases in mitochondria that have a high capacity to oxidise cytosolic NADPH and can modulate whole cell NADPH status during abiotic and biotic stress. We used potato and *A. thaliana* NDB1 as model, yeast ScND1 crystal structure as template, to study these enzymes by combining molecular modelling with evolutionary analysis, focusing on NAD(P)H substrate specificity and  $Ca^{2+}$  binding. The results suggest that  $Ca^{2+}$ -dependent external NADPH oxidation is an ancient process, indicating that it has a fundamental importance for eukaryotic cellular redox metabolism. In contrast, the external NADH dehydrogenases in plants are products of a recent expansion, mirroring the expansion of the alternative oxidase family.

### PC6.35 CYTOLOGICAL AND BIOCHEMICAL ASSESSMENT OF SOMATIC EMBRYOGENESIS AND CELL SUSPENSIONS OF POTATO AFTER LONG-TERM EXPOSURE TO SALT STRESS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ADEL M ELMAGHRABI (BIOTECHNOLOGY RESEARCH CENTER, LIBYA), ELMUNDER A BOGNIA (BIOTECHNOLOGY RESEARCH CENTER, LIBYA), GADA A EL-REGIAY (BIOTECHNOLOGY RESEARCH CENTER, LIBYA)

@ MAGHRABIAM@HOTMAIL.COM

Somatic embryogenesis occurred from shoot tips of potato after 6 months of in vitro culture. Calli that were obtained were then grown for another five months in MANA medium (MS with 2 mg/L NAA and 0.5 mg/L BAP) in a stepwise protocol comprising 50-to-100-to-150-to-250-to-350 mM NaCl. The rate of callus growth was determined monthly in all treatments. The mean growth rate of

callus increased significantly from month to month for 50 to 100 to 150 mM NaCl. However, growth was significantly less after 3 months following transfer to either 250 or 350 mM NaCl.  $Na^+$  accumulation in all callus cultures increased significantly and was proportionate to the concentration on NaCl, with the highest amount detected in the 350 mM NaCl treatment. Cell suspensions were also established in a modified MS medium, used to culture tobacco BY-2 cells, and grown continuously with 50, 100, 250 or 350 mM NaCl. Nuclear and cell size in these suspensions was highest in the 50 mM treatment compared with both the controls, 100, 150, 250 and 350 mM NaCl treatments. This type of cellular behaviour was also observed in the BY-2 cell line under these stress treatments. Flow cytometry results shows that callus was established on 50 mM NaCl. When NaCl was increased, most cells were delayed in S phase on 150 mM. Notably, 250 or 350 mM, proliferative diploid cells in G1 were clearly observable

### PC6.39 SIMULTANEOUS DROUGHT STRESS AND RALSTONIA SOLANACEARUM INFECTION INDUCES DISTINCT AND COMMON TRANSCRIPTOMIC RESPONSE IN CHICKPEA

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 RANJITA SINHA (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), AARTI GUPTA (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), MUTHAPPA SENTHIL-KUMAR (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA)

@ RANJITA@NIPGR.AC.IN

Plants under field condition often exist with drought and pathogen infection and their concurrence in various instances severely impacted yield. We studied the effect of drought stress on interaction of chickpea with vascular pathogen *Ralstonia solanacearum* (Rs), its net-effect and transcriptomic response of plants towards simultaneous stress. We imposed *R. solanacearum* infection with varied levels of drought stress on chickpea to evaluate the influence of stress intensities on plant-pathogen interaction. We observed decreased in-plant bacterial number at severe drought stress. Cell death decreased in plants under combined stress compared to only pathogen stress. Chlorophyll content in combined stress was more compared to pathogen only infected plants. Further, we studied the transcriptomic changes in response to combined stress at short duration (SD, fast drought stress with 2 days of Rs infection) and long duration (LD, slow drought stress with 4 days of Rs infection) stress by microarray analysis and compared it with only pathogen (SD-pathogen, LD-pathogen) and only drought stress (SD-drought, LD-drought). We found that 821 (31.8%) and 1039 (31.5%) differentially expressed genes (DEGs) were unique to SD-combined stress and LD-combined stress over their individual stresses respectively. SD-combined stress showed induced expression of genes involved in lignin biosynthesis, phytoalexin biosynthesis, abscisic acid, salicylic acid and ethylene signaling and genes involved in defense response and thus resulted in decreased bacterial multiplication. Here, we present that drought stress decreases the Rs multiplication in chickpea. Concomitantly, lignin deposition and phytoalexin production have increased likely as defense strategy to reduce bacterial infection under combined stress.

## PC6.41 SEGREGATION OF HOMOZYGOUS JAZ9 MUTANTS FROM CRISPR/CAS9-TRANSFORMED RICE

TUESDAY 4 JULY, 2017 POSTER SESSION

YANG DO CHOI (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), SANG YOOL LEE (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), GEUPIL JANG (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), TAE YOUNG UM (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), SUN HYUN CHANG (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), JU-KON KIM (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH))

@ CHOIYNGD@SNU.AC.KR

The CRISPR/Cas9 technology is useful for genome editing to generate targeted mutants. We have attempted targeted mutagenesis of rice JAZ9 gene to understand its function in stress response. Target region and gRNA spacer sequence were designed from CRISPR-PLANT website (<http://www.genome.arizona.edu/crispr>) to minimize off-target effect. The gRNA spacer was recombined into the CRISPR/Cas9 binary vector pRGE31 containing rice U3 promoter and gRNA scaffold3. In this system, Cas9 is expressed under the control of 2x35S promoter. Embryonic calli from the mature seeds of rice were cocultivated with transformed *Agrobacterium tumefaciens* and hygromycin-resistant transgenic plants were selected. Cas9-mediated A/T addition occurred at -3 nucleotide position from protospacer adjacent motif, whereas G addition at -5 nucleotide position from the PAM in transgenic rice. Analysis of individual T<sub>0</sub> plants showed coexistence of edited sequences and unedited wild-type sequence, and this phenomenon was broadly observed in most of the transgenic plants, suggesting genetic chimerism of T<sub>0</sub> plants. The ratio of edited sequence to unedited wild-type sequence in the chimeras was variable among the transgenic lines. Nucleotide sequence analysis of T<sub>1</sub> plants showed segregation of those edited sequences from transgenes including CRISPR/Cas9 genes and hygromycin selection marker. These results demonstrate that homozygotic mutant plants could be isolated from chimeric transgenic plants in T<sub>1</sub> generation. Characterization of these mutants will provide better chance to understand the function of JAZ9 in stress response.

## PC6.42 TRANSCRIPTOMIC ANALYSIS OF DORMANCY BREAK OF RICE SEEDS BY HEAT STRESS

TUESDAY 4 JULY, 2017 POSTER SESSION

IN SUN YOON (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), SU YEON KIM (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), BEOM GI KIM (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), TAEK YUN KWON (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH))

@ ISYOON@KOREA.KR

Seed dormancy is a highly variable agronomic trait affected by genetic and environmental factors. Temperature is known to affect both induction and termination of seed dormancy. However, the molecular mechanism underlying the thermal regulation of seed

dormancy is largely unknown. In many Korean rice cultivars, the primary seed dormancy is not maintained until harvest, but gradually released during the seed ripening stage. The dormancy of immature rice seeds was found to be released by heat stress (42°C). In the present study, we have investigated the effect of heat stress on the transcriptome profiling in the embryos of rice seeds. Thirty five percent of total 40,309 probes showed at least two fold changes by heat stress in seed embryos at 25 DAH, indicating that global transcriptomal changes were induced by heat stress. It was found that 34% of DEGs by heat stress was overlapped with the genes changed from 25 DAH to 60 DAH seeds. The most enriched GO terms were 'metabolic process' in biological process domain and 'catalytic activity' in molecular function domain (P < 0.05). Additional transcriptome profiling of 1,712 transcription factors showed that 483 TFs were differentially expressed by heat stress in 25 DAH seed embryo. It is noted that DREB family members were highly up-regulated. Supported by a grant (PJ011942032017) from BG21 program of RDA.

## PC6.43 BULK SEGREGANT ANALYSIS (BSA) FOR THE IMPROVEMENT OF DROUGHT RESISTANCE IN MAIZE (ZEA MAYS L.) INBRED LINES REVEALED BY SSR MOLECULAR MARKERS

TUESDAY 4 JULY, 2017 POSTER SESSION

MUHAMMAD QUDRAT ULLAH FAROOQI (KANGWON NATIONAL UNIVERSITY, KOREA (SOUTH))

@ MQFAROOQI@KANGWON.AC.KR

In this study, maize inbred lines were used to evaluate the performance under drought stress. Based on germination ability, twenty four lines were screened out with tolerance or susceptible to drought stress. Genetic diversity, relationship and population structure were evaluated by using 100 SSR molecular markers linked with drought tolerance in maize. A total 465 alleles were identified with an average of 4.65 alleles per locus using bulk segregant analysis (BSA). Out of them, 199 were specific alleles for tolerant and 35 were susceptible to drought, while the remaining 231 were shared between the two types. The major allele frequency varied from 0.39 to 0.61 with an average of 0.47. The gene diversity (GD) and polymorphic information content (PIC) average values from all lines were 0.64 and 0.59, respectively. Based on UPGMA analysis, four main cluster groups were identified as most of the drought tolerant lines were clearly discriminated from drought susceptible lines with 33% genetic similarity. On population structure analysis, 24 inbred lines of tolerant and susceptible were divided into 3 groups; based on probability membership threshold of 0.8. In addition to BSA, a total of 88 SSR's were identified as specific to tolerant and 29 SSR markers were specific to susceptible lines. Among these, bnlgl1627, umc1946, dupssr30b, bnlgl1812 and dupssr24 SSR markers had great potential for the improvement of drought resistance in maize. Our results were in good agreement with previous studies for drought tolerance using SSR molecular marker and will be useful for further maize breeding studies.

## PC6.44 HEAT STRESS TOLERANCE LIMIT IN NORWAY SPRUCE (*PICEA ABIES*) SEEDLINGS

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 ANE V VOLLSNES (UNIVERSITY OF OSLO, NORWAY),  
AUD B ERIKSEN (UNIVERSITY OF OSLO, NORWAY)

@ A.V.VOLLSNES@IBV.UIO.NO

Millions of Norway spruce (*Picea abies* L. Karst) seedlings are planted on clear-cut fields in large parts of Europe every year. The solar radiation to such open sites may induce high soil surface temperatures, impacting the stem temperatures of these seedlings. In addition, spruce seedling stems are exposed to heat in some forest plant nurseries during application of a protective layer of wax against pine weevil feeding on the stem bark in the forest. Melted wax which holds temperatures of 70-80°C is applied to the stem, and subsequently cooled with cold water, keeping the seedlings in good vigour. We have found that seedlings exposed to excess heat stress may not display any visible signs of this for the first seven weeks, even though the injury is lethal. Seedlings with dead phloem cells on the lower stem due to heat are not able to transport photosynthates to the root, although transport of water and mineral nutrients up to the shoot may be normal as long as the root is alive. Thus, injured seedlings may seem normal, but eventually die when the root has exhausted its resources. Growing seedlings for eight weeks or more to identify lethal heat injuries is not a viable method for evaluating a cooling procedure after wax application or for evaluating survival rates of seedlings on a hot forest site. We will present results from experiments aimed at identifying less time-consuming measures of survival rates after heat exposure on spruce seedling stems.

## PC6.45 DECIPHERING THE TOR SIGNALLING PATHWAY CONTROLLING PLANT GROWTH

📅 TUESDAY 4 JULY, 2017 POSTER SESSION

👤 CELINE FORZANI (INRA VERSAILLES, FRANCE),  
CHRISTIAN MEYER (INRA VERSAILLES, FRANCE)

@ CELINE.FORZANI@INRA.FR

The TOR (Target of Rapamycin) kinase signalling pathway, which has been identified in all eukaryotes, has emerged as a central hub controlling growth and metabolic pathways in response to nutrient availability. Dysfunction of the TOR kinase leads to cancer and metabolic diseases in humans. TOR is a member of the phosphatidylinositol 3-kinase (PI3K)-related kinase (PIKK) family. In plants the TOR kinase functions in a complex with two other proteins: RAPTOR (regulatory-associated protein of mTOR) and LST8 (lethal with SEC13 protein 8). The Arabidopsis genome encodes one TOR gene whereas RAPTOR and LST8 are encoded by 2 genes. Arabidopsis mutants arrest growth early during embryo development. This essential role played by TOR in plant growth has stalled the deciphering of TOR functions in Arabidopsis. On the other hand, *lst8-1* and *raptor* mutants are viable and show an overlap between their phenotypes. *lst8-1* mutants are hypersensitive to long days and have a reduced growth rate. This *lst8-1* phenotype provided the basis for an *lst8-1* suppressor screen. *lst8-1* revertants were isolated by screening a population of EMS (ethylmethanesulfonate) mutagenized M2 seeds directly in long-day greenhouse conditions. The fastest growing M2 mutant plants were selected. Six putative *lst8* suppressors were so far isolated and sequenced by NGS (next generation sequencing) technologies. Further characterization of the isolated *lst8* suppressors will be presented. This approach should lead to the identification of new molecular components of the plant TOR signalling pathway.

# PC7 PHOTOSYNTHETIC RESPONSE TO A CHANGING ENVIRONMENT - TOWARDS SUSTAINABLE ENERGY PRODUCTION

**ORGANISED BY:** CORNELIA SPETEA WIKLUND (UNIVERSITY OF GOTHENBURG, SWEDEN), PETER NIXON (IMPERIAL COLLEGE LONDON, UK) AND WOLFGANG SCHRÖDER (UMEÅ UNIVERSITY, SWEDEN)

**SESSION SPONSORED BY:** INTERNATIONAL SOCIETY OF PHOTOSYNTHESIS RESEARCH AND GOTHENBURG CENTRE FOR ADVANCED STUDIES IN SCIENCE AND TECHNOLOGY (GoCAS)

## PC7.1 MAINTENANCE OF THE PHOTOSYNTHETIC APPARATUS IN CHANGING ENVIRONMENTS

📅 WEDNESDAY 5 JULY, 2017 ⌚ 09:00

👤 EVA-MARI ARO (UNIVERSITY OF TURKU, FINLAND), SARI JÄRVI (UNIVERSITY OF TURKU, FINLAND), MARJAANA RANTALA (UNIVERSITY OF TURKU, FINLAND)

@ EVAARO@UTU.FI

Linear electron transfer chain of oxygenic photosynthetic organisms is rather similar from cyanobacteria to higher plants. On the contrary, the light harvesting systems and various regulation mechanisms of excitation energy distribution and electron flow, crucial for maintenance of the photosynthetic apparatus under varying environmental conditions, show distinct evolution. Development of chlorophyll b-containing light harvesting systems and complex regulatory networks of energy and electron transfer reactions led to the development of distinct lateral heterogeneity of the thylakoid membrane. Light-induced dynamics in lateral heterogeneity of higher plant thylakoid membrane allows fluent photosynthetic electron transfer and equal light harvesting capacity as well as efficient photoprotection of both photosystems in response to changes in the light environment. On the contrary, the fluency of electron flow in cyanobacteria thylakoid membrane is largely dependent on a broad range of electron valves that have gradually disappeared during evolution of plant chloroplasts. After a great breakthrough in demonstration of the lateral heterogeneity of the thylakoid membrane in higher plant chloroplasts in 1980, our knowledge on light-induced dynamics of such a heterogeneity has slowly evolved in parallel with the development of isolation and characterization methods of thylakoid subdomains.

## PC7.2 SYSTEMS BIOLOGY OF LEAF ONTOGENESIS IN TOBACCO

📅 WEDNESDAY 5 JULY, 2017 ⌚ 09:40

👤 MARK AUREL SCHÖTTLER (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), KAROLINA BELKIUS (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), SABINE KAHLAU (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), ELISA SCHULZ (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), EUGENIA MAXIMOVA (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), WOLFRAM THIELE (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), RALPH BOCK (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

@ SCHOETTLER@MPIMP-GOLM.MPG.DE

We report a comprehensive systems biology approach to follow leaf development and chloroplast biogenesis in tobacco. We determined changes in photosynthetic parameters, chloroplast structure, gene expression, and metabolism during the development of the fifth true leaf. The leaf was fully expanded after three weeks of development. At the same time, its chlorophyll content and the contents of both photosystems peaked, and then immediately started to decrease. In contrast, leaf assimilation and linear electron transport capacity began to decrease even prior to full leaf expansion, and this correlated with decreases in ATP synthase and cytochrome b6/f complex content. After full leaf expansion, only the contents of the light harvesting complexes increased, which resulted in a drastic increase in grana stacking, as revealed by electron microscopy. Besides of the re-modelling of the photosynthetic apparatus within each chloroplast, entire chloroplasts were degraded with increasing leaf age. The remaining chloroplasts accumulated high amounts of starch and lipid bodies, in line with a shift from galactolipids towards storage lipids. On the level of gene expression, within one week of leaf development, expression of the chloroplast transcription and translation machinery peaked, followed by genes for chlorophyll biosynthesis, photosynthetic complex assembly, and structural subunits of the complexes, which reached their expression maximum around day twelve. During senescence, mainly proteases reached their expression maximum. While transcript

levels of genes encoding photosystem subunits were strongly correlated among each other, gene expression levels and the actual complex content rarely correlated, indicating a predominant role of post-transcriptional regulation during leaf development.

### PC7.3 VESICLES ARE PERSISTENT FEATURES OF DIFFERENT PLASTIDS

WEDNESDAY 5 JULY, 2017 09:55

HENRIK ARONSSON (INSTITUTION OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES UNIVERSITY OF GOTHENBURG, SWEDEN), EMELIE LINDQUIST (INSTITUTION OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES UNIVERSITY OF GOTHENBURG, SWEDEN), KATALIN SOLYMOSSI (DEPARTMENT OF PLANT ANATOMY UNIVERSITY OF EÖTVÖS, HUNGARY)

@ HENRIK.ARONSSON@BIOENV.GU.SE

Peripheral vesicles in plastids have been observed repeatedly, primarily in proplastids and developing chloroplasts, in which they are suggested to function in thylakoid biogenesis. Previous observations of vesicles in mature chloroplasts have mainly concerned low temperature pre-treated plants occasionally treated with inhibitors blocking vesicle fusion. Here we show that such vesicle like structures occur not only in chloroplasts and proplastids but also in etio-, etio-chloro-, leuco-, chromo- and even transforming desiccoplasts without any specific pre-treatment. Observations are made both in C3 and C4 species, in different cell types (meristematic, epidermis, mesophyll, bundle sheath and secretory cells) and different organs (roots, stems, leaves, floral parts and fruits). Until recently not much focus has been given to the idea that vesicle transport in chloroplasts could be mediated by proteins, but recent data suggest that the vesicle system of chloroplasts has similarities with the cytosolic COPII system. All current data taken together support the idea of an on-going, active and protein-mediated vesicle transport not only in chloroplasts but in other plastids as well, obviously occurring regardless of chemical modifications, temperature and plastid developmental stage.

### PC7.10 REMODELLING OF A CYANOBACTERIAL CHLOROPHYLL-SYNTHASE COMPLEX BY HIGH-LIGHT INDUCIBLE PROTEINS

WEDNESDAY 5 JULY, 2017 11:00

ROMAN SOBOTKA (INSTITUTE OF MICROBIOLOGY CZECH ACADEMY OF SCIENCES, CZECH REPUBLIC)

@ SOBOTKA@ALGA.CZ

Cyanobacteria contain a family of one-helix high-light-inducible proteins (Hlips), which are widely accepted as ancestors of plant light harvesting antennae. Hlips are known to bind chlorophyll and carotenoids and to play an essential role in the photoprotection; however the mechanism is not explained. The cyanobacterium *Synechocystis* 6803 contains four small Hlips (HliA-D) and the expression of all of them is stress-induced. Nonetheless, the HliD seems to be constantly present in cells to play probably a

housekeeping role in chlorophyll synthesis/recycling. This protein forms a pigment-protein complex with chlorophyll-synthase (ChlG) and, via YidC insertase, the ChlG-HliD complex connects chlorophyll biosynthesis with protein translation. Moreover, the HliD promotes docking of the putative alcohol dehydrogenase Ycf39 to the ChlG complex. Under stress conditions the accumulated HliC binds tightly to HliD and, interestingly, the formed HliC-HliD pairs have rather opposite effect than the HliD. They monomerize ChlG and prevent Ycf39 to associate with ChlG but they are also required for the attachment of ChlG to an enigmatic form of monomeric photosystem I. The re-organization of ChlG complex appears to be particularly important during cold stress because *Synechocystis* strain lacking HliC is not viable at 17°C; most likely due to completely inhibited chlorophyll biosynthesis.

### PC7.11 REPAIR AND THE EVOLUTION OF PHOTOSYSTEM II

WEDNESDAY 5 JULY, 2017 11:30

SHENGXI SHAO (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), JIANFENG YU (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), JANA KNOPPOVÁ (INSTITUTE OF MICROBIOLOGY ACADEMY OF SCIENCES, CZECH REPUBLIC), TANAI CARDONA (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), JOSEF KOMENDA (INSTITUTE OF MICROBIOLOGY ACADEMY OF SCIENCES, CZECH REPUBLIC), PETER J NIXON (IMPERIAL COLLEGE LONDON, UNITED KINGDOM)

@ S.SHAO11@IMPERIAL.AC.UK

Despite the limited conservation in primary structure, the close similarity in tertiary structures of the PSI and PSII reaction centres suggests that they have evolved from a common ancestor. In the case of PSI, the PsaA and PsaB reaction centre subunits each consist of 11 transmembrane helices; while in PSII, the D1 and D2 reaction centre subunits, plus the inner antenna CP43 and CP47 subunits, form "5+6" counterparts to PsaA and PsaB. Why nature has selected separate D1/D2 and CP47/CP43 subunits remains unclear. In this work, we have constructed a more "PSI-like" PSII reaction centre by fusing the C-terminus of CP43 to the N-terminus of D1. We show that the resulting CP43-D1 fusion protein is able to form active oxygen-evolving PSII complexes with electron transfer properties similar to normal wild type PSII complexes. However, photoautotrophic growth of the CP43-D1 fusion strain was inhibited at high light irradiances due to an impaired ability to replace the damaged CP43-D1 fusion protein. In a phylogenetic analysis, we showed that FtsH proteases closely related to the repair of PSII diverged at a very early stage during the evolution. Our results therefore provide evidence to support the idea that detachment of CP43 is important for prompt and efficient degradation of damaged D1 by FtsH protease and that the efficient repair of damaged PSII is an important constraint in the evolution of PSII.

## PC7.12 MOLECULAR SWITCHES IN THE THYLAKOID MEMBRANE

📅 WEDNESDAY 5 JULY, 2017 ⌚ 13:50

👤 ROBERTA CROCE (VU UNIVERSITY AMSTERDAM, NETHERLANDS)

@ R.CROCE@VU.NL

Photosynthetic organisms evolved a natural capacity to modulate photosynthetic activity in response to varying light and other environmental conditions. In low light they need to harvest every available photon to sustain life, while in high light they dissipate the energy absorbed in excess to avoid photodamage. Light-Harvesting Complexes (LHCs) are pigment-protein systems responsible for photon absorption and transfer of the excitation energy to the reaction center, where charge separation occurs. It is generally believed that LHCs can change their function from a light harvesting to a photoprotective mode by switching between different conformations. However, the underlying molecular picture has not been elucidated yet. A series of experiments in which we study the molecular mechanisms of quenching in LHCs and their putative triggers will be discussed.

## PC7.13 FUNCTIONAL CHARACTERIZATION OF CURT1A—A MAJOR PLAYER IN THYLAKOID MEMBRANE PLASTICITY

📅 WEDNESDAY 5 JULY, 2017 ⌚ 14:30

👤 ANURAG SHARMA (UNIVERSITY OF COPENHAGEN, DENMARK), OMAR SANDOVAL-IBÁÑEZ (UNIVERSITY OF COPENHAGEN, DENMARK), MATHIAS LABS (LUDWIG-MAXIMILIANS-UNIVERSITÄT, GERMANY), DARIO LEISTER (LUDWIG-MAXIMILIANS-UNIVERSITÄT, GERMANY), MATHIAS PRIBIL (UNIVERSITY OF COPENHAGEN, DENMARK)

@ ASHARMA@PLEN.KU.DK

The thylakoid membrane system of the chloroplast consists of grana, cylindrical discs of membrane stacked over each other, and stroma lamellae, unstacked membranes that interconnect the grana stacks. The formation of the three-dimensional network of thylakoid membranes involves forces that mediate the stacking and bifurcation of membranes, as well as mechanisms that promote bending of the membranes at the margins of the grana stacks. In *Arabidopsis* four Curvature thylakoid1 proteins (CURT1 A-D) were identified which localize to the highly curved region of the thylakoid membrane, the grana margins. CURT1 proteins form high molecular weight oligomeric complexes among themselves and can facilitate membrane bending in a dosage dependent manner. CURT1A, the major isoform of the CURT1 protein family is sufficient to induce thylakoid membrane bending both in planta and in vitro. Structurally CURT1 proteins consist of two transmembrane domains, allowing the insertion into the thylakoid membrane, and N- and C-terminal domains that are exposed to the chloroplast stroma. However, the specific roles of CURT1A domains have not been explored, therefore, I aim to characterize the roles of the N-, C-terminal and transmembrane domains of CURT1A with respect to thylakoid membrane bending and CURT1 complex-formation. We have generated stable lines of *Arabidopsis thaliana* expressing

a variety of mutant CURT1A proteins in *curt1* mutant and wild-type genetic backgrounds. We are using various range of techniques from molecular cell biology to protein biochemistry to unravel the role of the CURT1 domains in shaping the thylakoid ultrastructure.

## PC7.14 ION FLUXES WITH ROLE IN REGULATION OF PMF AND PHOTOSYNTHESIS

📅 WEDNESDAY 5 JULY, 2017 ⌚ 14:45

👤 ANDREI HERDEAN (UNIVERSITY OF GOTHENBURG, SWEDEN), HUGUES NZIENGUI (UNIVERSITY OF GOTHENBURG, SWEDEN), OTILIA CHEREGI (UNIVERSITY OF GOTHENBURG, SWEDEN), CORNELIA SPETEA WIKLUND (UNIVERSITY OF GOTHENBURG, SWEDEN)

@ ANDREI.HERDEAN@OUTLOOK.COM

**Introduction:** In natural habitats, plants frequently experience rapid changes in the intensity of sunlight. In response to these changes, plants adjust photosynthetic light utilization in electron transport and photoprotective mechanisms. This involves a proton motive force (pmf) across the thylakoid membrane, which is affected by ion fluxes ( $H^+$ ,  $K^+$ ,  $Cl^-$ ,  $Mg^{2+}$ ). The identity of the genes involved in  $K^+$  and  $Cl^-$  fluxes have been unraveled in *Arabidopsis* (TPK3, KEA3, CLCe and VCCN1) as well as their effects on regulation of pmf and photosynthesis.

**Methods:** The current knowledge is based on characterization of single knockout mutants of the studied genes in *Arabidopsis*, which often do not display strong phenotypic differences from wild type. To investigate possible functional relationships between  $K^+$  and  $Cl^-$  fluxes, we isolated homozygous double and triple mutant lines from single *Arabidopsis* mutants of KEA3, CLCe and VCCN1 genes.

**Results:** Phenotypic kinetic analyses reveal an interdependent action of the encoded proteins in regulation of photosynthesis upon transition from dark to light (VCCN1), during changes in light intensity (VCCN1, KEA3), and upon transition from light to dark (CLCe).

**Conclusions:** Our findings contribute to understanding the thylakoid network of ion fluxes and how they help plants to adjust photosynthesis in variable light environments.



## PC7.15 OXIDATIVE REGULATION IN PHOTOSYNTHETIC HOMEOSTATIC MECHANISMS

WEDNESDAY 5 JULY, 2017 15:00

AVIHAI DANON (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL), VIVEKANAND TIWARI (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL), BAT CHEN WOLF (WEIZMANN INSTITUTE OF SCIENCE, ISRAEL)

AVIHAI.DANON@WEIZMANN.AC.IL

Homeostasis is a self-regulating process by which organisms tend to maintain stability while adjusting to small changes in conditions that are otherwise optimal for growth. A role for signaling by low-levels of reactive oxygen species (ROS) has been implicated by an increasing number of reports also under growth promoting conditions. Yet, the mechanistic details of ROS perception, transduction and regulation in homeostasis are only beginning to unravel.

Photosynthesis is perhaps the important biology processes in nature converting sunlight to chemical energy. If not regulated properly, photosynthesis side reactions, which produce toxic ROS can turn harmful in a short time period. ROS production by the photosynthetic reactions is a daily phenomenon that occurs even under optimal growth conditions. We identified a pathway of low-level oxidative signal, which attenuates AGPase, the first committed enzyme of starch synthesis, in homeostatic conditions (PNAS 2015, 112:12876). Notably, the attenuating oxidative signal counterbalanced an independent activating reductive signal, and both signals combined to adjust a dynamic equilibrium of AGPase activity to small but abrupt changes in light intensity. Recently, we found that the thylakoid protein PGRL1, a photosynthesis regulator, is also modulated in vivo by an attenuating homeostatic oxidative signal. Notably, the oxidative attenuation of PGRL1 decreases photosynthetic thermal dissipation and, thus, may increase photosynthesis efficiency. While our findings illustrate a beneficial role for homeostatic oxidative signals regulatory mechanisms, a more thorough extension of these studies is required for our general understanding of low oxidative signals transduction.

## PC7.16 HOW CAN SPRUCE NEEDLES BE GREEN IN THE WINTER?

WEDNESDAY 5 JULY, 2017 15:45

STEFAN JANSSON (UPSC DEPT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN)

STEFAN.JANSSON@UMU.SE

Most conifer species are evergreens, and face the challenge of keeping its photosynthetic tissues during the winter, dealing with the combination of low temperature and light. Excited chlorophylls can transfer energy to other molecules, including oxygen and plants have evolved several control systems that allow the photosynthesis machinery to harmlessly dissipate a variable fraction of the excitation energy, and during the winter, needles of many conifers enters a state of sustained quenching. We would like to understand the dissipation mechanism used by evergreen conifers in the winter, and have recently started a project to study the structure and function of the photosynthetic apparatus of conifers (in particular Norway spruce) in the winter.

We have used followed the photosynthetic properties of Norway spruce (and Scots pine) needles over the winter using various kinds of fluorescence studies, to characterize the process. We have sequenced the Norway spruce genome and built genomics and transcriptomics tools, and have subjected over wintering needles to transcript profiling using RNAseq. We have also initiated studies on thylakoid protein complexes (using BN electrophoresis) and thylakoid structure (using electron microscopy) to obtain additional information, and are also developing genome editing tools to be able to manipulate the protein composition of the needles. The data generated so far will be summarized in this talk.

## PC7.17 A NEW PROTOCOL FOR IMPROVED H<sub>2</sub> PHOTOPRODUCTION YIELDS IN C. REINHARDTII

WEDNESDAY 5 JULY, 2017 16:15

YAGUT ALLAHVERDIYEVA (UNIVERSITY OF TURKU, FINLAND), SERGEY KOSOurov (UNIVERSITY OF TURKU, FINLAND)

ALLAHVE@UTU.FI

Some microalgae under microoxic conditions are able to utilize solar energy harnessed by photosynthesis to produce molecular hydrogen (H<sub>2</sub>), which is considered as a clean and environmentally friendly energy carrier. One of the main challenges in H<sub>2</sub> photoproduction by microalgae is oxygen sensitivity of the enzymes involved in H<sub>2</sub> metabolism. Currently, for induction of efficient and sustained H<sub>2</sub> photoproduction in the model green alga *Chlamydomonas reinhardtii* a two-stage protocol is applied. According to this protocol, H<sub>2</sub> production occurs during anaerobiosis induced in the second stage upon nutrient deprivation of the cells. The cells under nutrient (e.g. S- or Mg-) deprivation switch to anaerobic cell metabolism due to (i) reversible down-regulation of photosynthetic O<sub>2</sub> evolution and (ii) stimulation of respiration. We have developed a new protocol for improved H<sub>2</sub> photoproduction yields in *C. reinhardtii* cultures, which does not require severe stress conditions. Efficient H<sub>2</sub> production in green alga occurs under periodic light conditions

and could be sustained for at least two days with the maximum rate of about 12 mmol(mg Chl h)<sup>-1</sup> during the first 8h. The protocol, thus, can be adapted to the natural light-dark cycle. Moreover, novel approaches permitting higher light-to-hydrogen conversion efficiency and efficient H<sub>2</sub> photoproduction in heterocystous N<sub>2</sub>-fixing cyanobacteria will be discussed.

### PC7.18 LIGHT REMODELS THE PHOTOSYNTHETIC APPARATUS AND CARBON PARTITIONING BETWEEN ORGANELLES IN *NANNOCHLOROPSIS GADITANA* LEADING TO SUSTAINED LIPID ACCUMULATION

WEDNESDAY 5 JULY, 2017 16:30

ALESSANDRO ALBORESI (UNIVERSITÀ DI PADOVA, ITALY), GIORGIO PERIN (UNIVERSITÀ DI PADOVA, ITALY), ANDREA MENEHESSE (UNIVERSITÀ DI PADOVA, ITALY), ERIC MARÉCHAL (CNRS-CEA-UNIVERSITÉ GRENOBLE ALPES, FRANCE), NICOLA VITULO (UNIVERSITÀ DI PADOVA, ITALY), GIORGIO VALLE (UNIVERSITÀ DI PADOVA, ITALY), GIANFRANCO DIRETTO (ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES ENERGY AND SUSTAINABLE DEVELOPMENT CASACCIA RESEARCH, ITALY), GIULIANO GIOVANNI (ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES ENERGY AND SUSTAINABLE DEVELOPMENT CASACCIA RESEARCH, ITALY), TOMAS MOROSINOTTO (UNIVERSITÀ DI PADOVA, ITALY)

@ ALESSANDRO.ALBORESI@UNIPD.IT

The seawater oleaginous microalga *Nannochloropsis gaditana* has the ability to accumulate large amounts of lipids and it is raising as a model organism for the production of next generation biofuels. The study of metabolism regulation is an essential step toward the optimization of algae productivity.

In order to decipher light-driven changes, we performed a combined analysis of transcriptome, lipidome and metabolome of *Nannochloropsis gaditana* cultures exposed for five days to three different light intensities: low (LL), medium (ML) or high light (HL). The growth of LL cells was limited by light availability while HL algae were impaired because of the toxic effect of the excess light. Clustering analysis of RNA-seq data revealed that light induced significantly the up-regulation of reductive tricarboxylic acid cycle and monosaccharide biosynthetic process and the down-regulation of genes involved in DNA integration, chlorophyll and carotenoid biosynthetic process, unsaturated fatty acid biosynthetic process and photosynthesis/light harvesting.

Coherently with transcriptomic data, we measured a generalized reduction of *Nannochloropsis gaditana* photosynthetic apparatus in HL and also chloroplast polar lipids were decreased. Moreover, strong illumination stimulated the biosynthesis of carbohydrates and lipids but also compounds associated to oxidative stress response. This situation correlated with the induction of genes coding for a putative cytosolic fatty acid synthase of type 1 (FAS1) and polyketide synthase (PKS) and the down-regulation of the chloroplast fatty acid synthase of type 2 (FAS2). These results highlight the high flexibility of lipid biosynthesis in *Nannochloropsis gaditana* and the importance of metabolite allocation at the subcellular level.

### PC7.19 SPECIES- AND STRAIN-SPECIFIC STRATEGIES OF MICROALGAL STRAINS (DESMIDS, GENUS *COSMARIUM*, ZYGNEMATOPHYCEAE, STREPTOPHYTA) AS PROTECTION AGAINST EXCESSIVE PHOTOSYNTHETICALLY ACTIVE RADIATION

WEDNESDAY 5 JULY, 2017 16:45

MARIJA STAMENKOVIC (INSTITUTE FOR BIOLOGICAL RESEARCH "SINIŠA STANKOVIĆ", SERBIA AND MONTENEGRO), KAI BISCHOF (MARINE BOTANY FACULTY BIOLOGY/CHEMISTRY, GERMANY), DIETER HANELT (BIOZENTRUM KLEIN FLOTTBEK, GERMANY)

@ MARIE110@GMAIL.COM

We examined the influence of irradiance on protection strategies of four microalgal strain belonging to the genus *Cosmarium* (Zygnematophyceae, Streptophyta), which were isolated from various climatic zones and cultured long term (>15 years). The photosynthetic behaviour and composition of photosynthetic pigments of the *Cosmarium* strains were examined under low, moderate and photoinhibitory white light by means of PAM fluorometry, and high-performance liquid chromatography. Generally, the *Cosmarium* strains displayed the photosynthetic performance and pigment composition corresponding to that of high-light adapted plants and algae. Yet, all the *Cosmarium* strains demonstrated physiological responses that were consistent with the light intensity prevailing at their source location as concluded from chlorophyll fluorescence and changes of pigment composition, confirming that these responses are genetically preserved. Addition of inhibitors of chloroplast-encoded protein synthesis (chloramphenicol and streptomycin) and violaxanthin de-epoxidase (dithiothreitol) indicated that the *Cosmarium* strains developed "sun- or shade-plant" protection strategies, in accordance with the climate at their sampling sites. Strikingly, the typical arctic taxon, *C. crenatum* var. *boldtianum*, displayed the incomplete violaxanthin cycle yielding an accumulation of antheraxanthin during high light stress, which is considered as an adaptation to occasional high irradiances in the polar zone due to the albedo. Antheraxanthin actively participated in the heat dissipation from PSII centres in *C. crenatum*, as concluded from a significant positive correlation between non-photochemical quenching and the quantity of antheraxanthin. The species- and strain-specific ecophysiological adaptations discussed in this study may enable desmids to cope with excessive and fluctuating light conditions in their environment.

## PC7.20 REGULATION OF PROTON MOTIVE FORCE BY ALTERNATIVE ELECTRON TRANSPORT

THURSDAY 6 JULY, 2017 09:00

TOSHIHARU SHIKANAI (KYOTO UNIVERSITY, JAPAN)

SHIKANAI@PMG.BOT.KYOTO-U.AC.JP

In the light reactions of photosynthesis, electron transport from water to NADP<sup>+</sup> is coupled with proton translocation across the thylakoid membrane. Resulting proton motive force (*pmf*) contributes to ATP synthesis. This linear electron transport does not satisfy the ATP/NADPH production ratio required by the Calvin-Benson cycle. Additional ATP synthesis is supplemented by alternative electron transport including cyclic electron transport around PSI. In *Arabidopsis*, PSI cyclic electron transport consists of PGR5/PGRL1-dependent and chloroplast NDH-dependent pathways. The chloroplast NDH complex mediates ferredoxin-dependent plastoquinone reduction, likely coupled with proton translocation through the complex. The NDH complex is associated with two copies of the PSI supercomplex via linker proteins, Lhca5 and Lhca6. In addition to proton concentration gradient, membrane potential contributes to *pmf*. In addition to ATP synthesis, luminal acidification also down-regulates electron transport by inducing thermal dissipation of absorbed light energy from PSII and down-regulation of electron transport through the Cytochrome *b<sub>6</sub>f* complex. To optimize photosynthesis under fluctuating light, the compartments of *pmf* factors need to be regulated by controlling iron movement across the thylakoid membrane.

## PC7.21 THE INTERACTION BETWEEN PHOTOSYNTHETIC ELECTRON TRANSPORT AND CHLOROPLAST ELECTRON CONSUMPTION; PROTECTION AND SIGNALLING FOR PLANT HEALTH AND PRODUCTIVITY

THURSDAY 6 JULY, 2017 09:40

PETER J GOLLAN (UNIVERSITY OF TURKU, FINLAND), YUGO LIMA-MELO (FEDERAL UNIVERSITY OF CEARÁ, BRAZIL), MIKKO TIKKANEN (UNIVERSITY OF TURKU, FINLAND), EVA-MARI ARO (UNIVERSITY OF TURKU, FINLAND)

PETGOL@UTU.FI

The photosynthetic light reactions provide energy that is consumed and stored in electron sinks, the products of photosynthesis. A balance between light reactions and electron consumption in the chloroplast is vital for plants, and is protected by several photosynthetic regulation mechanisms. Photosystem I is particularly susceptible to photoinhibition when these factors become unbalanced, which can occur in low temperatures or in high light. In this study we used the *pgr5* *Arabidopsis* mutant that lacks' pH-dependent regulation of photosynthetic electron transport as a model to study the consequences of PSI photoinhibition under high light. We found that PSI damage severely inhibits carbon fixation and starch accumulation, and attenuates enzymatic oxylipin synthesis

and chloroplast regulation of nuclear gene expression after high light stress. This work shows that modifications to regulation of photosynthetic light reactions, which may be designed to improve yield in crop plants, can negatively impact metabolism and signalling, and thereby threaten plant growth and stress tolerance.

## PC7.22 EVOLUTION OF PHOTOSYNTHESIS REGULATION: LESSONS FROM THE MOSS *PHYSCOMITRELLA PATENS*

THURSDAY 6 JULY, 2017 09:55

CATERINA GEROTTO (DEPT. OF BIOCHEMISTRY MOLECULAR PLANT BIOLOGY UNIVERSITY OF TURKU, FINLAND), ALESSANDRO ALBORESI (DEPT. OF BIOLOGY UNIVERSITY OF PADOVA, ITALY), ANDREA MENEGHESSE (DEPT. OF BIOLOGY UNIVERSITY OF PADOVA, ITALY), MARTINA JOKEL (DEPT OF BIOCHEMISTRY MOLECULAR PLANT BIOLOGY UNIVERSITY OF TURKU, FINLAND), MARJAANA SUORSA (DEPT OF BIOCHEMISTRY MOLECULAR PLANT BIOLOGY UNIVERSITY OF TURKU, FINLAND), EVA-MARI ARO (DEPT OF BIOCHEMISTRY MOLECULAR PLANT BIOLOGY UNIVERSITY OF TURKU, FINLAND), TOMAS MOROSINOTTO (DEPT. OF BIOLOGY UNIVERSITY OF PADOVA, ITALY)

CATERINA.GEROTTO@UTU.FI

Sun light provides energy supporting life of photosynthetic organisms but when in excess it can also lead to the formation of harmful reactive oxygen species and cell damage. Natural environments are characterized by highly variable light conditions, therefore the flow of excitation energy and electrons in the photosynthetic apparatus needs to be continuously modulated. Photosynthetic organisms evolved several photoprotective processes to respond to the dynamics of the environment. Intriguingly, studies on model species from cyanobacteria, algae and flowering plants showed that changes in the proteins involved in those important protection mechanisms have taken place during evolution. Flavodiiron (FLV) proteins are seminal components of this regulatory machinery in cyanobacteria. FLVs were lost during evolution by flowering plants but are still present in non-vascular plants, as the moss *Physcomitrella patens*. To gain insights on the evolution of photosynthesis regulatory processes during the key step of land colonization, we generated *P. patens* mutants depleted in FLV proteins exploiting *P. patens* ability to perform homologous recombination in the nuclear genome. *flv* knock-out (KO) mutants phenotype demonstrated FLVs function as an electron sink downstream of Photosystem I after any increase in irradiation intensity. *flv* KO plants also showed impaired growth and strong PSI photo-inhibition when exposed to fluctuating light, demonstrating FLV biological role as a safety valve from excess electrons in dynamic light. The lack of FLVs was partially compensated by an increased cyclic electron flow, suggesting that in flowering plants FLVs biological role was likely taken by other alternative electron routes.

### PC7.23 THE ENERGY BUDGET IN C<sub>4</sub> PHOTOSYNTHESIS: QUANTITATIVE INSIGHTS FROM AN ANALYTICAL MODEL OF CELL-TYPE SPECIFIC LINEAR AND CYCLIC ELECTRON TRANSPORT

THURSDAY 6 JULY, 2017 10:10

XINYOU YIN (WAGENINGEN UNIVERSITY, NETHERLANDS),  
PAUL C STRUIK (WAGENINGEN UNIVERSITY, NETHERLANDS)

@ XINYOU.YIN@WUR.NL

The operation of CO<sub>2</sub> concentrating mechanism (CCM) in C<sub>4</sub> photosynthesis from the coordinated functioning of mesophyll (M) and bundle-sheath (BS) cells requires ATP and probably NADPH. This extra ATP most likely comes from cyclic electron transport around photosystem I (CET) because linear electron transport (LET) produces a correct ATP:NADPH ratio only for the Calvin cycle. As relative metabolic ATP:NADPH requirements in M and BS cells differ among C<sub>4</sub> subtypes, the subtypes may differ in the extent to which CET operates in these cells. We report an analytical model for calculating cell-type specific NADPH and ATP production in C<sub>4</sub> photosynthesis. This model was then used to quantify the energy balance by matching modelled cell-type specific NADPH and ATP production with the metabolic requirements. Our analysis supports that a mixed decarboxylation is needed to achieve a balanced NADPH and ATP budget in both M and BS cells. In a typical NADP-ME subtype, ca 25% of C<sub>4</sub> acids are converted into aspartate for transport to BS cells. In a typical NAD-ME subtype, ca 25% of C<sub>4</sub> acids follow a secondary decarboxylation by phosphoenolpyruvate-carboxykinase (PEP-CK), in a facultative manner. For both the ME subtypes, ca 50% of total electron flux is CET, whereas CET requirement is negligible in the standard PEP-CK subtype. As a consequence, photosynthetic quantum yield is theoretically higher in types involving PEP-CK. These results have implications for engineering to bring C<sub>4</sub> mechanisms into C<sub>3</sub> crops, as low intrinsic quantum yields of C<sub>4</sub> crop plants are the major constraint to canopy productivity.

### PC7.24 BIOCHEMICAL CHARACTERIZATION AND PHYSIOLOGICAL ROLE OF THE PLASTID TERMINAL OXIDASE PTOX

THURSDAY 6 JULY, 2017 10:55

ANJA KRIEGER (CEA SACLAY, FRANCE)

@ ANJA.KRIEGER-LISZKAY@CEA.FR

Protein levels of the plastid terminal oxidase PTOX increase upon abiotic stress. PTOX may protect the photosynthetic apparatus when electron transport is limited. However, overexpression of PTOX in *Nicotiana tabacum*, increased the production of reactive oxygen species and exacerbated photoinhibition.

The active site of PTOX comprises a non-heme diiron centre that catalyses the oxidation of plastoquinol and the reduction of O<sub>2</sub> to H<sub>2</sub>O. We have performed for the first time a biochemical characterization of purified PTOX. The activity of PTOX was determined to be much higher than what had been previously

estimated from chlorophyll fluorescence. The main reaction of PTOX is the reduction of O<sub>2</sub> to H<sub>2</sub>O but PTOX generates O<sub>2</sub><sup>•-</sup> in a side reaction in a substrate- and pH-dependent manner. PTOX activity *in vivo* was investigated using tobacco and *Synechocystis* PCC 6803 overexpressing PTOX. PTOX competed efficiently with photosynthetic electron flow. In tobacco high CO<sub>2</sub> concentrations inactivated PTOX most likely because of an acidification of the stroma. Immunoblots showed that PTOX detached from the membrane in dark-adapted leaves or in the presence of uncouplers. A model is proposed in which the membrane association of PTOX is controlled by stromal pH. When the stromal pH is neutral, PTOX exists as a soluble form and is enzymatically inactive avoiding the interference of PTOX with linear electron flow. When the stromal pH is alkaline and the photosynthetic electron chain is highly reduced under stress conditions, PTOX binds to the membrane, has access to its substrate and can serve as safety valve.

### PC7.25 CHLOROPLAST THIOREDOXIN SYSTEMS IN THE REGULATION OF LIGHT AND CARBON ASSIMILATION REACTIONS – PROSPECTS FOR IMPROVING PHOTOSYNTHESIS

THURSDAY 6 JULY, 2017 11:25

EEVI RINTAMÄKI (UNIVERSITY OF TURKU, FINLAND),  
LAURI NIKKANEN (UNIVERSITY OF TURKU, FINLAND),  
JOUNI TOIVOLA (UNIVERSITY OF TURKU, FINLAND),  
MANUEL GUINEA DIAZ (UNIVERSITY OF TURKU, FINLAND)

@ EVIRIN@UTU.FI

Thioredoxins (TRXs) are protein oxidoreductases that control the structure and function of cellular proteins by cleavage of a disulphide bond between the side chains of two cysteine residues. Oxidized thioredoxins are reactivated by thioredoxin reductases (TR) and a TR-dependent reduction of TRXs is called a thioredoxin system. Of the two plant plastid thioredoxin systems the ferredoxin-dependent system relays reducing equivalents from photosystem I via ferredoxin and ferredoxin-thioredoxin reductase (FTR) to chloroplast proteins, while NADPH-dependent thioredoxin reductase (NTRC) forms a complete thioredoxin system including both reductase and thioredoxin domains in a single polypeptide. Based on photosynthetic characterization of Arabidopsis plants overexpressing or lacking a functional NTRC we have shown that NTRC system participates in regulation of primary photosynthetic reactions and is particularly important in the dark/light transitions and under conditions where light limits photosynthesis. We have demonstrated an improvement of leaf photosynthetic activity by an elevated chloroplast thiol-redox state through NTRC overexpression, especially at low light intensities. Elevation of photosynthetic activity is based on (i) increase in the quantum yield of CO<sub>2</sub> fixation, (ii) decrease in the acceptor site limitation of PSI under low light and (iii) reduced the need to dissipate extra energy via non-photochemical quenching. (iv) Increased chloroplast thiol-redox state also promoted the activity of the chloroplast antioxidant system independently of light conditions. Our results highlight the importance of the regulatory mechanisms of photosynthesis and render chloroplast TRX systems as tempting targets for future genetic engineering projects aiming at enhancement of photosynthesis in crop plants.

## PC7.26 THE TRIPLE-EDGED SWORD OF THE THYLAKOID PROTON MOTIVE FORCE: ENERGY, PROTOPROTECTION AND PHOTODAMAGE

THURSDAY 6 JULY, 2017 13:50

DAVID M KRAMER (MICHIGAN STATE UNIVERSITY, UNITED STATES)

@ KRAMERD8@MSU.EDU

The talk will describe how new enabling technologies (including [www.photosynq.org](http://www.photosynq.org) and the dynamic environmental phenotype imager (DEPI)) to bridge the gaps between the lab and the field, and thus allow exploration of photosynthetic processes under real world conditions. The results thus far identify important limitations and tradeoffs to photosynthesis involving the interactions of the electron transfer reactions with the thylakoid proton motive force ( $pmf$ ) and its storage in the electric field ( $\Delta\psi$ ) and  $\Delta pH$  components. At one extreme, making the ATP synthase too fast does not increase photosynthetic rates, but instead inhibits the buildup of  $\Delta pH$  that normally governs light capture and electron flow, allowing electrons to accumulate on photosystem I, leading to photodamage. At the other extreme, under fluctuating light, excessive  $\Delta\psi$  destabilizes charge separated states in the photosynthetic reaction centers, leading to recombination and the production of toxic  $^1O_2$ , a process we call "Field Recombination Induced Photodamage" (FRIP). I will present data from the that these processes represent substantial limitations to photosynthesis under field conditions, and likely drove the evolution of a large number of "ancillary regulatory components" of photosynthesis that modulate  $\Delta\psi$  and  $\Delta pH$  to fine tune regulatory balance in response to environmental conditions. Finally, I will discuss the prospects and caveats of using this basic knowledge and new phenotyping tools to improve the breeding and management of crops.

## PC7.27 EFFECT OF GROWTH IRRADIANCE AND LEAF AGE ON PHOTOSYNTHETIC PARAMETERS

THURSDAY 6 JULY, 2017 14:30

MOUALEU NGANGUE DANY PASCAL (LEIBNIZ UNIVERSITÄT HANNOVER, GERMANY)

@ MOUALEU@GEM.UNI-HANNOVER.DE

The photosynthetic acclimation is a complex process evolving changes in different leaf traits that might lead to changes in the leaf gross photosynthetic rate ( $P_{cmax}$ ). The acclimation process of  $P_{cmax}$  was found to be dynamical, and influenced by current growing conditions such as growing irradiance as well as the amount of nitrogen available for the leaf. These factors can vary greatly within a crop canopy.

A model combining the dynamics of the maximum carboxylation rate ( $V_{cmax}$ ), potential maximum electron transport rate ( $J$ ), mesophyll conductance ( $g_m$ ), daytime respiration rate ( $R_d$ ), apparent quantum yield ( $Q_y$ ) and the triose-phosphate utilization rate into (TPU) into the biochemical photosynthetic model to described dynamically the change in the photosynthetic capacity as result of growing irradiance and thermal time was developed and presented.

Statistically significant growing irradiance and thermal time effects, as well as interaction between these two factors were found for the gross photosynthesis rate under saturated light condition ( $P_{max}$ ),  $V_{cmax}$ , TPU,  $J$ ,  $Q_y$ , and  $R_d$ . No thermal time effect was found on the proportion of PPFD harvested by Photosystem II, and on the stomatal conductance to water vapour. The model allowed a quantification of down regulation of photosynthesis in response to change in growing irradiance and thermal time.

## PC7.28 COMPARATIVE ANALYSIS OF MUTANT PLANTS IMPAIRED IN THE MAIN REGULATORY MECHANISMS OF PHOTOSYNTHETIC LIGHT REACTIONS - FROM BIOPHYSICAL MEASUREMENTS TO MOLECULAR MECHANISMS

THURSDAY 6 JULY, 2017 14:45

MIKKO TIKKANEN (MOLECULAR PLANT BIOLOGY DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF TURKU, FINLAND), SANNA RANTALA (MOLECULAR PLANT BIOLOGY DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF TURKU, FINLAND), MICHELE GRIECO (MOLECULAR PLANT BIOLOGY DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF TURKU, FINLAND), EVA-MARI ARO (MOLECULAR PLANT BIOLOGY DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF TURKU, FINLAND)

@ MISATI@UTU.FI

Chlorophyll fluorescence emission by photosystem II (PSII) and light absorption by P700 reaction center chlorophyll a of photosystem I (PSI) provide easy means to probe the function of the photosynthetic machinery. The exact relationship between the measured optical variables and the molecular processes have, however, remained elusive. Today, the availability of mutants with distinct molecular characterization of photosynthesis regulatory processes should make it possible to gain further insights into this relationship, yet a systematic comparative analysis of such regulatory mutants has been missing. Here we have systematically compared the behavior of Dual-PAM fluorescence and P700 variables from well-characterized photosynthesis regulation mutants. The analysis revealed a very convincing relationship between the given molecular deficiency in the photosynthetic apparatus and the original fluorescence and P700 signals obtained by using varying intensities of actinic light and by applying a saturating pulse. Importantly, the specific information on the underlying molecular mechanism, present in these authentic signals of a given photosynthesis mutant, was largely nullified when using the commonly accepted parameters that are based on further treatment of the original signals. Understanding the unique relationship between the investigated molecular process of photosynthesis and the measured variable is an absolute prerequisite for comprehensive interpretation of fluorescence and P700 measurements. The data presented here elucidates the relationships between the main regulatory mechanisms controlling the photosynthetic light reactions and the variables obtained by fluorescence and P700 measurements. It is discussed how the full potential of optical photosynthesis measurements can be utilized in investigation of a given molecular mechanism.

## PC7.29 THE ROLE SnRK2 KINASES IN REGULATION OF PLANT RESPONSE TO LONG TERM SALT STRESS

📅 THURSDAY 6 JULY, 2017 ⌚ 15:00

👤 ANNA KULIK (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND), RADOSLAW MAZUR (FACULTY OF BIOLOGY UNIVERSITY OF WARSAW, POLAND), OLGA SZTATELMAN (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND), MACIEJ GARSTKA (FACULTY OF BIOLOGY UNIVERSITY OF WARSAW, POLAND), GRAZYNA DOBROWOLSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS POLISH ACADEMY OF SCIENCES, POLAND)

@ ANJA@IBB.WAW.PL

Salinity and water deficit are among the most commonly occurring stress factors for plants all over the globe. Both of them cause considerable disturbance of photosynthesis, which is the source of energy for cellular processes and metabolism in plants. Disorders in photosynthesis lead to immediate reduction of plant growth, development and reproduction. Because the mechanisms which regulate photosynthesis under salinity are still not fully described, the aim of our research was to identify pathways and signaling proteins involved in regulation of this process. One of proteins of our interest were kinases belonging to the SnRK2 family (Sucrose non-fermenting-1 Related Kinases 2) from group 1 (not activated by abscisic acid), which are known as signaling proteins regulating plant response to osmotic stress. Our study revealed that two kinases belonging to this group, SnRK2.4 and SnRK2.10, are activated within minutes in leaves of hydroponically grown *Arabidopsis thaliana* plants in response to treatment with NaCl. In plants treated with salt for few days, we identified several photosynthesis efficiency parameters, photosynthesis-related proteins, and genes regulated in SnRK2's-dependent manner. The insertion mutants *snrk2.4* and *snrk2.10* showed reduced dry mass in comparison to wild type plants when grown under salt stress conditions. Summing up, we demonstrate a role of ABA-not activated SnRK2s in regulation of photosynthesis, plant metabolism and growth efficiency under salt stress.

## PC7.30 DEG PROTEASES - SURVIVAL AT ABIOTIC STRESS

📅 THURSDAY 6 JULY, 2017 ⌚ 15:45

👤 CHRISTIANE FUNK (UMEÅ UNIVERSITY, SWEDEN), RAIK WAGNER (UMEÅ UNIVERSITY, SWEDEN), OTILIA CHEREGI (UMEÅ UNIVERSITY, SWEDEN)

@ CHRISTIANE.FUNK@UMU.SE

In natural environment photosynthetic organisms are exposed to tremendous changes in light intensities and temperatures. To cope with this stress they contain a variety of chaperones and proteases that monitor proper folding and function of proteins. Particularly interesting candidates are enzymes of the multifunctional Deg family of ATP-independent serine endopeptidases. The three Deg proteases of the cyanobacterium *Synechocystis* sp. PCC 6803, called HtrA (high temperature requirement A), HhoA and HhoB (HtrA homologues A and B, respectively), are important for survival under high light and temperature stresses.

We investigated the function of these three Deg proteases comparing wild type cells with mutants containing single or multiple deg-deletions. Proteomic and metabolomic studies revealed hypothetical substrates and unique functions of the three Deg proteases. Protein expression as well as protease activity differs between the three proteases with HhoA being most active. Cells lacking all three Deg proteases are impaired in the outer cell layers, which might reduce the protection of the mutant cells and explain their higher sensitivity toward light and oxidative stress. Furthermore, expression of putative secreted proteins is affected in this strain.

### PC7.31 HOW TO DEAL WITH HEAT - PROTECTIVE MECHANISMS OF HEAT ACCLIMATION IN ARABIDOPSIS REVEALED THROUGH TRANSCRIPTOME ANALYSIS

THURSDAY 6 JULY, 2017 16:15

GE GAO (KING ABDULLAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, SAUDI ARABIA), YONG WOO (KAUST, SAUDI ARABIA), CHRIS GEHRING (KAUST, SAUDI ARABIA), MARK TESTER (KAUST, SAUDI ARABIA)

GE.GAO@KAUST.EDU.SA

Heat stress has a profound impact on many cellular functions and metabolic processes in plants, it adversely affects plants' photosynthesis, impairs the translocation of assimilates, and reduce carbon gain, leading to altered growth and reproduction. On the other hand, plants exhibit heat acclimation, where pre-exposure to increased temperatures ensures higher survival in otherwise lethal heat stress conditions. To investigate the molecular processes underlying heat acclimation, we examined changes in Arabidopsis transcriptome throughout the acclimation period and heat shock treatment. We observed significant up-regulation of genes involved in processes important for heat tolerance, such as protein folding and response to oxidative stress, as well as down-regulation of phosphorylation, membrane signaling, and cell cycle. Next, we compared the heat shock response of acclimated to the unacclimated plants to identify gene clusters showing 'transcriptional memory' of heat acclimation. The acclimation enhanced expression of genes involved in flavonoid biosynthesis, lipid transport and repressed transcription of photosynthesis and cell death related genes. Finally, we identified a gene module with reduced expression during heat shock that was enriched for genes targeted to the chloroplast. Interestingly, those genes were co-regulated similarly in Arabidopsis, Oryza, and Chlamydomonas. The chloroplast-targeted genes were also negatively correlated with cell death suppressor genes in all of the three species, suggesting they are part of a transcriptional network that has remained conserved across various species since radiation of plant species. The gene clusters and transcriptional responses observed in our study will provide a framework for future detailed genetic studies of heat acclimation.

### PC7.33 DESPITE PHYLOGENETIC EFFECTS, C<sub>3</sub>-C<sub>4</sub> LINEAGES BRIDGE THE ECOLOGICAL GAP TO C<sub>4</sub> PHOTOSYNTHESIS

THURSDAY 6 JULY, 2017 16:45

MARJORIE R LUNDGREN (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), PASCAL-ANTOINE CHRISTIN (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

MARJORIE.LUNDGREN@SHEFFIELD.AC.UK

C<sub>4</sub> photosynthesis is a physiological innovation involving several anatomical and biochemical components that emerged recurrently in flowering plants. This complex trait evolved via a series of physiological intermediates, broadly termed "C<sub>3</sub>-C<sub>4</sub>", which have been widely studied to understand C<sub>4</sub> origins. While this research program focused on biochemistry, physiology, and anatomy, the ecology of these intermediates remains largely unexplored. Here, we use global occurrence data and local habitat descriptions to characterize the niche of multiple C<sub>3</sub>-C<sub>4</sub> lineages, as well as their close C<sub>3</sub> and C<sub>4</sub> relatives. While C<sub>3</sub>-C<sub>4</sub> taxa tend to occur in warm climates, their abiotic niches are spread along other dimensions, making it impossible to define a universal C<sub>3</sub>-C<sub>4</sub> niche. Phylogeny-based comparisons suggest that, despite shifts associated with photosynthetic types, the precipitation component of the C<sub>3</sub>-C<sub>4</sub> niche is particularly lineage specific, being highly correlated with that of closely related C<sub>3</sub> and C<sub>4</sub> taxa. Our large-scale analyses suggest that C<sub>3</sub>-C<sub>4</sub> lineages converged toward warm habitats, which may have facilitated the transition to C<sub>4</sub> photosynthesis, effectively bridging the ecological gap between C<sub>3</sub> and C<sub>4</sub> plants. The intermediates retained some precipitation aspects of their C<sub>3</sub> ancestor's habitat, and likely transmitted them to their C<sub>4</sub> descendants, contributing to the diversity among C<sub>4</sub> lineages seen today.

## PC7.4 APPLYING SMALL-ANGLE SCATTERING METHODS TO INVESTIGATE THYLAKOID MEMBRANES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DAINIUS JAKUBAUSKAS (NIELS BOHR INSTITUTE UNIVERSITY OF COPENHAGEN, DENMARK), POUL ERIK JENSEN (COPENHAGEN PLANT SCIENCE CENTER DEPT. OF PLANT AND ENVIRONMENTAL SCIENCES UNIVERSITY OF COPENHAGEN, DENMARK), KELL MORTENSEN (NIELS BOHR INSTITUTE UNIVERSITY OF COPENHAGEN, DENMARK), JACOB KIRKENSGAARD (NIELS BOHR INSTITUTE UNIVERSITY OF COPENHAGEN, DENMARK)

@ DAINIUS@NBI.KU.DK

Small-angle scattering is a non-invasive technique which provides structural information of the nanometer length scale. Although this technique is extensively used in structural biophysics to investigate lipid-protein systems in solutions, its application in plant membrane biology is extremely limited due to inherent system complexity and lack of mathematical apparatus. Using small-angle scattering techniques we investigated plant and cyanobacterial species with various degrees of thylakoid stacking and membrane bending. We show that scattering data recorded from measurements *in vivo* correlate with the thylakoid membrane stacking - highly stacked membranes yield pronounced and periodic scattering patterns. Furthermore, we are able to follow thylakoid membrane dynamics *in vivo* during illumination and heat treatments. By correlating data from small-angle scattering and electron microscopy for the same sample, our study provides a biologically-relevant mathematical model of thylakoid ultrastructure explaining all the observed scattering peaks. Applying such mathematical models will lay the foundation for using small-angle scattering as a quantitative structural tool for *in vivo* studies of plant membranes.

## PC7.5 PHOTOSYNTHETIC COMPLEX ADJUSTMENTS IN TOBACCO PSI MUTANTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

SONJA V BERGNER (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MARK A SCHÖTTLER (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), WOLFRAM THIELE (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), RALPH BOCK (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

@ BERGNER@MPIMP-GOLM.MPG.DE

The photosynthetic complexes of the thylakoid membrane are well characterized in terms of their structure and properties. However, a deeper understanding of how these complexes are dynamically adjusted to optimize light harvesting and excitation energy transfer efficiency is still missing. In particular, the PSI contents of higher plants have been found to be relatively stable throughout adjustments in assimilation capacity in response to changes in metabolic state and environmental cues. We studied the consequences of reduced/subunit-altered PSI on protein supercomplex accumulation and discuss our results with regard to redox-regulated changes in the antenna cross.

## PC7.6 ROLE OF PLASTID TERMINAL OXIDASE (PTOX) IN HEAT TEMPERATURE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MARIELA P AGUILERA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GILES N JOHNSON (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ MARIELA.AGUILERAMIRANDA@POSTGRAD.MANCHESTER.AC.UK

Plastid Terminal Oxidase (PTOX) is a nuclear encoded protein, located on the stromal side of the thylakoid. PTOX has been reported to play a role in some higher plants, acting as an alternative electron sink, resulting in a protection of the plastoquinone pool from over-reduction in a variety of environmental stress conditions. This is due to the ability of this enzyme to transport electrons from plastoquinol to molecular oxygen, generating water.

We are examining the ability *Hordeum vulgare* (Cassata variety), to adjust to different environmental conditions, in particular a range of temperatures, focusing on the role of plastid terminal oxidase (PTOX) acting as a safety valve for photosynthesis. Using physiological techniques to estimate photosynthetic parameters (gas exchange, chlorophyll fluorescence, and absorption spectroscopy), chlorophyll content and the amount of protein through western blot technique.

Measurements of electron transport rate (ETR) of Photosystem II (PSII) show sensitivity to the concentration of oxygen in *H. vulgare*, above 20°C, suggesting PTOX activity in response to heat stress. This is supported by the reduction in PSII ETR after infiltrating leaves with Propyl gallate (PTOX inhibitor).

Response of PTOX to different environmental conditions is being explored using q-PCR and protein expression analysis.



## PC7.7 PHYSIOLOGICAL INTEGRATION AND INDUCIBLE CAM AS POSSIBLE DROUGHT TOLERANCE MECHANISMS IN THE CLONAL INVASIVE PLANT, CARPOBROTUS EDULIS (ICE PLANT)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

PETER T BRAUN (CALIFORNIA STATE UNIVERSITY SAN BERNARDINO, UNITED STATES), JOHN B SKILLMAN (CALIFORNIA STATE UNIVERSITY SAN BERNARDINO, UNITED STATES)

@ BRAUP300@COYOTE.CSUSB.EDU

Outside of its native South Africa, *Carpobrotus edulis*, a stoloniferous succulent plant, is highly invasive in Mediterranean climate ecosystems. Physiological integration of connected *C. edulis* ramets allows water sharing among ramets in different hydrological microsites. Droughted *C. edulis* can switch from C<sub>3</sub> photosynthesis to crassulacean acid metabolism (CAM), presumably improving plant water conservation. We were interested in whether water savings through inducible CAM, and water sharing through physiological integration, were interactive or independent drought tolerance mechanisms in this important invasive species. Both stolon-connected and stolon-severed paired ramets were grown for four weeks in paired pots where in one ramet in each pair was watered daily and the other ramet in each pair was continuously droughted. Watered ramets, whether connected or severed, maintained high rates of C<sub>3</sub> photosynthesis and shoot growth throughout the study. Shoot growth stopped in the connected-droughted ramets but leaf-level physiology in these plants was indistinguishable from that of ramets in either of the regularly watered treatments. Severed-droughted ramets showed drought-stress symptoms at the leaf (e.g. reduced photosynthesis) and whole plant (e.g. growth cessation) levels of organization. CAM induction was not observed in any treatment. These findings indicate that water-sharing via physiological integration operates independent of inducible CAM in *C. edulis* and serves as the primary drought tolerance mechanism for this invasive species. Whether or not water-saving via inducible CAM plays a role in *C. edulis* drought tolerance under more severe conditions remains unknown.

## PC7.8 INDUCIBLE RNAi REPRESSION OF GALACTOLIPID BIOSYNTHESIS IN TOBACCO REVEALS A STRICT COORDINATION OF THYLAKOID MEMBRANE CONSTITUENT ACCUMULATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MONIKA SUCHOSZEK (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MARTA HOJKA (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), WERONIKA GAJZIK (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), ELISA SCHULZ (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MOHAMED ABD ALLAH SALEM (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), PATRICK GIAVALISCO (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), EUGENIA MAXIMOVA (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MARK AUREL SCHÖTTLER (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

@ SUCHOSZEK@MPIMP-GOLM.MPG.DE

To examine the impact of galactolipid deficiency on the photosynthetic apparatus in tobacco, we have used an ethanol-inducible RNAi approach against two key enzymes of galactolipid biosynthesis in the chloroplast, MGD1 and DGD1. Using this approach, it is possible to track changes in lipid composition and photosynthesis at different time points after RNAi induction. Our studies revealed very similar changes in both MGDG- and DGDG-deficient mutants. While no changes of photosynthetic parameters and only minor changes in lipid content were observed in mature leaves of transgenic lines, which had fully established their photosynthetic apparatus prior to RNAi induction, drastic differences occurred in newly developing leaves after induction. Total chlorophyll content per leaf area and the accumulation of all photosynthetic complexes were reduced by more than 50%, relative to the wild type. Because of the reduced demand for membrane lipids in young leaves, fatty acids were repartitioned into storage lipids, as shown by the accumulation of triacylglycerols and the appearance of lipid droplets in the cytosol of the mutants. Our data indicate that both investigated galactolipids mainly serve as structural lipids since changes in photosynthetic parameters were mostly due to proportional reductions of all photosynthetic constituents. In response to restricted synthesis of a specific galactolipid species, thylakoid biogenesis is precisely readjusted to keep the proper stoichiometry and functionality of photosynthetic apparatus.

## PC7.9 ASSESSING DROUGHT TOLERANCE OF SUNFLOWER INBRED LINES BY PSII PHOTOCHEMICAL EFFICIENCY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• NURAN ÇIÇEK (HACETTEPE UNIVERSITY, TURKEY), VELİ PEKCAN (TRAKYA AGRICULTURAL RESEARCH INSTITUTE, TURKEY), ÖZLEM ARSLAN (GİRESUN UNIVERSITY, TURKEY), ŞEKÜRE ÇULHA ERDAL (HACETTEPE UNIVERSITY, TURKEY), AYŞE SUNA BALKAN NALÇAYI (HACETTEPE UNIVERSITY, TURKEY), AYŞE NURAN ÇİL (ÇUKUROVA AGRICULTURAL RESEARCH INSTITUTE, TURKEY), VAKAS ŞAHİN (ÇUKUROVA AGRICULTURAL RESEARCH INSTITUTE, TURKEY), YASEMİN EKMEKÇİ (HACETTEPE UNIVERSITY, TURKEY)

@ NCICEK@HACETTEPE.EDU.TR

Drought is an important environmental factor for plant growth and development which affecting agricultural yield. Therefore, the identification/selection of plant species tolerant to drought (arid) conditions is a primary requisite. Sunflower (*Helianthus annuus* L.) is one of the most important oil plant in the world. As well as being a plant tolerant to drought, sunflower genotypes might exhibit different photochemical responses under arid conditions. In the study, we purposed to investigate the drought stress effects on fluorescence kinetics of PSII in sunflower, screen the genotypes and find out potentially underlying mechanisms. Experiment was established in the agricultural field of Çukurova Agricultural Research Institute in Adana. Twenty six inbred lines were sown and harvested in the end of March and August 2016, respectively. Control plants were irrigated regularly, whereas stressed plants were not irrigated and were left to natural conditions. To evaluate the drought effects, polyphasic chlorophyll fluorescence measurements were made at the three growth stages as R-3 (vegetative), R5-1 (head formation) and R-6 (milk seed) referred to mild (R-3), moderate (R5-1) and severe (R6) stress levels, respectively. Genotypes were sorted and classified according to drought factor index (DFI) calculated by photosynthetic performance index (PI<sub>abs</sub>). Based on DFI values, as 9444AX9947R and 9444AX8129R might be classified the most drought tolerant, while 7751AXTT135R and 9814AX9979R as the most sensitive. Potential tolerance mechanisms of genotypes have been evaluated using photochemical efficiency of PSII.

## PC7.34 YEAR ROUND BIODIESEL PRODUCTION IN MICROALGAE ON THE SWEDISH WEST COAST

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• OTILIA CHEREGI (UNIVERSITY OF GOTHENBURG, SWEDEN), MATS X ANDERSSON (UNIVERSITY OF GOTHENBURG, SWEDEN), ANNA GODHE (UNIVERSITY OF GOTHENBURG, SWEDEN), CORNELIA SPETEA WIKLUND (UNIVERSITY OF GOTHENBURG, SWEDEN)

@ OTILIA.CHEREGI@BIOENV.GU.SE

Biodiesel from microalgae has the potential of providing a viable and sustainable alternative to fossil fuels. Compared to energy crops, which are cultivated seasonally, the microalgal biomass can be obtained all year round, thus representing a stable staple of fuel. However, year-round production of microalgal biomass using outdoor cultivation has been rarely reported. In our study we aim to screen and select species and strains of marine microalgae from existing local collections and compare their growth, photosynthetic activity and lipid production when cultivated in the dynamic environmental conditions representative for the climate seasons on the Swedish west coast. The ecological records of microalgae blooms in different seasons in the sea allow us to hypothesize that microalgae species/strains isolated from the west coast have an inherited mechanism of acclimation to the local environmental conditions, and will be most productive in biomass and lipids in those conditions. Seasonal succession of microalgae strains prompts us to propose a rotation culture strategy: local strains that produce lipids at low temperatures in low light would be cultured during the cold season, and replaced in the warm season by strains performing better at high temperatures and light intensities. We will describe the concept and present preliminary results.

# PC8 CROP MODELS IMPROVEMENT WITH BIOLOGICAL KNOWLEDGE: WHICH, WHY AND HOW?

**ORGANISED BY:** BERTRAND MULLER (INRA, FRANCE)  
AND PIERRE MARTRE (INRA SUPAGRO, FRANCE)

## PC8.1 INTEGRATING AND ACCOUNTING FOR MULTIPLE STRESSES AND EXTREME EVENTS

📅 TUESDAY 4 JULY, 2017 ⌚ 13:40

👤 FRANK EWERT (LEIBNIZ CENTRE FOR AGRICULTURAL LANDSCAPE RESEARCH (ZALF), GERMANY), HEIDI WEBBER (UNIVERSITY OF BONN, GERMANY)

@ FRANK.EWERT@ZALF.DE

Recent progress in crop modelling has resulted in the development of models for more crops, regions and different scales. The range of applications is likewise expanding, including for decision support, support of breeding and impact assessment studies of climate change among others. While an increasing range of environmental and management factors is considered in modelling of effects of climate change and variability on crops, effects of stresses caused by extreme weather events such as heat and drought have until recently received comparably little attention. The modelling of multiple stresses and how to properly integrate these into crop models remains largely unexplored.

This paper provides an overview of stresses and extreme events as they are accounted for in crop models. Emphasis is on heat and drought and effects of tropospheric ozone. Typical characteristics of multiple stresses on crops are also reviewed and results of investigations are presented in which multiple stresses are included into crop models.

Based on selected examples, progress and challenges in modelling multiple stresses are critically discussed. Specific attention is given to challenges related to the required modelling detail and model complexity, the relative importance of interactions among stresses and methods of upscaling stresses for large area impact assessments. The paper concludes with suggestions to identify generic characteristics of stresses including their interactions to simplify modelling and to utilize recent developments in modular programming to support the modelling of multiple stresses across crops, environments and regions.

## PC8.2 CANOPY TEMPERATURE MODEL ROBUSTNESS FOR HEAT STRESS SIMULATION

📅 TUESDAY 4 JULY, 2017 ⌚ 14:20

👤 HEIDI WEBBER (UNIVERSITY OF BONN, GERMANY), JEFFREY W WHITE (ARS-USDA, UNITED STATES), BRUCE A KIMBALL (ARS-USDA, UNITED STATES), FRANK EWERT (ZALF, GERMANY), SENTHOLD ASSENG (UNIVERSITY OF FLORIDA, UNITED STATES), EHSAN EYSHI REZAEI (UNIVERSITY OF BONN, GERMANY), PAUL J PINTER (USDA-ARS, UNITED STATES), JERRY L HATFIELD (USDA-ARS, UNITED STATES), MATTHEW P REYNOLDS (CIMMYT, MEXICO), BEHNAM ABABAEI (INRA, FRANCE), MARCO BINDI (UNIVERSITY OF FLORENCE, ITALY), JORDI DOLTRA (CIFA, SPAIN), ROBERTO FERRISE (UNIVERSITY OF FLORENCE, ITALY), HENNING KAGE (CHRISTIAN-ALBRECHTS-UNIVERSITY, GERMANY), BELAY T KASSIE (UNIVERSITY OF FLORIDA, UNITED STATES), KURT C KERSEBAUM (ZALF, GERMANY), ADAM LUIG (CHRISTIAN-ALBRECHTS-UNIVERSITY, GERMANY), JØRGEN E OLESEN (AARHUS UNIVERSITY, DENMARK), MIKHAIL A SEMENOV (ROTHAMSTED RESEARCH, UNITED KINGDOM), PIERRE STRATONOVITCH (ROTHAMSTED RESEARCH, UNITED KINGDOM), ARNE M RATJEN (CHRISTIAN-ALBRECHTS-UNIVERSITY, GERMANY), ROBERT L LAMORTE (USDA-ARS, UNITED STATES), STEVEN W LEAVITT (UNIVERSITY OF ARIZONA, UNITED STATES), DOUGLAS J HUNSAKER (USDA-ARS, UNITED STATES), GERARD W WALL (USDA-ARS, UNITED STATES), PIERRE MARTRE (INRA, FRANCE)

@ HWEBBER@UNI-BONN.DE

Process-based crop models are commonly used in climate change impact assessments, though most use air temperature to estimate impacts of heat stress, rather than crop canopy temperature ( $T_c$ ), ignoring the complex interactions among air temperature, crop and soil water status,  $CO_2$  concentration and atmospheric conditions. A comparison of the  $T_c$  simulations of nine crop models was conducted for two experiments: the China Wheat experiment, conducted over two years at five North American locations included drained and irrigated treatments and the FACE-Maricopa experiment, conducted over four years with ambient and elevated atmospheric  $CO_2$  concentrations, and two years each of nitrogen and water stress treatments. Each of the nine models implemented an empirical (EMP), an energy balance assuming neutral stability (EBN) or an energy balance correcting for atmospheric stability conditions (EBSC)  $T_c$  approach. The EBSC models' simulations had the highest  $R^2$  and lowest RMSE values for measured vs. simulated differences between  $T_c$  and air temperature ( $\Delta T$ ). The portion of variation in observed  $\Delta T$  explained by modeled values of  $\Delta T$ , ranged from 34% for the EBSC models to 19% and 20% for the EBN or EMP

types, respectively in the FACE-Maricopa data. All models but one explained a significant portion of variation in observed  $\Delta T$ , ranging between 20, 7 and 34% for EMP, EBN and EBSC models respectively in the China Wheat experiment. At least one model from each approach was able to simulate response to  $\text{CO}_2$  concentration. These results suggest that energy balance approaches that consider atmospheric stability conditions should be used.

### PC8.3 INTERACTIVE EFFECTS OF ELEVATED $\text{CO}_2$ , DROUGHT AND HIGH TEMPERATURE ON PHOTOSYNTHESIS, WATER RELATION AND GRAIN YIELD IN WHEAT

TUESDAY 4 JULY, 2017 14:35

EVA ROSENQVIST (DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES UNIVERSITY OF COPENHAGEN, DENMARK), DORTHE H LARSEN (DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES, DENMARK), XIANGNAN LI (DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES, DENMARK), KARINA N KRISTENSEN (DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES, DENMARK), FULAI LIU (DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES, DENMARK)

ERO@PLEN.KU.DK

Rising atmospheric  $\text{CO}_2$  concentration and temperature along with changing precipitation patterns affect crop physiological processes and productivity. The interactions between the abiotic factors induce complex responses that challenge our understanding of the effects on crop performance.

Two years experiments were conducted in a glasshouse on the wheat (*Triticum aestivum* L.) cultivars 'Gladius' and 'Paragon'. Drought (25% of pot water holding capacity) and high temperature (36/30°C day/night temperature) were applied during anthesis, alone or in combination, together with ambient (400 ppm) and elevated (800 ppm)  $\text{CO}_2$ .

Drought and heat stress decreased photosynthesis, while elevated  $\text{CO}_2$  alleviated the stresses by maintaining higher leaf water potential and relative water content sustaining the photosynthetic rate of the flag leaf, though differently between the cultivars and stresses. The leaf ABA concentration increased by drought and heat, while elevated  $\text{CO}_2$  reduced the drought induced increase in leaf ABA concentration in 'Gladius'. Spike weight was reduced by drought and heat stress in both cultivars, whereas elevated  $\text{CO}_2$  increased the spike weight in 'Gladius' alone. The grain number per spike and thousand-kernel weight were lowered by drought and heat. This was alleviated by elevated  $\text{CO}_2$  in some of the treatments.

The stress responses including the biomass allocation patterns into grains and the alleviating effects of elevated  $\text{CO}_2$  differed between the wheat cultivars, which has significant implications for modeling of combined stresses in wheat.

### PC8.4 IMPROVING MODELS AND PLANT PHENOTYPING PIPELINES FOR A SMART AGRICULTURE UNDER ABIOTIC STRESS COMBINATION AND ELEVATED $\text{CO}_2$

TUESDAY 4 JULY, 2017 14:50

CARL-OTTO OTTOSEN (DEPARTMENT OF FOOD SCIENCE AARHUS UNIVERSITY, DENMARK), BENITA HYLDGAARD (DEPARTMENT OF FOOD SCIENCE AARHUS UNIVERSITY, DENMARK), THAYNA MENDANHA (DEPARTMENT OF FOOD SCIENCE AARHUS UNIVERSITY, DENMARK), STEVEN DRIEVER (CENTRE FOR CROP SYSTEMS ANALYSIS WAGENINGEN UNIVERSITY WAGENINGEN, NETHERLANDS)

COO@FOOD.AU.DK

Reliable crop models are of key importance in the innovation and breeding process towards creation of "climate efficient" or tolerant varieties of crops. In the ERANET+ project MODCARBOSTRESS we focus on acclimation of wheat. The experimental setup with multiple climatic factors enables us to create knowledge on the interacting effects of stresses on photosynthesis and to improve model parameterization.

A  $\text{CO}_2 \times$  drought  $\times$  temperature experiment with two wheat cultivars, including swapping of plants between the temperature treatments was performed with in depth analysis of  $A/C_i$  curves, photosynthetic temperature responses and morphological analysis to clarify the effects of the interactions of abiotic factors on the acclimation of the photosynthetic apparatus.

One cultivar, Paragon, showed a shift in temperature optimum, when grown at ambient  $\text{CO}_2$ , but not at elevated  $\text{CO}_2$ . The other cultivar Gladius did not show a curve shift. Both cultivars showed a small upward shift of the curve in response to increased  $\text{CO}_2$  when grown at 18°C, but not at 28°C. The drought treatment resulted in different responses between cultivars, with different effects of both temperature and/or  $\text{CO}_2$ .

When combined stresses caused different cultivar responses. Drought treatment reduced  $V_{c,max}$  when combined with both  $\text{CO}_2$  and elevated temperature separately and all climate treatments combined, while  $J_{max}$  was reduced equally under combined stresses. These findings will form part of an extensive analysis, to support the models for regulation of photosynthesis in wheat under future climates to assess combined moderate stresses (temperature and drought) and elevated  $\text{CO}_2$ .

## PC8.12 ERRORS AND UNCERTAINTIES IN CROP MODELS – WHERE BIOLOGICAL MECHANISMS COULD HELP?

📅 TUESDAY 4 JULY, 2017 ⌚ 16:00

👤 PIERRE MARTRE (INRA, FRANCE), BERTRAND MULLER (INRA, FRANCE), ENLI WANG (CISRO, FRANCE), JOHN R PORTER (MONTPELLIER SUPAGRO, FRANCE), FRANK EWERT (ZALF, GERMANY), SENTHOLD ASSENG (UNIVERSITY OF FLORIDA, UNITED STATES)

@ PIERRE.MARTRE@INRA.FR

Process-based crop simulation models are popular tools to project climate and crop management impact on crop performance. Crop models are also increasingly used to analyse and predict the response of genotypes to the environment. Recently a number of multi-model intercomparison studies have analysed the extent of error and uncertainty in simulating crop growth response to environmental changes. These studies pinpoint processes for which model improvement is needed. The uncertainty of wheat models response to temperature was related to their temperature response functions for key physiological processes. Temperature response functions for these processes were derived from field experiments with a wide range of temperature. The implementation of these new temperature functions reduced the error for simulating grain yield response to temperature by nearly half on average. Simulation error for grain yield usually correlate poorly with error for other variables, such as leaf area index, anthesis date, total above ground N mass, or water use. It is thus important to evaluate and improve models not just for yield but also for individual processes. We discuss how resource use efficiency identities can be used to deconstruct model errors and identify processes, the descriptions of which should be improved in models. This analysis of model error stresses the need for detailed experimental data sets with in-season crop and soil measurements.

## PC8.13 SHOULD THERMAL ACCLIMATION OF PHOTOSYNTHESIS BE CONSIDERED IN CROP MODELS?

📅 TUESDAY 4 JULY, 2017 ⌚ 16:30

👤 MAEVA BAUMONT (INRA MONTPELLIER, FRANCE), BORIS PARENT (INRA MONTPELLIER, FRANCE), BERTRAND MULLER (INRA MONTPELLIER, FRANCE), PIERRE MARTRE (INRA MONTPELLIER, FRANCE)

@ MAEVA.BAUMONT@INRA.FR

Temperature is one of the most important abiotic factor affecting plant development, grow and carbon assimilation. Several physiological processes such as photosynthesis acclimate to temperature, which may significantly contribute to the continuous success of an individual under fluctuating temperature. However, most crop models do not consider the change of physiological processes regarding the plant climatic history. Here we analyzed the time courses of acclimation of photosynthesis to temperature for wheat. Plants were grown in growth chambers with day time temperatures ranging from 8°C to 33°C. Plants were transferred during the vegetative growth period to cooler or warmer

environments, with amplitudes of 5°C or 10°C. Temperature response (between 10°C and 38°C) of photosynthesis at saturating light was monitored over time before and after the transfer of plants to their new thermal environment. Our results show that photosynthesis optimal temperature changes within a day after the transfer, while it takes up to 5 days at infra and supra optimal temperatures. Moreover, the rate of acclimation depends on growth temperature. For instance, photosynthesis acclimation takes longer (expressed in thermal time) for plants transferred from 13°C to 23°C than for plants transferred from 18°C to 28°C. Following this study, acclimation to changing temperatures could be modeled according to a ratio and a velocity depending on the growth temperature and on the amplitude of variation. This can be an important step to improve photosynthesis models in fluctuating thermal environments and, therefore, to reduce the uncertainty of climate change impact analyses.

## PC8.14 SOLVING THE OPTIMUM NITROGEN PARTITIONING AMONG PHOTOSYNTHETIC COMPOUNDS: TOWARDS MODELLING PLANT ACCLIMATION TO GROWTH ENVIRONMENT

📅 TUESDAY 4 JULY, 2017 ⌚ 16:45

👤 XINYOU YIN (WAGENINGEN UNIVERSITY, NETHERLANDS), AD HCM SCHAPENDONK (PHOTOSYNTAX BV, NETHERLANDS), STEVEN M DRIEVER (WAGENINGEN UNIVERSITY, NETHERLANDS), PAUL C STRUIK (WAGENINGEN UNIVERSITY, NETHERLANDS)

@ XINYOU.YIN@WUR.NL

The distribution of leaf nitrogen among photosynthetic protein responds to environmental changes, and we hypothesise that this response may underlie plant acclimation to growth environment. We describe an analytical method to solve optimum leaf nitrogen partitioning for maximised leaf photosynthesis in  $C_3$  plant species. Photosynthetic nitrogen in leaves is assumed to distribute among pools for chlorophyll-protein complex, electron transport system, Rubisco, and other soluble protein related to the activities of the Calvin cycle enzymes. The model predicts that a high investment of nitrogen in Rubisco is required under the conditions that lead to an excessive energy supply relative to energy demand by stromal metabolism (e.g. low temperature, high light, low nitrogen supply, low  $CO_2$ ). Conversely, more nitrogen is invested in the chlorophyll complex when energy supply is limiting. At a doubling  $CO_2$  concentration, an increased electron transport capacity relative to Rubisco carboxylation velocity is required. An increase of the optimum temperature for photosynthesis is predicted not only with increasing temperature, light or  $CO_2$  level, but also with decreasing leaf nitrogen content. Results of optimisation are qualitatively in line with reported acclimation experiments in the literature. The model is also parameterised from our growth chamber experiments on photosynthetic acclimation in wheat. As the optimum partitioning under any specific environmental condition can be fast determined, the approach can be implemented in plant models simulating plant acclimation and growth under fluctuating environmental conditions.

### PC8.15 NEW TOOLS TO ACCOUNT FOR ROOT WATER UPTAKE IN CROP MODELS : SCALING UP FROM 2D CELL WATER FLOW TO 4D SOIL-PLANT WATER DYNAMICS AND SIMPLIFYING COMPLEX BIOLOGICAL MODELS DOWN TO CROP MODEL-COMPATIBLE SOLUTIONS

WEDNESDAY 5 JULY, 2017 09:00

XAVIER DRAYE (UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM), VALENTIN COUVREUR (UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM), FÉLICIE MEUNIER (UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM), GUILLAUME LOBET (FZ JUELICH, GERMANY), MATHIEU JAVAUX (UNIVERSITÉ CATHOLIQUE DE LOUVAIN, BELGIUM)

XAVIER.DRAYE@UCLouvain.BE

Over the last ten years, novel models of water flow in the soil-root domain have been developed. These models, which account for root architecture and root hydraulics down to the organ scale, have revealed a number of interesting root water uptake behaviors that were not intuitive to most plant biologists. From a computational point of view, these models are not compatible with crop models. Recently, however, analytical solutions have been established which speed up the solving of flux equations considerably. We have used a similar strategy to simulate the composite water transport model at the sub-cell scale with a complete anatomical description of the root cross section. Here again, unexpected behaviors emerge from simulations which call for a re-interpretation of conflicting results. Analytical solutions are now being developed which should allow, on a medium term, to incorporate molecular aspects of water transport (e.g. aquaporin and apoplastic barriers regulation) into crop models.

### PC8.16 NO FURTHER STIMULATION OF WHEAT YIELD BY CO<sub>2</sub> ABOVE 600 PPM?

WEDNESDAY 5 JULY, 2017 09:40

MALIN C BROBERG (UNIVERSITY OF GOTHENBURG, SWEDEN), PETRA HÖGY (UNIVERSITY OF HOHENHEIM, GERMANY), HÅKAN PLEIJEL (UNIVERSITY OF GOTHENBURG, SWEDEN)

MALIN.BROBERG@BIOENV.GU.SE

Elevated carbon dioxide (eCO<sub>2</sub>) is well known to stimulate plant photosynthesis and growth. Effects of eCO<sub>2</sub> on crop yield are of particular interest due to the strong concern for future food security. We compiled data from 102 field experiments (Free-Air Carbon Dioxide Enrichment, FACE; Open-Top Chamber, OTC; field tunnel, FT) where wheat was exposed to different CO<sub>2</sub> concentrations [CO<sub>2</sub>]. Data on grain yield, grain mass, grain number, harvest index, and grain protein concentration were analysed by meta-analysis to estimate average effects, and response functions were derived to assess the effect size in relation to [CO<sub>2</sub>].

Grain yield was increased by 27% on average due to eCO<sub>2</sub>, mainly as a result of an increase in grain number per area. Grain mass and harvest index remained unaffected by eCO<sub>2</sub>, while grain protein concentration was significantly decreased by almost 10%, which is serious with respect to human nutrition.

The response function for grain yield with [CO<sub>2</sub>] strongly suggests a non-linear response, where the gradual increase in yield saturates at ~600 ppm. This result is supported by the meta-analysis, which does not indicate any significant increase in yield stimulation of wheat grown at [CO<sub>2</sub>] > 600 ppm compared to [CO<sub>2</sub>].

### PC8.17 MODELLING PLANT GROWTH: WHAT ARE THE LIMITATIONS TO CARBON ALLOCATION?

WEDNESDAY 5 JULY, 2017 09:55

BETHANY HOLLAND (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), RICHARD CLAYTON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), NICK MONK (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), COLIN OSBORNE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

BHOLLAND1@SHEFFIELD.AC.UK

Improving crop yield is essential to meet increasing global food demands. Boosting crop yield requires the coordination of carbon acquisition by leaves and carbon utilisation by roots and seeds. Simple modelling approaches may be used to explain how this coordination is achieved within plant growth. Here, the limits to allocation strategies are explored by analysing the sensitivity of a simple root-shoot carbon allocation model. The model is formulated based on fundamental constraints on plant growth and therefore can be applied to all plants. This general approach shows that parameters defining the cost of root and leaf respiration alter the relationship between carbon allocation and final plant size by enabling a range of allocation strategies to produce a similar plant mass. This plasticity is enhanced by increasing assimilation rate and reduced by increasing the effect of shading within the model.

### PC8.18 LIMITATIONS OF CARBON SOURCE DRIVEN CROP MODELS UNDER WATER STRESS CONDITIONS

WEDNESDAY 5 JULY, 2017 10:10

MORITZ KUPISCH (UNIVERSITY OF BONN INSTITUTE OF CROP SCIENCE AND RESOURCE CONSERVATION, GERMANY), FRANK EWERT (UNIVERSITY OF BONN INSTITUTE OF CROP SCIENCE AND RESOURCE CONSERVATION, GERMANY), MATTHIAS LANGENSIEPEN (UNIVERSITY OF BONN INSTITUTE OF CROP SCIENCE AND RESOURCE CONSERVATION, GERMANY)

MKUPISCH@UNI-BONN.DE

Most vegetation and crop models assume a positive feedback loop between carbon assimilation and leaf area growth, influenced by temperature dependent phenology and environmental factors acting on the carbon source process. These models accurately simulate growth at the canopy level under optimal growing conditions and even fairly well under environmental stress when carbon source limiting functions are calibrated and only a small subset of all model variables are compared with observations. Such model design stays in contrast to common physiological knowledge about plant growth under non-optimal conditions, which is often not carbon source limited

but reduced due to inhibitions of tissue formation and expansion. This presentation compares the predictive qualities of three crop models with different carbon assimilation submodels under drought conditions in winter wheat. The evaluated model variables comprise different temporal and organizational scales such as instantaneous leaf and canopy assimilation, daily canopy assimilation and transpiration, total dry matter and LAI. Results show that all models are unable to simulate plant growth under drought when this larger set of variables was considered. Reasonably correct simulated variables like total dry matter and total daily carbon assimilation turned out to be a false compromise between an overestimation of LAI and an underestimation of instantaneous carbon assimilation at the leaf level. Simulations were improved by applying adapted water stress functions on simulated LAI growth and photosynthesis which mimic response curves of cell elongation and leaf photosynthesis to leaf water potential.

### PC8.19 THE ALPHA AND OMEGA OF PLANT GROWTH

WEDNESDAY 5 JULY, 2017 11:00

CHRISTIAN KÖRNER (UNIVERSITY OF BASEL, SWITZERLAND)

CH.KOERNER@UNIBAS.CH

For a plant to grow, it needs three things to come together: (1) the resources needed to build new tissue (primary metabolism), (2) conditions that permit the process of tissue formation to proceed (secondary metabolism) and (3) plant internal settings that 'say' go (developmental controls). Past growth research has largely built upon (1), while taking (2) and (3) as a given. I will advocate a view at growth controls that roots in ideas by Boyer, Hsiao, Bradshaw, Evans and others back to the 1980s that suggest that (2) plays a more central role than we wish, from a modelling perspective. At least for C uptake viewed in isolation, we have perfect algorithms, but we have, at best, some statistical functions for (2) and (3). Even for (1), C uptake cannot be explained without concurrent nutrient uptake, which facilitates a proportional resource supply to building sites, meeting stoichiometric rules. Any advance in a mechanistic understanding in plant growth will thus, require advances in the understanding of the coupling of the nutrient cycle with the carbon cycle, and the coupling of meristematic activity with both, above- and below-ground resource supply. In most cases, CO<sub>2</sub> uptake runs on demands set by (2) and (3), hence plant growth cannot be understood from primary controls of leaf photosynthesis. Yet, source- and sink-activity cannot be decoupled in the long run, so knowing either one implies the rate of the other one, with the causality only revealed through manipulation.

### PC8.20 GROWTH REPRESSORS REVEAL PLANT GROWTH IS SINK-NOT SOURCE-LIMITED

WEDNESDAY 5 JULY, 2017 11:30

NICK PULLEN (JOHN INNES CENTRE, UNITED KINGDOM),  
ALBOR DOBON (JOHN INNES CENTRE, UNITED KINGDOM),  
NAICHAO ZHANG (JOHN INNES CENTRE, UNITED KINGDOM),  
STEVE PENFIELD (JOHN INNES CENTRE, UNITED KINGDOM)

NICK.PULLEN@JIC.AC.UK

Understanding plant growth and carbon fixation is essential for understanding global climate systems and agricultural productivity. In plant ecophysiology the difference between carbon fixed by photosynthesis and carbon respired, known as Net Primary Productivity (NPP), is presumed to be a key factor limiting plant growth. Classical models for plant growth thus assume growth is source-limited. We have new evidence that supports the view that growth is mainly sink driven and we are attempting to model this mathematically. By doing so we aim to more accurately predict the carbon fluxes in dynamic global vegetation models and to improve models of crop yield. We have been working with Arabidopsis growth repressor mutants which produce phenotypically bigger plants but with a similar relative growth rate to wild type. Counter-intuitively we found that these mutants photosynthesize no more than wild-type plants. In our conditions the mutant plants have larger leaves and have greater fresh and dry weight. Thus the mutant plants create more biomass than wild type despite similar NPP. Presumably therefore, wild type plants use only a fraction of the available NPP for growth. We are building a model to discover and quantify how much of a gap exists between maximum potential NPP (due to net photosynthesis) and actual NPP due to growth regulation. We have also started to measure how these physiological variables are affected by environmental conditions, particularly temperature. By modelling environmental effects on tissue growth we propose that genetics regulates photosynthesis and that plants are therefore sink-limited in many circumstances.

### PC8.21 BIOLOGICAL REALITY AND PARSIMONY IN CROP MODELS - WHY WE NEED BOTH IN CROP IMPROVEMENT!

WEDNESDAY 5 JULY, 2017 13:50

GRAEME HAMMER (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

G.HAMMER@UQ.EDU.AU

The potential to add significant value to the rapid advances in plant breeding technologies associated with statistical whole genome prediction methods is a new frontier for crop physiology and modelling. Yield advance by genetic improvement continues to require prediction of phenotype based on genotype, and this remains challenging for complex traits despite recent advances in genotyping and phenotyping. Crop models that capture physiological knowledge and can robustly predict phenotypic consequences of genotype-by-environment-by-management (G\*E\*M) interactions have demonstrated potential as an integrating tool. But does this biological reality come with a degree of complexity that restricts applicability in crop improvement? Simple, high speed, parsimonious

models are required for dealing with the thousands of genotypes and environment combinations in modern breeding programs. In contrast, it is often considered that greater model complexity is needed to evaluate potential of putative variation in specific traits in target environments as knowledge on their underpinning biology advances. Is this a contradiction leading to divergent futures? Here it is argued that biological reality and parsimony do not need to be separable. It is further asserted that the structure needed for the next generation of crop models to be most effective perhaps requires both jointly, along with the capacity to evolve in crop growth and development process algorithms. Specific examples in modelling photosynthesis from biochemical scale and canopy development from plant scale are used to highlight the concepts presented.

### PC8.22 OPTIMIZING THE PHENOTYPING PROTOCOLS OF PERENNIAL RYEGRASS THROUGH PRACTICAL IDENTIFIABILITY ANALYSIS - CASE STUDY WITH THE PASTURE SIMULATION MODEL

WEDNESDAY 5 JULY, 2017 14:30

TOM DE SWAEF (THE INSTITUTE FOR AGRICULTURAL AND FISHERIES RESEARCH (ILVO), BELGIUM), GIANNI BELLOCCHI (INRA - CLERMONT-FERRAND, FRANCE), JONAS APER (THE INSTITUTE FOR AGRICULTURAL AND FISHERIES RESEARCH (ILVO), BELGIUM), PETER LOOTENS (THE INSTITUTE FOR AGRICULTURAL AND FISHERIES RESEARCH (ILVO), BELGIUM), ISABEL ROLDÁN-RUIZ (THE INSTITUTE FOR AGRICULTURAL AND FISHERIES RESEARCH (ILVO), BELGIUM)

TOM.DESWAEF@ILVO.VLAANDEREN.BE

Crop models representing the interactions between plant, weather, soil and agricultural practices tend to be very complex. They explain crop growth and yield formation under a range of environmental conditions via several interlinked bio-physical processes. The parameterization of these models can be problematic as it may require extended experimental data. Practical identifiability analysis is recognized as an elegant way to assess which parameters in a model can be estimated independently from experimental data. A parameter set is 'identifiable' if every parameter has a substantial effect on the model output and can be independently determined by the observations. The application of practical identifiability analysis holds great potential for proper parameter estimation and for optimizing experimental designs but its application to crop models is still limited. Using the FME package for calibration, sensitivity and identifiability analysis in R, we applied practical identifiability analysis to PaSim, a complex model specifically developed to simulate grassland systems. In a first step, we used a breeding programme dataset, where dry matter yield and forage quality data were collected at five cutting events during one growing season of perennial ryegrass. In a second step, we evaluated virtual datasets with additional hypothetical measurements (i.e. increased frequency, increased number of variables and additional locations) to satisfy the minimum data requirement for proper estimation of 19 influential parameters in PaSim. Using the real dataset, 12-13 parameters can be estimated, but additional measurements taken between cutting dates would enable the estimation of up to 18 parameters.

### PC8.23 SENSITIVITY TO DROUGHT DURING REPRODUCTIVE DEVELOPMENT WILL LIMIT INCREASE IN WHEAT YIELD POTENTIAL IN EUROPE UNDER CLIMATE CHANGE

WEDNESDAY 5 JULY, 2017 14:45

MIKHAIL A SEMENOV (ROTHAMSTED RESEARCH, UNITED KINGDOM), MATTHEW J PAUL (ROTHAMSTED RESEARCH, UNITED KINGDOM)

MIKHAIL.SEMENOV@ROTHAMSTED.AC.UK

Drought prior flowering can trigger grain abortion resulting in yield losses. (Nuccio et al. 2015) showed that it was possible to modify genetically maize to enable drought "tolerance" around flowering by increasing the concentration of sucrose in ear spikelets. In field experiments, genetically modified maize performed better under non-drought and drought conditions. Ji et al. (Ji et al. 2010) demonstrated that in wheat yield responses to drought stress around flowering vary greatly between cultivars. Our objective was using modelling approach to assess importance of tolerance to drought in wheat during reproductive development for achieving high yield potential and yield stability under future climates. We used the refined Sirius wheat mode with incorporated wheat responses to drought prior flowering, to optimise wheat ideotypes for future climate scenarios at selected European sites representing wheat growing areas. At those sites where water could be limited, drought-sensitive (DS) ideotypes produce substantially lower mean yields. For example, at SL yield for a drought-tolerant (DT) ideotype was 37% higher compared with a DS ideotype. Moreover, yield variability expressed as yield CV was substantially higher (up to 2.5 times) for DS ideotypes. Therefore, tolerance to drought during reproductive development is likely to be an essential trait for wheat in the future in order to achieve high yield potential and stability in Europe to underpin food security.



## PC8.24 USING NUMERICAL PLANT MODELS AND PHENOTYPIC CORRELATION SPACE TO DESIGN ACHIEVABLE IDEOTYPES

WEDNESDAY 5 JULY, 2017 15:00

PIERRE CASADEBAIG (INRA, FRANCE), VICTOR PICHENY (INRA, FRANCE), RONAN TRÉPOS (INRA, FRANCE), ROBERT FAIVRE (INRA, FRANCE), DAVID DA SILVA (INRA, FRANCE), PATRICK VINCOURT (INRA, FRANCE), EVELYNE COSTES (INRA, FRANCE)

PIERRE.CASADEBAIG@INRA.FR

Numerical plant models can predict the outcome of plant traits modifications resulting from genetic variations, on plant performance, by simulating physiological processes and their interaction with the environment. Optimization methods complement those models to design ideotypes, i.e. ideal values of a set of plant traits resulting in optimal adaptation for given combinations of environment and management, mainly through the maximization of a performance criteria (e.g. yield, light interception). As use of simulation models gains momentum in plant breeding, numerical experiments must be carefully engineered to provide accurate and attainable results, rooting them in biological reality.

Here, we propose a multi-objective optimization formulation that includes a metric of performance, returned by the numerical model, and a metric of feasibility, accounting for correlations between traits based on field observations. We applied this approach to two contrasting models: a process-based crop model of sunflower and a functional-structural plant model of apple trees. In both cases, the method successfully characterized key plant traits and identified a continuum of optimal solutions, ranging from the most feasible to the most efficient. The present study thus provides successful proof of concept for this enhanced modeling approach, which identified paths for desirable trait modification, including direction and intensity.

## PC8.25 APPLYING THE ARABIDOPSIS FRAMEWORK MODEL TO LINK SNPS TO CLINES

WEDNESDAY 5 JULY, 2017 15:45

ANDREW J MILLAR (SYNTHSYS AND SCHOOL OF BIOLOGICAL SCIENCES UNIVERSITY OF EDINBURGH, UNITED KINGDOM), YIN HOON CHEW (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), DANIEL D SEATON (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), URIEL URQUIZA (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ALASTAIR HUME (EPCC UNIVERSITY OF EDINBURGH, UNITED KINGDOM), EILIDH TROUP (EPCC UNIVERSITY OF EDINBURGH, UNITED KINGDOM), TOMASZ ZIELINSKI (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ARGYRIS ZARDILIS (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ROBERT MUETZELFELDT (SIMULISTICS LTD., UNITED KINGDOM)

ANDREW.MILLAR@ED.AC.UK

The 24-hour circadian clock controls the sleep-wake cycle, the cell cycle and seasonal reproduction through photoperiodism. Breeders have selected alleles of clock-associated genes in multiple crops. We seek to understand how the clock gene circuit controls plant growth, biomass and life history in *Arabidopsis thaliana*. This work links genotype to phenotype, quantitatively and mechanistically. However, the proof of concept for a 'crops *in silico*' approach should ideally start from increasingly-available crop genome sequences and reach up to field traits, in a transparent and accessible fashion.

We previously built mechanistic, mathematical models of the clock gene circuit, and of each clock-regulated process between germination and flowering, including the nightly utilisation of starch carbon stores. Linking specific aspects of the clock model to genome sequence (SNPs) is one current challenge. We also combined three further models to form the Arabidopsis Framework Model (FMv1; [www.plasmo.ed.ac.uk/plasmo/models/model.shtml?accession=PLM\\_76](http://www.plasmo.ed.ac.uk/plasmo/models/model.shtml?accession=PLM_76)), which predicts rosette biomass quantitatively in simple reference conditions (Chew et al. PNAS 2014). In the FMv2 (Chew et al. *bioRxiv* 2017, <https://doi.org/10.1101/105437>), we modelled the clock-regulated processes in the whole-plant context, to understand quantitatively the pleiotropic phenotypes of a 'slow' clock mutant, from the gene circuit dynamics to biomass. Testing the FMv2 under multiple environments now suggests the potential to link from SNPs to clinal variation.

To address the challenge of making such models accessible, I will contrast a very simple, online simulator (<http://turnip.bio.ed.ac.uk/fm/>) with previous approaches. The FAIRDOM data management system ([www.fair-dom.org](http://www.fair-dom.org)) promises to organise relevant data and models, and seems easily applicable to crop models.

## PC8.26 DISSECTING THE GENETIC VARIABILITY OF LIGHT INTERCEPTION AND LIGHT USE EFFICIENCY IN COMPLEX MAIZE CANOPIES VIA HIGH-THROUGHPUT PHENOTYPING AND MODELLING

WEDNESDAY 5 JULY, 2017 16:15

TSU-WEI CHEN (LEPSE INRA MONTPELLIER, FRANCE), CHRISTIAN FOURNIER (LEPSE INRA MONTPELLIER, FRANCE), SIMON ARTZET (LEPSE INRA MONTPELLIER, FRANCE), CHRISTOPHE PRADAL (CIRAD MONTPELLIER, FRANCE), LLORENÇ CABRERA-BOSQUET (LEPSE INRA MONTPELLIER, FRANCE), CLAUDE WELCKER (LEPSE INRA MONTPELLIER, FRANCE), FRANÇOIS TARDIEU (LEPSE INRA MONTPELLIER, FRANCE)

CHEN@GEM.UNI-HANNOVER.DE

Understanding genetic control and variability in traits related to radiation interception efficiency (RIE) and radiation use efficiency (RUE), e.g. leaf expansion, architectural traits and photosynthetic parameters, may accelerate crop improvement. Based on the daily images of 255 well-watered maize hybrids in three experiments from the PhenoArch high-throughput phenotyping platform, we reconstructed dynamic 3D architecture of the platform canopy to estimate daily radiation interception of each plant in the platform. This allows 1) dissecting RIE and RUE into genetic and environmental components valid for complex canopy with varietal mixtures, as in any phenotyping platform condition; 2) extracting environmental terms from RIE and RUE to better associate detected QTL of biomass to their genetic components having high heritability, naming leaf area, competitiveness and dependency of RUE to light; 3) assessing the contribution of genetic variability in genetic components on variation in biomass quantitatively; 4) simulating virtual fields with single genotype or any varietal mixture to assess the complexity of a canopy that changes behavior of individual genotypes and 5) estimating parameters using crop models, e.g. light extinction coefficient. Our results suggest that 1) genetic variability in biomass is three times more determined by leaf expansion and competitiveness to light than by RUE 2) the differences in biomass between canopies consisting of single or multiple genotypes can be predicted by the competitiveness of the genotypes. Our analyses and simulations allow studying light-related traits in a complex canopy, determining their genetic controls and predicting behavior of genotypes for crop model improvement.

## PC8.5 MODIFICATION OF SOURCE LEAF STARCH METABOLISM IN TRANSGENIC *ARABIDOPSIS THALIANA* INCREASES PLANT BIOMASS AND DOUBLES OILSEED PRODUCTION

TUESDAY 4 JULY, 2017 POSTER SESSION

IAN J TETLOW (UNIVERSITY OF GUELPH, CANADA), FUSHAN LIU (UNIVERSITY OF GUELPH, CANADA), LIPING WANG (UNIVERSITY OF GUELPH, CANADA), ZAHEER AHMED (UNIVERSITY OF GUELPH, CANADA), NOEL MANO (UNIVERSITY OF GUELPH, CANADA), MICHAEL J EMES (UNIVERSITY OF GUELPH, CANADA)

ITETLOW@UOGUELPH.CA

We have identified a novel means to achieve substantially increased vegetative biomass and oilseed production in the model plant *Arabidopsis thaliana*. Specific endogenous starch biosynthetic enzymes were substituted with corresponding maize (*Zea mays*) enzymes. Transformants were compared with the starch-free background and with the wild-type plants. Each of the maize-derived enzymes restored starch biosynthesis but both morphology and structure of starch particles were markedly altered. Altered starch metabolism in the transformants is associated with enhanced biomass formation and more-than-doubled oilseed production while maintaining seed oil quality. Enhanced oilseed production is primarily due to an increased number of siliques per plant whereas oil content and seed number per silique is essentially unchanged. Introduction of specific cereal starch biosynthetic enzymes into oilseed plants represents a potentially useful strategy to increase biomass and oil production in related oilseed crops and manipulate the structure and properties of leaf starch.

## PC8.6 LUSH SPIKE - TOWARDS THE GENETICS AND MECHANISM OF SPIKELET SURVIVAL IN BARLEY

TUESDAY 4 JULY, 2017 POSTER SESSION

THIRULOGACHANDAR VENKATASUBBU (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), AHMAD MOHAMMAD I ALQUDAH (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), RAVI KOPPOLU (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), TWAN RUTTEN (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), JOCHEN KUMLEHN (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), THORSTEN SCHNURBUSCH (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY)

VENKATASUBBU@IPK-GATERSLEBEN.DE

Improving grain yield is a major objective of crop breeding, and a promising avenue for maximizing yield is through enhanced spikelet survival during pre-anthesis development. However, little is known about spikelet survival and its impact in grain yield in cereals. In barley, the growth period from primordium to tipping is considered as the most critical pre-anthesis phase determining spikelet/spikelet primordia abortion and grain yield since ~70% of spikelet abortion occurs during this phase, regardless of growth

conditions and row-type. Mortality of spikelet primordia may begin with the onset of rapid stem and spike growth and lasts until heading. Interestingly, the high broad-sense heritability (>80%) of spikelet survival in barley underscores that there are major genetic players regulating this trait. Therefore, we aim to discover QTL for spikelet survival in a GWAS panel and validate them in biparental double haploid (DH) mapping populations. Furthermore, interesting QTL will be Mendelized, functionally characterized, and the underlying gene will be identified using a map-based approach. A histological analysis of spikelet/spikelet primordia during pre-anthesis phase will be performed to describe the developmental process of spikelet survival/abortion in barley. Additionally, the histologic studies will be complemented with the tissue-specific transcriptome, metabolome and phytohormone analysis. Finally, the spatio-temporal patterns of transcript, metabolite and phytohormone distribution/modulation in the spike may illustrate the mechanistical regulation of spikelet survival.

### PC8.7 ASSESSING MULTIPLE STRESS EFFECTS ON WHEAT SPIKE MORPHOLOGY AND GRAIN PRODUCTION USING microCT SCANNING

TUESDAY 4 JULY, 2017 POSTER SESSION

FIONA CORKE (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), NATHAN HUGHES (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), CALLUM SCOTSON (UNIVERSITY OF SOUTHAMPTON, UNITED KINGDOM), KAREN ASKEW (NATIONAL PLANT PHENOMICS CENTRE ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), CANDIDA NIBAU (NATIONAL PLANT PHENOMICS CENTRE ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), EVA ROSENQVIST (UNIVERSITY OF COPENHAGEN, DENMARK), CARL-OTTO OTTOSEN (AARHUS UNIVERSITY, DENMARK), JOHN DOONAN (NATIONAL PLANT PHENOMICS CENTRE IBERS ABERYSTWYTH UNIVERSITY, UNITED KINGDOM)

@ FIC5@ABER.AC.UK

Yield components include spike number per unit area, grain number per spike and grain size while grain shape contributes to quality. Measuring traits such as number and size is straightforward in harvested loose grain, but developmental and spatial information that may be useful in breeding or experimental analyses is lost. To retain this trait information in its developmental context, we have adapted established biomedical imaging techniques, including X-ray computed tomography (CT) scanning to provide accurate estimates of grain parameters in intact spikes of wheat and applied it to examine the combined effects of 2 independently applied stresses, mild drought from 6th leaf stage and heat stress for 4 days from GS39 (during meiosis).

Plants were harvested at maturity and the primary ear (tagged at extension) were kept separate from other ears. The ear length and weight were measured. Grain numbers were ground truthed from reconstructed 3D scans to provide a training set for automated software development. Information extracted from 3-D CT models will be combined with other phenotypic data and used to parameterize predictive crop models.

This high-content medium-throughput CT method has a range of other potential applications including dissecting varietal variation in wheat, oats and forage and wild grasses as well as other crops such as Brassica and pulses. The procedure is inherently non-destructive and seed can be recovered for further analysis or growth tests.

Open source software for 3-D visualisation and feature extraction allow non-experienced users to interrogate the data and will be released in due course.

### PC8.10 PHENOTYPING FROM CLIMATE CHAMBERS TO FIELD. TOMATO GENOTYPES PHENOTYPED FOR HIGH $F_v/F_m$ DURING HEAT STRESS IN CONTROLLED ENVIRONMENT MAINTAIN HIGH FRUIT YIELD DURING HEAT STRESS IN THE FIELD

TUESDAY 4 JULY, 2017 POSTER SESSION

CARL-OTTO OTTOSEN (AARHUS UNIVERSITY, DENMARK), DAMODAR POUDYAL (SEAN SEED SERVICE CENTRE LIMITED, NEPAL), EVA ROSENQVIST (COPENHAGEN UNIVERSITY, DENMARK)

@ COO@FOOD.AU.DK

The aim of the study was to correlate the heat responses of young tomato (*Solanum lycopersicum* L.) from controlled environments to field using the maximum photochemical efficiency of PSII ( $F_v/F_m$ ) and gas exchange. Initially 28 tomato genotypes, selected for good performance in the field in Nepal were phenotyped in 40/28°C day/night temperature for two days in controlled environments followed by a second screening of four genotypes with high and four with low  $F_v/F_m$  for four days in 38/28°C, followed by 5-day recovery in 26/20°C.

The genotypes with high  $F_v/F_m$  maintained higher rates of net photosynthesis ( $P_N$ ) at 38°C, due to high stomatal conductance leading to efficient cooling of the leaf. The genotypes had identical biomass during control but after heat stress a difference between the sensitive and tolerant lines were seen in biomass accumulation and pollen viability, similar to  $F_v/F_m$  and  $P_N$ . Two tolerant and two sensitive genotypes were grown with irrigation in the field in Nepal during a natural heat wave (38/26°C). The tolerant genotypes had significantly higher biomass accumulation; lower heat injury index and higher fruit yield than the sensitive genotypes.

To our knowledge this is the first study where screening of tomatoes for heat tolerance by  $F_v/F_m$  in controlled environments have been followed by a field trial for agricultural traits under natural high temperature stress. The results confirm  $F_v/F_m$  as an effective physiological marker for early detection of heat tolerance correlating to the yield under heat stress in the field.

### PC8.11 CAN PHYSIOLOGICAL MAPS GUIDE GENETIC SELECTION FOR IMPROVED RESPONSES TO ENVIRONMENTAL STRESSES

TUESDAY 4 JULY, 2017 POSTER SESSION

MARCUS DAVID BELLETT-TRAVERS (IM GEOSPATIAL, UNITED KINGDOM)

MTRAVERS@IMGEOSPATIAL.COM

Plants respond to environmental stress through a combination of biochemical, physiological and phenological responses, controlled by a number of genes. The genetic control of such responses can be manipulated to improve crop survival or yield under suboptimum growing conditions but often changes in genetic response to stress bring about penalties as well as benefits. These come about through, sometimes unappreciated, indirect consequences of the genetic manipulation or the requirement for multiple gene expression to bring about the desired outcome. Mapping physiological processes and the associated genes gives insight into the consequences changes in gene expression and the likely outcomes to physiology, phenology and yield. A map of over 3000 physiological and phenological processes linked to water stress will be presented using mapping software. This will be used, as an example, to show how changes to key genes can have some desired and undesired effects.

### PC8.9 MODELING CEREAL METABOLISMS FOR ELUCIDATING STRESS RESPONSES AND GUIDING CROP IMPROVEMENT

TUESDAY 4 JULY, 2017 POSTER SESSION

BIJAYALAXMI MOHANTY (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE), MEIYAPPAN LAKSHMANAN (BIOPROCESSING TECHNOLOGY INSTITUTE, SINGAPORE), DONG-YUP LEE (NATIONAL UNIVERSITY OF SINGAPORE BIOPROCESSING TECHNOLOGY INSTITUTE, SINGAPORE)

CHEBIJAY@NUS.EDU.SG

Rice, maize, wheat, sorghum and barley are the major food crops which constitute the most of world's nutrient requirements. Although the overall yield of such cereals has been increasing, the growing population and adverse climatic changes pose huge challenges for their sustained production in the future. Therefore, systematic approaches are highly required to explore their effects on cereal crops' phenotypic and cellular responses. It could be achieved by combining the available multiple high throughput data such as genomics, metabolomics, proteomics and transcriptomics, thereby analyzing the possible biochemical adaptations to several abiotic stresses, and subsequently improving the crop yield. Concurrently, the advent of constraint-based metabolic reconstruction and analysis paves way to characterize the cereals cellular physiology under various stresses via the mathematical network models, which is relatively new. Since early 2000s, the plant metabolic modeling studies have started to appear in the literature on Arabidopsis (~40%) possibly because it is the first plant to have its genome sequenced. Although, plant research on Arabidopsis will still uncover much complex biological process, it is also important to elucidate the molecular mechanisms that are specific to cereal plants such as rice, maize, wheat, and barley

via their abundant NGS data. In this regard, a handful of constraint-based modeling studies have appeared in the past decade on rice (~18%), maize (~11%) and barley (~5%). We summarize the recent developments of cereal metabolic models and in silico analysis to characterize their stress responses and identify agronomic traits for crop improvement.

### PC8.8 EVALUATION OF THE CROPGRO MODEL TO SIMULATE THE GROWTH AND DEVELOPMENT OF THE PEANUT CROP

TUESDAY 4 JULY, 2017 POSTER SESSION

BRUNO A ALVES (ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - ESALQUSP, BRAZIL), FABIO R MARIN (ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - ESALQUSP, BRAZIL), RUBENS D COELHO (ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - ESALQUSP, BRAZIL), DANILLO G GOMES (ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - ESALQUSP, BRAZIL)

BRUNOARAUJO@USP.BR

Peanut is one of the most cultivated oilseeds in the world, with high protein and energy potential. Crop simulation models are defined as a set of equations related to biophysical processes to estimate growth, development and production of a crop from genetic factors and environmental variables, which allows the analyses of various components of production. The goal was to evaluate the CROPGRO-Peanut model to estimate the growth and development of peanut cultivar Runner IAC 886. One experiment was conducted in a greenhouse in the experimental area of the Biosystems Engineering Department of ESALQ/USP, Piracicaba, SP, in boxes of 100L filled with Red-Yellow Latosol (LV) soil, in the period between September 2015 and January 2016. The experimental outline used was randomized blocks with four repetitions. The treatments were composed by biometric and productivity assessments in five seasons (77, 93, 100, 106 and 130 DAP - days after planting) using surface drip irrigation system. The genetic coefficients to cultivate have been modified to obtain the best adjustment between observed and model-simulated data, seeking desirable values for the correlation coefficient ( $R^2$ ) and Willmott's index (d). The calibration of the coefficients related to phenology was satisfactory when compared to the dates of occurrence of the phenological events observed with the simulation. Thus, the model simulated satisfactorily the growth and development of peanut cultivar Runner IAC 886.

# PC9 IMAGING PLANT PHENOTYPING

**ORGANISED BY: GEORGE LITTLEJOHN (PLYMOUTH UNIVERSITY, UK)  
AND MICHAEL DEEKS (UNIVERSITY OF EXETER, UK)**

## PC9.1 EXPLORING DEFENCE AND DISEASE DYNAMICS DURING PLANT PATHOGEN INTERACTIONS, FROM THE WHOLE PLANT TO SUB-CELLULAR RESPONSES

📅 MONDAY 3 JULY, 2017 ⌚ 14:00

👤 MURRAY GRANT (UNIVERSITY OF WARWICK, UNITED KINGDOM), TRUPTI GAIKWAD (UNIVERSITY OF EXETER, UNITED KINGDOM), GEORGE LITTLEJOHN (UNIVERSITY OF PLYMOUTH, UNITED KINGDOM), SATISH KULASEKARAN (UNIVERSITY OF WARWICK, UNITED KINGDOM), HANNAH BROWN (JOHN INNES INSTITUTE, UNITED KINGDOM), MIKE DEEKS (UNIVERSITY OF EXETER, UNITED KINGDOM), PETER WINLOVE (UNIVERSITY OF EXETER, UNITED KINGDOM), STEPHEN GREEN (UNIVERSITY OF EXETER, UNITED KINGDOM), DAVID HORSALL (UNIVERSITY OF EXETER, UNITED KINGDOM), MARTA DE TORRES ZABALA (UNIVERSITY OF EXETER, UNITED KINGDOM)

@ M.GRANT@WARWICK.AC.UK

Given that 25% of crop losses are attributable to pathogens, an area that holds real potential in the collective global challenge to ensure food security in the coming decades is to develop new plant lines with broad spectrum resistance to pathogens. Significant progress has been made over the past 25 years in identifying classical plant disease resistance genes, and subsequently components of plant basal defence - the two key defence mechanisms deployed by plants. However, our knowledge of the dynamics of plant-pathogen interactions are limited, especially our understanding of the processes occurring post recognition of the pathogen (basal defence) or its effector proteins (gene-for-gene resistance) and how the host responds at the cellular level. We take a multidisciplinary approach to dissect host responses to pathogens exploiting genetics, whole plant imaging, subcellular imaging and electrophysiological responses. This talk will look at the dynamics of the early molecular events underpinning two key defence responses; (i) the induction and subsequent suppression of plant basal immunity in *Arabidopsis thaliana* by virulent *Pseudomonas syringae* focussing on an emerging role of the chloroplast in plant immunity and (ii) the rapid early events in the initiation and propagation of systemic signals following gene-for-gene recognition. Finally, we examine how generic these responses are to other host-pathogen systems and exploit knowledge of the timing of responses to re-engineer plant defence responses.

## PC9.2 HOW DOES PHYTOPHTHORA DELIVER EFFECTORS TO HOST PLANT CELLS?

📅 MONDAY 3 JULY, 2017 ⌚ 14:40

👤 PETRA C BOEVINK (THE JAMES HUTTON INSTITUTE, UNITED KINGDOM), SHUMEI WANG (UNIVERSITY OF DUNDEE, UNITED KINGDOM), HAZEL MCLELLAN (UNIVERSITY OF DUNDEE, UNITED KINGDOM), QIN HE (UNIVERSITY OF DUNDEE, UNITED KINGDOM), SHAISTA NAQVI (UNIVERSITY OF DUNDEE, UNITED KINGDOM), INGO HEIN (THE JAMES HUTTON INSTITUTE UNIVERSITY OF DUNDEE, UNITED KINGDOM), ELEANOR M GILROY (THE JAMES HUTTON INSTITUTE, UNITED KINGDOM), STEVE C WHISSON (THE JAMES HUTTON INSTITUTE, UNITED KINGDOM), PAUL R J BIRCH (UNIVERSITY OF DUNDEE THE JAMES HUTTON INSTITUTE, UNITED KINGDOM)

@ PETRA.BOEVINK@HUTTON.AC.UK

Phytophthora infestans utilises a large array of secreted, translocated RXLR class effectors to infect its host. Relatively few of the hundreds of potential RXLR effectors have been demonstrated to have specific host targets and activities but many are able to enhance colonisation and accumulate at distinct sites within the plant cell. Not surprisingly the characterised effectors have been demonstrated to target known defence pathways, either inhibiting protein activities that promote defence or stimulating activities that down-regulate the defence response.

RXLR effector translocation into host cells remains a controversial area and previous attempts to detect translocation in living tissue with fluorescent reporters were unsuccessful. In revisiting this area we have focussed on effectors that locate to the nucleolus in an effort to concentrate the low level of translocated effector in a small volume. With this approach we are able to detect live fluorescently tagged effector translocation in infected cells. This opens the way to investigating the mechanism of effector secretion from the pathogen and uptake by the host cells. We demonstrate that the haustorium is a site of delivery of both apoplastic and cytoplasmic effectors by distinct secretory pathways.

### PC9.3 ROLE OF MOLECULAR PATTERN IN ACTIN MEDIATED VESICLE TRAFFICKING OF *ARABIDOPSIS THALIANA* HYPOCOTYL CELLS

📅 MONDAY 3 JULY, 2017 ⌚ 15:10

👤 STEFAN SASSMANN (UNIVERSITY OF EXETER, UNITED KINGDOM), KAYLEE WORLOCK (UNIVERSITY OF EXETER, UNITED KINGDOM), MARK HEATH (UNIVERSITY OF EXETER, UNITED KINGDOM), DAVID RICHARDS (UNIVERSITY OF EXETER, UNITED KINGDOM), DAVID HORSELL (UNIVERSITY OF EXETER, UNITED KINGDOM), MIKE DEEKS (UNIVERSITY OF EXETER, UNITED KINGDOM)

@ S.SASSMANN@EXETER.AC.UK

Plants are constantly exposed to a variety of biotic (e.g. bacteria, fungi) and abiotic (e.g. temperature, mechanical) stress factors. Some do not pose a threat, others however might inflict disastrous damage. In the field these two different stress types must be differentiated as mechanical stress to the cell wall can also be inflicted by random abrasion. How do plant cells perceive the difference?

*A. thaliana* detects Microbial Associated Molecular Patterns (MAMP) during attacks from a broad range of pathogens. This leads to focal transport of the Penetration resistance 3 (PEN3) ATP binding cassette transporter and other cargoes to the interaction site to fight the infection. This also leads to a change in actin filament organisation and transport mediated by actin filaments is critical for successful response to the stimulus. But the rearrangement of actin microfilaments can also be induced within five minutes by touching the surface of epidermal cells with microneedles.

We are integrating interdisciplinary approaches to separate the effects of mechanical stress and molecular patterns on actin-mediated transport directed by the plant immune system. We have found vesicle exchange at nascent immune sites to be dependent upon actin filament dynamics, and that a complex sub-division of immune cargoes occurs at the plasma membrane following actin-driven distribution. We are currently testing the hypothesis that the biophysics associated with locally applied pressure acts cooperatively to focus vesicle dynamics to the epicenter of pathogen contact.

### PC9.4 RXLR-EFFECTOR AVR1 OF *PHYTOPHTHORA INFESTANS* TARGETS PLANT EXOCYST COMPLEX SUBUNIT SEC5 TO SUPPRESS IMMUNITY

📅 MONDAY 3 JULY, 2017 ⌚ 15:25

👤 ELYSA OVERDIJK (WAGENINGEN UNIVERSITY, NETHERLANDS), YU DU (WAGENINGEN UNIVERSITY, NETHERLANDS), JOEP SMITS (WAGENINGEN UNIVERSITY, NETHERLANDS), KLAAS BOUWMEESTER (WAGENINGEN UNIVERSITY, NETHERLANDS), TIJS KETELAAR (WAGENINGEN UNIVERSITY, NETHERLANDS), FRANCINE GOVERS (WAGENINGEN UNIVERSITY, NETHERLANDS)

@ ELYSA.OVERDIJK@WUR.NL

Previously, we have shown that RXLR-effector AVR1 of the late blight pathogen *Phytophthora infestans* targets exocyst subunit Sec5 in the host plant potato, thereby suppressing plant defense. The exocyst is a protein complex involved in exocytosis by mediating

initial vesicle tethering to the plasma membrane. Exocytosis is one of several cellular processes playing an essential role in plant defense. To mechanistically study the effect of AVR1 on the exocyst complex dynamics on a subcellular level, we use the model moss species *Physcomitrella patens*. We showed that different *Phytophthora* species are able to initiate a defense response in this moss and that this system is ideal for high-resolution live-cell imaging of defense processes such as actin cytoskeleton dynamics and exocytosis. Furthermore, we showed that several moss exocyst subunits localize at the site of attack by *Phytophthora capsici*. Besides binding to potato Sec5, we showed with yeast-two-hybrid assays that AVR1 is also binding to Sec5 homologs of *P. patens*. Ectopic expression of AVR1 in *P. patens* causes a growth phenotype and we are currently studying the effect of AVR1 on the subcellular localization of Sec5 and other exocyst subunits of *P. patens* by fluorescence microscopy. Together, this study gives more insight in how pathogen effectors modulate plant cellular processes.

### PC9.5 THE IMPACT OF ENVIRONMENT ON PLANT DEFENCE

📅 MONDAY 3 JULY, 2017 ⌚ 16:10

👤 KATHERINE DENBY (UNIVERSITY OF YORK, UNITED KINGDOM), PRESTA CONSORTIUM (UNIVERSITY OF WARWICK, UNITED KINGDOM), EMILY BREEZE (UNIVERSITY OF WARWICK, UNITED KINGDOM), KRZYSZTOF POLANSKI (UNIVERSITY OF WARWICK, UNITED KINGDOM), CLAIRE STOKER (UNIVERSITY OF WARWICK, UNITED KINGDOM), LAURA RODEN (UNIVERSITY OF CAPE TOWN, SOUTH AFRICA), ROB INGLE (UNIVERSITY OF CAPE TOWN, SOUTH AFRICA)

@ KATHERINE.DENBY@YORK.AC.UK

The outcome of plant-pathogen interaction is governed by the genotype of the pathogen and genotype of the plant in combination with environmental conditions. We will explore some of the ways in which environment impacts on disease susceptibility informed by transcriptome studies. Plant immunity is a multi-layered response including large-scale transcriptional reprogramming. Within the PRESTA project we generated high-resolution time series expression data from *Arabidopsis* leaves following infection with bacterial and fungal pathogens, during senescence and after drought and high light treatment. These data sets have enabled us to identify overlapping responses to these conditions on a more nuanced level than single time point expression data and point to decisions that must be made by plants in fine-tuning responses to pathogen infection. Analysis of the time series transcriptome data after fungal infection led to investigation of the effect of time of day of inoculation on subsequent infection outcome. We demonstrated that the circadian clock impacts on the plant defence response and ultimately the susceptibility of the host to infection. We have identified a mechanism (JAZ6) driving this daily rhythmic variation in immunity and are currently investigating the regulatory control points at which JAZ6 acts. Small changes in early transcriptional responses appear to drive significant differences in lesion development over a much longer time frame highlighting the importance of environment in assessing disease susceptibility.

## PC9.6 LIFE CELL IMAGING OF THE CYTOSKELETON IN *PHYTOPHTHORA* REVEALS NOVEL ACTIN AND TUBULIN CONFIGURATIONS

MONDAY 3 JULY, 2017 16:55

TIJS KETELAAR (WAGENINGEN UNIVERSITY, THE NETHERLANDS), KIKI KOTS (WAGENINGEN UNIVERSITY, THE NETHERLANDS), HAROLD MEIJER (WAGENINGEN UNIVERSITY, THE NETHERLANDS), FRANCINE GOVERS (WAGENINGEN UNIVERSITY, THE NETHERLANDS)

@ TIJS.KETELAAR@WUR.NL

The cytoskeleton is essential for proper functioning of eukaryotic cells. We study the cytoskeleton in *Phytophthora* species, oomycete plant pathogens that cause devastating crop losses. Oomycete diseases are difficult to control and there is an urgent need to find novel oomycete specific drug targets. We used *Phytophthora infestans* expressing Lifeact-eGFP for live cell imaging of the actin cytoskeleton during development to identify oomycete specific actin configurations. Firstly, we identified actin plaques as unique oomycete structures. Secondly, we studied cyst germination and appressorium formation and we found actin configurations to be associated with plug deposition in germ tubes and with appressorium formation. These actin configurations strongly suggest a role for the actin cytoskeleton in plug formation and plant cell penetration. To investigate how microtubule organization and dynamics relate to *Phytophthora* functioning we have generated *Phytophthora palmivora* transformants expressing GFP- $\alpha$ -tubulin. These transformants allow live cell imaging of the microtubule cytoskeleton in oomycetes for the first time. Although actin and tubulin are conserved proteins, many structural and regulatory proteins interacting with the cytoskeleton are not. The oomycete specific cytoskeletal organizations that we observed are potential targets for oomycete specific drugs.

## PC9.7 HOW MEMBRANE TRAFFICKING REGULATES IMMUNITY

TUESDAY 4 JULY, 2017 10:30

SILKE ROBATZEK (THE SAINSBURY LABORATORY, UNITED KINGDOM), SARA BEN KHALED (THE SAINSBURY LABORATORY, UNITED KINGDOM), GILDAS BOURDAIS (THE SAINSBURY LABORATORY, UNITED KINGDOM), MICHAELA KOPISCHKE (THE SAINSBURY LABORATORY, UNITED KINGDOM), LYDIA RICKETT (THE SAINSBURY LABORATORY, UNITED KINGDOM), MARY TETLOW (THE SAINSBURY LABORATORY, UNITED KINGDOM)

@ ROBATZEK@TSL.AC.UK

Our main research focus has been how cells defend themselves against infection of their extracellular space by microbial pathogens. Conserved microbial patterns activate cell surface receptors that are essential for host immunity, and induce their internalization. Over the years we have characterized immune receptor-mediated endocytosis, a process conserved across different receptor family members. Yet, the significance of receptor-mediated endocytosis in the regulation of immune signalling remained controversial. We recently have revealed that endocytosis of cell surface immune receptors is a mechanism

for sustaining cellular responsiveness to microbial patterns, as it exists in natural encounters of plants with their biotic environment, to confer long-term anti-bacterial immunity.

This work is supported by the Gatsby Charitable Foundation and a grant by the European Research Council (ERC).

## PC9.8 WHAT 'R' YOU DOING HERE? INVESTIGATING THE ROLE OF S-ACYLATION IN PLANT DISEASE RESISTANCE PROTEIN SIGNALLING

TUESDAY 4 JULY, 2017 11:10

DIONNE TURNBULL (UNIVERSITY OF DUNDEE, UNITED KINGDOM), PAUL RJ BIRCH (UNIVERSITY OF DUNDEE, UNITED KINGDOM), PIERS A HEMSLEY (UNIVERSITY OF DUNDEE, UNITED KINGDOM)

@ D.W.TURNBULL@DUNDEE.AC.UK

Plants are constantly surrounded by potential pests and pathogens, threatening the health of the plant and the security of our food production. Unable to move to escape challenge, plants must defend themselves in situ, and possess a complex, multi-layered innate immune system. A crucial aspect of the plant's immune response is the recognition of specific pest and pathogen 'effector proteins' - secreted molecules that manipulate plant processes to promote the pest or pathogen's lifestyle, increasing disease potential. Effector recognition is facilitated by plant resistance (R) proteins, largely belonging to the nucleotide-binding leucine-rich repeat (NB-LRR) family, and analogous to immune receptors found in animal systems. The modular structure, and interaction between domains, enables effector recognition and subsequent activation of these R-proteins, but the mechanisms of activation and downstream immune signalling remain poorly understood. Recent work on a group of R-proteins from potato, required for resistance to the notorious potato late blight pathogen *Phytophthora infestans*, has revealed that several of these undergo S-acylation - a reversible fatty acid based post-translational modification. S-acylation is particularly known for its role in membrane anchoring and localisation but, due to its reversibility, is also linked to regulating aspects of protein function such as activation, trafficking, and protein-protein interaction. Ongoing investigation has shown that S-acylation occurs at multiple sites, in multiple domains, within R proteins, suggesting a complex role for this dynamic post-translational modification in plant R-protein function. Our latest data on the functional consequences of R-protein S-acylation during plant defence responses will be presented.

## PC9.9 MECHANISMS THAT CONTROL CHITIN-TRIGGERED CHANGES TO CELL-TO-CELL CONNECTIVITY VIA PLASMODESMATA

TUESDAY 4 JULY, 2017 11:25

CECILIA CHEVAL (JOHN INNES CENTRE, UNITED KINGDOM),  
XIAO KUN LIU (JOHN INNES CENTRE, UNITED KINGDOM),  
CHRISTINE FAULKNER (JOHN INNES CENTRE, UNITED KINGDOM)

@ CECILIA.CHEVAL@JIC.AC.UK

Recognition of chitin from fungal pathogens by pattern recognition receptors triggers a range of immune responses in plants. Among these responses is the regulation of symplastic continuity and molecular flux between cells. Recent studies have demonstrated that a plasmodesmata (PD)-located, GPI anchored receptor protein LYM2 perceives chitin and triggers PD closure in *Arabidopsis*. This contributes to resistance to a fungal pathogen. Significantly, LYM2 signalling occurs independently of other chitin-activated response pathways.

LysM receptor proteins form complexes with LysM receptor kinases. We have identified two LysM receptor kinases that interact with and are necessary for chitin-triggered PD closure. Chitin-triggered signalling is known to activate the NADPH oxidase RBOHD and our data indicates that similar to non-PD chitin signalling, RBOHD is required for signal transmission and PD closure. However, chitin-triggered PD signalling is transmitted via a specific mechanism of RBOHD activation. Ultimately, chitin perception triggers callose deposition at PD, and this is correlated with PD closure. This data enables a context to dissect the hierarchy of molecular events that ultimately induce PD closure.

PD closure is a newly identified plant defence response and raises questions relating to how cells communicate during pathogen attack. The data/tools generated in this project will enable us to understand how cells symplastically co-ordinate their defence responses.

## PC9.10 INVESTIGATING APPRESSORIUM-MEDIATED PLANT INFECTION BY THE RICE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

TUESDAY 4 JULY, 2017 11:40

MIRIAM OSES-RUIZ (UNIVERSITY OF EXETER, UNITED KINGDOM), WASIN SAKULKOO (UNIVERSITY OF EXETER, UNITED KINGDOM), GEORGE R LITTLEJOHN (PLYMOUTH UNIVERSITY, UNITED KINGDOM), MAGDALENA MARTIN-URDIROZ (UNIVERSITY OF EXETER, UNITED KINGDOM), NICHOLAS J TALBOT (UNIVERSITY OF EXETER, UNITED KINGDOM)

@ MO256@EXETER.AC.UK

To cause rice blast disease, the fungal pathogen *Magnaporthe oryzae* develops a specialised infection structure called an appressorium. The appressorium is a dome-shaped cell that accumulates enormous internal turgor that is translated into mechanical force by reorientation of septin-dependent F-actin cytoskeleton at the base of the infection cell. Septin-dependent polarity determinants reorganize at the base of the appressorium to

produce a rigid and narrow penetration peg that ruptures the tough, waxy leaf cuticle to allow colonization of the plant tissue. Here, we show that appressorium mediated plant infection by *M. oryzae* is tightly linked with cell cycle control and more specifically, requires two independent S-phase cell cycle checkpoints. The first checkpoint occurs during initial formation of appressoria on the rice leaf surface and acts through the DNA damage response (DDR) pathway, involving the Cds1 kinase. By contrast, appressorium repolarization involves a novel, DDR-independent S-phase checkpoint, triggered by appressorium turgor generation and melanisation. This second S-phase checkpoint regulates septin-dependent, NADPH oxidase-regulated F-actin dynamics to organise the appressorium pore and facilitate entry of the fungus into a plant cell. We show that a minimum turgor threshold in the appressorium, which depends on melanin production, is necessary to trigger this unusual S-phase cell cycle checkpoint and is necessary for the appressorium to function.

## PC9.11 ELUCIDATING MECHANISMS OF PLANT AND NECROTROPHIC FUNGAL INTERACTIONS

TUESDAY 4 JULY, 2017 POSTER SESSION

ELSPETH RANSOM (UNIVERSITY OF WARWICK, UNITED KINGDOM), ADAM TALBOT (UNIVERSITY OF YORK, UNITED KINGDOM), SASCHA OTT (UNIVERSITY OF WARWICK, UNITED KINGDOM), JOHN CLARKSON (UNIVERSITY OF WARWICK, UNITED KINGDOM), KATHERINE DENBY (UNIVERSITY OF YORK, UNITED KINGDOM)

@ E.M.RANSOM@WARWICK.AC.UK

Necrotrophic fungal pathogens cause devastating damage to crops globally, with lettuce (*Lactuca sativa*) losses up to 50% pre-harvest reported as a result of infection. Currently fungicide control methods are expensive and unsustainable, stressing the need for alternative management strategies. The development of new lettuce varieties with fungal resistance has the potential to save growers £7million per annum.

Analysis of novel dual RNAseq timecourse data, generated from lettuce leaves inoculated with *Sclerotinia sclerotiorum*, has captured the pattern of host and pathogen genes differentially expressed over 48 hours post infection. Functional analysis of the time series has shown the chronology of lettuce gene expression changes, including initial fungal perception, the activation of pathogenesis related genes and transcriptional signalling. Using genes with known *Arabidopsis thaliana* orthologs, core conserved defence responses to necrotroph infection in Lettuce and *Arabidopsis* can be observed. Furthermore, *S. sclerotiorum* pathogenesis genes including those involved in the oxalic acid pathway, carbohydrate active enzymes and effector candidates were identified.

During the defence response a large scale regulatory reprogramming occurs before visible symptoms. Network modelling of differentially expressed transcription factors has identified key lettuce regulatory hub genes, which are up-regulated in response to *S. sclerotiorum*. To elucidate the impact of these key hub genes upon *S. sclerotiorum* resistance we are using CRISPR-Cas9 knockouts, generated through the regeneration of transformed lettuce protoplasts, and over-expression mutants. Examining these results alongside disease resistance QTL data, will establish whether network analysis can be used to enhance the identification of candidate genes underlying resistance for breeding programs.



# PC10 GENERAL CELL AND PLANT BIOLOGY

**ORGANISED BY:** KATHERINE DENBY (PLANT SECTION CHAIR, SEB)  
AND JOHN LOVE (CELL SECTION CHAIR, SEB)

## PC10.1 LINKS BETWEEN SUB-CELLULAR BODIES, RNA BIOLOGY AND HUMAN DISEASE

📅 TUESDAY 4 JULY, 2017 ⌚ 10:30

👤 JUDITH E SLEEMAN (UNIVERSITY OF ST ANDREWS, UNITED KINGDOM)

@ JES14@ST-ANDREWS.AC.UK

The nucleus of the mammalian cell contains numerous distinct structures, all formed without the use of membranes. Roles in various stages of the regulation of gene expression have been proposed for several of these structures including the Cajal body, which is implicated in splicing snRNP biogenesis. Many of the characteristic proteins found in sub-nuclear structures are multi-functional RNA binding proteins (RBPs) and also accumulate in cytoplasmic structures. The SMN protein is a key example of this, with proposed roles in splicing snRNP assembly, pre-mRNA splicing and mRNA trafficking in the cytoplasm among others. The inherited neurodegenerative condition, Spinal Muscular Atrophy (SMA), results from low levels of expression of SMN. In the nucleus, SMN accumulates in gems (Gemini of Cajal bodies), which are separable from their twin structure, Cajal bodies, only under certain conditions and in some cell types. In the cytoplasm, SMN accumulates in highly mobile structures associated with microtubules and implicated in mRNA trafficking. It is increasingly clear that the cellular pathologies of a number of human diseases including Amyotrophic Lateral Sclerosis (ALS) and Myotonic Dystrophy Type 1 (DM1) as well as SMA, involve defects in multi-functional RBPs and the sub-cellular structures in which they accumulate. A fuller understanding of these structures, their functions and the relationship between nuclear and cytoplasmic activities of multi-functional RBPs has the potential to shed light on these diseases.

## PC10.2 DYNAMICS OF PROTEIN PHOSPHORYLATION DURING FILAMENTATION IN CANDIDA ALBICANS

📅 TUESDAY 4 JULY, 2017 ⌚ 11:10

👤 PRIYANKA GHORAI (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), ALKA NARULA (JAMIA HAMDARD, INDIA), ASIS DATTA (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA)

@ ME.PRIYANKA2800@GMAIL.COM

Phosphoregulation of proteins through phosphorylation and dephosphorylation events has gained importance in understanding the regulation of pathogenicity. Among several pathogens, *Candida albicans* is both a commensal and an opportunistic human pathogen, and its reversible transition between yeast (avirulent) and hyphal (virulent) form is regulated by several factors and pathways. In view of delineating the role of phosphoproteins in regulating the morphological transition, global analysis of phosphoproteome after hyphal induction with elevated temperature, serum and N-acetyl-D-glucosamine (GlcNAc) treatments was performed. A total of 60, 20 and 52 phosphoproteins were identified to be unique to temperature, serum and GlcNAc treated conditions, respectively, and the distribution of unique phosphorylation sites sorted by the modified amino acids showed higher phosphorylation in serine residues, followed by threonine and tyrosine. Further, 2-DE gel of phosphoproteins treated with different factors showed the presence of large number of protein spots, from which candidate spots are identified for peptide isolation and characterization. The interaction network-based functional annotation of protein kinases and phosphoproteins of *C. albicans* demonstrated the interaction of kinases with phosphoproteins during filamentous growth, indicating the activation of kinase cascades during hyphal morphogenesis. Altogether, these findings will assist further functional characterization of candidate proteins to gain an in-depth understanding on hyphal morphogenesis and fungal pathogenicity.

Keywords: *Candida albicans*, Hypha development, phosphoproteome, IMAC, LC-MS/MS, protein kinase.

### PC10.3 TRPV4 RECEPTOR EXPRESSION AND FUNCTION IN THE GUINEA-PIG URINARY BLADDER – A ROLE IN ATP RELEASE

TUESDAY 4 JULY, 2017 11:25

MAX ROBERTS (UNIVERSITY OF SURREY, UNITED KINGDOM), MICHAEL R RUGGIERI (TEMPLE UNIVERSITY, UNITED STATES), CHANGHAO WU (UNIVERSITY OF SURREY, UNITED KINGDOM)

MAX.ROBERTS.90@GMAIL.COM

The bladder urothelium is a newly recognized sensory structure that plays a key role in detecting bladder fullness. Central to this role is the release of ATP, however the receptor-mediated modulation and mechanisms underlying this release are poorly understood. Recent evidence suggests TRPV4 channels in the urothelium may serve as key sensory molecules in response to bladder stretch. We investigated the role of TRPV4 in urothelial ATP release and the underlying cell signalling pathways mediating the process.

Bladder tissues were obtained from young (2-5 months) guinea-pigs (GP) (male Dunkin-Hartley, schedule-1). TRPV4 expression was assessed by western blotting and functional organ bath technique used to evaluate mechanisms of TRPV4-mediated ATP release. Intracellular  $Ca^{2+}$  was measured in isolated urothelial cells with fura-2. TRPV4 expression was detected in both mucosal and smooth muscle lysates, with significantly higher levels in mucosa. Specific TRPV4 inhibition revealed this receptor to be responsible for a significant proportion of stretch-induced mucosal ATP release. TRPV4 activator GSK1016790A (GSK, 1 $\mu$ M) triggered significant increase in ATP release in mucosal preparations. Various inhibitors revealed this ATP was released via conductive pathways, with little contribution from vesicular release and relied upon extracellular  $Ca^{2+}$  and protein tyrosine kinase activity.

These findings demonstrate the importance of TRPV4 in the physiological release of ATP from the urothelium, where the identified underlying mechanisms provide a better understanding of the cellular signalling involved.

### PC10.4 VACUOLAR HYDROGEN ATPase PLAYS AN ESSENTIAL ROLE IN BIOMINERAL CELL WALL SYNTHESIS OF MARINE DIATOMS

TUESDAY 4 JULY, 2017 11:40

DANIEL P YEE (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), MARTIN TRESGUERRES (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES), MARK HILDEBRAND (SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNITED STATES)

DANIEL.P.YEE@GMAIL.COM

Diatoms are highly productive unicellular phytoplankton that synthesize silica cell walls, called frustules, through a process known as silicification. Silicification occurs in a compartment called the silicon deposition vesicle (SDV), and is coordinated by proteins facilitating biomineralization of silica at the nanoscale. Here we investigated the role of vacuolar hydrogen ATPase (VHA), a well conserved multi-subunit proton pump common to eukaryotes, in the biosynthesis of frustules in the marine diatom *Thalassiosira pseudonana*. Intracellular localization of VHA was analyzed by expressing eGFP fusion proteins, which revealed VHA localized to the SDV during the G2+M phase of the cell cycle. This observation was supported by peak RNA expression of multiple VHA subunits during this phase of the cell cycle, however, Western blot analysis of VHA-subunit B revealed a variable expression pattern that was uncoupled from transcript levels. Exposure of *T. pseudonana* to high levels of the VHA specific inhibitor, concanamycin A, resulted in cessation of silicification and cell division. Additionally, VHA inhibition affected the targeting and activity of a Silicalemma Associated Protein, SAP3, which aids in coordinating the detailed architecture of the frustule. Finally, scanning electron microscopy revealed that partial inhibition of VHA led to morphological changes in frustule architecture. These changes were likely due to disruption of pH within the SDV, affecting the normal activity of SAP3 and other silicification proteins. Our results implicate VHA as a key regulator of the biochemical environment for biomineral cell wall synthesis inside the SDV of diatoms.

## PC10.5 CELL WALL PECTIN CROSS-LINKING IMPLICATED IN PROTECTING AGAINST FREEZING STRESS

📅 TUESDAY 4 JULY, 2017 ⌚ 11:55

👤 PAIGE E PANTER (UNIVERSITY OF DURHAM, UNITED KINGDOM), IAN CUMMINS (UNIVERSITY OF DURHAM, UNITED KINGDOM), DAYAN SANHUEZA (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), STEPHEN C FRY (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), MARC R KNIGHT (UNIVERSITY OF DURHAM, UNITED KINGDOM), HEATHER KNIGHT (UNIVERSITY OF DURHAM, UNITED KINGDOM)

@ P.E.PANTER@DURHAM.AC.UK

Plant species vary enormously in their ability to tolerate freezing temperatures. Most temperate plants are able to increase their tolerance through a process known as cold acclimation, whereby low, non-freezing temperatures induce changes within the plant that allow it to survive in a sub-zero environment. The plant cell wall has been implicated as a target of these changes suggesting a role for the wall in protecting the plant against freezing, although as yet, no clear functional relationship has been established. Here we show that a mutant that has altered cell wall morphology also displays an increased sensitivity to sub-zero temperatures, indicating a role for the cell wall in protecting against freezing stress. We demonstrate that the *sensitive-to-freezing-8 (sfr8)* mutant has decreased activity of the enzyme that catalyses L-fucose synthesis, resulting in reduced fucosylation of the cell wall pectin Rhamnogalacturonan-II (RG-II). This prevents RG-II pectin monomers from dimerising via borate ester cross-links. Supplementing *sfr8* mutants with boric acid restores RG-II cross-linking and reverses freezing sensitivity, suggesting that RG-II dimerisation impacts on freezing tolerance. We are currently investigating the effect of cold acclimation on RG-II synthesis and dimerisation, to assess whether cold-responsive changes in either of these might contribute to increased freezing tolerance of the acclimated plant. An alteration to plant-water relations that occurs with decreased RG-II dimerisation could be one reason for this: excised leaves of *sfr8* plants lose water more rapidly than wild-type counterparts. The possible implications this may have for freezing tolerance will be discussed.

## PC10.6 REGULATION OF GROWTH, ION HOMEOSTASIS, PHOTOSYNTHESIS AND MITIGATION OF SALT-INDUCED OXIDATIVE STRESS IN MANGROVE SPECIES, KANDELIA OBOVATA: INSIGHT INTO THE ROLE OF NITRIC OXIDE

📅 TUESDAY 4 JULY, 2017 ⌚ 12:10

👤 MIRZA HASANUZZAMAN (CENTER OF MOLECULAR BIOSCIENCE UNIVERSITY OF THE RYUKYUS, JAPAN), MASASHI INAFUKU (CENTER OF MOLECULAR BIOSCIENCE UNIVERSITY OF THE RYUKYUS, JAPAN), HIROSUKE OKU (CENTER OF MOLECULAR BIOSCIENCE UNIVERSITY OF THE RYUKYUS, JAPAN)

@ MHZSAUAG@YAHOO.COM

We investigated the role of nitric oxide (NO) in regulating ion accumulation, improving growth and photosynthetic parameters and in reduction of oxidative stress in *Kandelia obovata* seedlings grown under 1.5 and 3.0% NaCl solution for further 2 months. To observe the role of NO, 100  $\mu$ M sodium nitroprusside (SNP) was used as NO donor, while same dose of Hemoglobin (Hb) and N-nitro-L-arginine methyl ester hydrochloride (L-NAME) were used as NO scavenger and NO synthase (NOS) inhibitor, respectively. The results showed that 3.0% salt decreased root length and shoot length. Both salt concentrations markedly increased the Na<sup>+</sup> content but decreased the K<sup>+</sup> and therefore the ratio of K<sup>+</sup>/Na<sup>+</sup> decreased. The content of Ca<sup>2+</sup> and Mg<sup>+</sup> decreased only at 3.0% salinity. Low (1.5% NaCl) salinity did not show any negative effect on photosynthetic parameters, rather it improved some parameters. Higher salinity (3.0%) decreased chlorophyll content (SPAD value), net photosynthetic rate (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>), internal CO<sub>2</sub> concentration (C<sub>i</sub>), the actual quantum efficiency of PSII ( $\Phi$ PSII) and electronic transport ratio (ETR) also. Salt stress also decreased transpiration and water use efficiency and increased a couple of oxidative stress markers i.e. lipid peroxidation and H<sub>2</sub>O<sub>2</sub> content where results were prominent only at 3.0% NaCl. Exogenous NO had little effect in improving such photosynthetic parameters and decreasing oxidative stress. However, specific scavenger and NOS inhibitor clearly reversed these effects that indicated that endogenous NO has an obvious role in enhancing photosynthesis and maintaining antioxidant defense in mangroves.

**PC10.15 WE SEED WEED SEEDS**

📅 WEDNESDAY 5 JULY, 2017 ⌚ 09:00

👤 DANA R MACGREGOR (DURHAM UNIVERSITY, UNITED KINGDOM)

@ DANA.R.MACGREGOR@DURHAM.AC.UK

The ability to control seed germination allows for management of when seedlings will emerge and therefore regulates when all subsequent development will take place. Seed dormancy prevents germination under what would otherwise be favourable conditions. The level of dormancy that a seed has is determined by both genetic and environmental factors. Research on the model plant *Arabidopsis thaliana* has provided a good understanding regarding how seed dormancy is established and what is required to break it. Although *Arabidopsis* is widespread in nature, it is of low agricultural relevance either as a crop or as an arable weed. *Alopecurus myosuroides* (blackgrass) is an annual grass-weed that causes considerable problems for arable farmers in Western Europe, including significant reduction of crop yield. Novel methods are required to control blackgrass because it has 1) a germination window overlapping winter crop sowing, 2) widespread multiple-herbicide resistance, and 3) seeds that are shed before winter crops are harvested. This project aims to directly alter blackgrass dormancy using the current knowledge about the establishment and maintenance of dormancy in *Arabidopsis*. In addition to establishing protocols for transient and stable blackgrass transformation, blackgrass-specific methods for analysis of hormones, gene expression, and various seed physiology traits will be developed in this project. By understanding how to regulate blackgrass seed dormancy in the lab, we can work toward altering it in seeds that are set in the field.

**PC10.16 GROWTH AND CARBON PARTITIONING IN A LEAF OIL CROP**

📅 WEDNESDAY 5 JULY, 2017 ⌚ 09:15

👤 MADELINE MITCHELL (CSIRO, AUSTRALIA), JENIFER PRITCHARD (CSIRO, AUSTRALIA), SURINDER SINGH (CSIRO, AUSTRALIA), JAMES PETRIE (CSIRO, AUSTRALIA), THOMAS VANHERCKE (CSIRO, AUSTRALIA), JEAN-PHILIPPE RAL (CSIRO, AUSTRALIA)

@ MADELINE.MITCHELL@CSIRO.AU

Demand for plant oil is increasing due to population growth, changing diets and increasing non-food applications. Accumulation of oil in vegetative rather than seed tissues has the potential for high yields and the production of novel crops for food, feed and the chemical industry. Previously, we generated transgenic tobacco lines that accumulate up to 35% oil (triacylglycerol or TAG) by dry weight in leaves with calculated yields equivalent to that of oil palm. Initial results identified a small biomass penalty and greatly reduced leaf starch content in high leaf oil plants. The aims of this study are to: (1) quantify germination and early growth; (2) characterise in detail the effects of TAG accumulation on leaf carbon and nitrogen partitioning; (3) investigate whether TAG breakdown can fuel metabolism. Although germination and relative growth rate were equivalent to wild-type in the high leaf oil plants, the plants were much smaller during early growth. Changes to plant development narrowed the gap between wild-type and high leaf oil plant biomass by flowering. TAG accumulation strongly influenced carbon and nitrogen partitioning and was also dependent on plant and leaf developmental stage. No evidence of synthesis and degradation of oil during dark/light cycles was found. Future experiments will test the influence of altered carbon reserves on the ability of high leaf oil plants to withstand abiotic stress. Understanding growth, development and TAG accumulation in high leaf oil plants will enable maximal oil production and help transfer this technology to other species.

### PC10.17 DOSAGE OF DUPLICATED AND ANTIFUNCTIONALIZED HOMEBOX PROTEINS INFLUENCES LEAF AND SPIKELET DEVELOPMENT IN BARLEY

WEDNESDAY 5 JULY, 2017 09:30

THIRULOGACHANDAR VENKATASUBBU (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), GEETHA GOVIND (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), TWAN RUTTEN (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), GOETZ HENSEL (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), JOCHEN KUMLEHN (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY), LENNART ESCHENLIPPOLD (LEIBNIZ INSTITUTE OF PLANT BIOCHEMISTRY, GERMANY), NESE SREENIVASULU (INTERNATIONAL RICE RESEARCH INSTITUTE, PHILIPPINES), MARKUS KUHLMANN (LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH, GERMANY)

VENKATASUBBU@IPK-GATERSLEBEN.DE

Spike architecture is one of the major domestication traits in barley (*Hordeum vulgare ssp. vulgare*), which also influences grain yield. Barley has two principal spike types: two-rowed forms and six-rowed forms governed by a single gene, *Six-rowed Spike 1* (*Vrs1*, *syn. HvHox1*) which belongs to homeodomain leucine zipper class I protein family. It was proposed that the *HvHox1* is only expressed in lateral spikelet meristems and act as a negative regulator of its development. It was also shown that the gene is the resultant of a recent gene duplication occurred only in tribe Triticeae and its paralogous gene is known as *HvHox2*. Here, we show *HvHox1* expression in central and lateral spikelet meristems but with a different dosage level. We also found that *HvHox1* and *HvHox2* have similar spatio-temporal but different dosage-dependent expression during spikelet and leaf development. Both proteins have similar dimerization and DNA binding properties, but exhibit differences in their transactivation property. Expression of these genes in barley leaves is the prime evidence of the wide leaf and narrow leaf phenotypes observed in six- and two-rowed barley plants, respectively. Overexpression of *HvHox2* under *HvHox1* promoter recovers (partially) the fertility of lateral spikelets in transgenic two-rowed barley, indicating that *HvHox2* is a positive regulator of spikelet development. Transcriptome analysis during early leaf development in six-rowed and two-rowed showed that *HvHOX1* suppresses cell proliferation in leaf cells. We exemplify that dosage of duplicated and antifunctionalized homeobox proteins influences leaf and spikelet development in barley.

### PC10.18 GENETICS OF ACHENE YIELD AND DROUGHT STRESS TOLERANCE RELATED TRAITS IN SUNFLOWER (HELIANTHUS ANNUUSL.)

WEDNESDAY 5 JULY, 2017 09:45

HUMERA RAZZAQ (PLANT BREEDING AND GENETICS UNIVERSITY OF AGRICULTURE FAISALABAD PAKISTAN, PAKISTAN), M. HAMMAD NADEEM TAHIR (PBG UNIVERSITY OF AGRICULTURE FAISALABAD, PAKISTAN), HAFEEZ AHAMD SADAQAT (PBG UNIVERSITY OF AGRICULTURE FAISALABAD, PAKISTAN), BUSHRA SADIA (CABB UNIVERSITY OF AGRICULTURE FAISALABAD, PAKISTAN)

HUMERARAZZAQ@GMAIL.COM

Sixty sunflower accessions were evaluated under Polyethylene glycol (PEG-6000) mediated drought stress. Selected tolerant and sensitive accessions were crossed in Line ' Tester fashion and resultant F1 along with their parents were evaluated for drought tolerance in field and PEG mediated drought in lab. Data were recorded on morphological and physiological parameters to estimate genetic variability, general and specific combining ability effects, heterosis manifestation, gene action, correlation and path analyses. Genetic variation among the entries under normal and drought stress treatments, indicated that this breeding material may be used for the development of drought tolerant types. Combining ability analysis exhibited variable direction and magnitude of general combining (GCA) effects among line and testers and specific combining ability effects (SCA) among crosses. The lines A-23, G-33 and 017583 and testers HA-133 and 017577 were best general combiners under normal and drought stress treatments. Results of SCA indicated that crosses A-79 ' HA-342, 017592 ' HA-133 and A-79 ' HA-124 were best specific combiners. These crosses had also mid parent, better parent and commercial heterosis for various traits under treatments. Additive type of gene action was observed for germination percentage, days to 50% flowering, days to 50% maturity, stem diameter and oil content while other traits showing non-additive gene action. Association of traits based on correlation and path analyses suggested that leaf area, seedling height and head diameter might be used as criteria for selection of sunflower for drought tolerance and high achene yield.

## PC10.19 CROP WILD RELATIVES AS A RESOURCE FOR GENERATING LOW-CYANIDE, DROUGHT-TOLERANT *SORGHUM*

📅 WEDNESDAY 5 JULY, 2017 ⌚ 10:00

👤 MAX COWAN (MONASH UNIVERSITY, AUSTRALIA), CECILIA BLOMSTEDT (MONASH UNIVERSITY, AUSTRALIA), ROS GLEADOW (MONASH UNIVERSITY, AUSTRALIA)

@ MAX.COWAN@MONASH.EDU

*Sorghum bicolor* is the 5<sup>th</sup> largest cereal crop in terms of global use, grown in semi-arid regions for the grain and as forage for livestock. Despite being relatively drought-tolerant, it is still susceptible to severe stress at terminal growth stages, and there is commercial interest to ensure the crop is resilient to more frequent and severe instances of drought under climate change. In addition, concentration of the cyanogenic glucoside dhurrin, precursor to the production of hydrogen cyanide, has been observed to increase in *S. bicolor* under drought stress, up to levels potentially toxic to cattle. Wild relatives of many crop genera have been used to improve crop tolerance to abiotic stress. Several wild *Sorghum* species occur natively in arid regions, suggesting high drought tolerance, and it is unknown whether they are also cyanogenic. Nineteen species of wild *Sorghum* were grown under controlled conditions and the cyanide potential tested at different developmental stages. All produced lower levels of dhurrin compared to *S. bicolor*, with some species being completely acyanogenic. Based on cyanogenic status and environmental distribution, seven species were selected to investigate drought tolerance and its effects on dhurrin production compared to *S. bicolor*. Plants were grown in pots under well-watered (control) and drought-stressed conditions (25-15% soil water content). Plants were monitored and weighed every 2<sup>nd</sup> day and destructively harvested 6 weeks post-germination. Biomass, transpiration rate, photosynthetic parameters and cyanide potential were measured for each species. Studies of wild relatives provide an alternative approach in identifying traits of agronomic interest.

## PC10.20 NITROGEN APPLICATION REVERSES HEAT-INDUCED RICE CHALKINESS: EVIDENCE FOR ORGANELLE REARRANGEMENT DUE TO THE RECOVERY OF PROTEIN SYNTHESIS IN ENDOSPERM CELLS

📅 WEDNESDAY 5 JULY, 2017 ⌚ 10:15

👤 YUTO HATAKEYAMA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), YAYOI ONDA (EHIME UNIVERSITY, JAPAN), HIROSHI NONAMI (EHIME UNIVERSITY, JAPAN), TAIKEN NAKASHIMA (HOKKAIDO UNIVERSITY, JAPAN), HIROSHI NAKANO (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), ROSA ERRA-BALSELLS (UNIVERSITY OF BUENOS AIRES, ARGENTINA), KENZO HIRAOKA (THE UNIVERSITY OF YAMANASHI, JAPAN), HIROSHI WADA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN)

@ HWADA@AFFRC.GO.JP

High temperature during grain filling increases rice chalkiness, although chalkiness is partially reversed by nitrogen application. Air spaces formed in the endosperm cells cause a random light reflection to create chalk; however, what occurs in the cells remains unclear. By combining newly developed pico pressure-probe-electrospray-ionization mass spectrometry (picoPPESI-MS) with a transmission electron microscopy, our hypothesis that heat preserves protein storage vacuoles (PSVs), and that PSVs might be reversed by nitrogen supply, resulting in normal development of protein body (PB-II) to partially reduce air spaces was tested. Microscopic observations showed that PSVs were preserved among amyloplasts heterogeneously developed in size, indicating an inhibition of protein synthesis. When nitrogen was supplied prior to the heat treatment, development of amyloplasts and PB-II recovered, resulting in a remarkable decline in air spaces, turning to translucent. Metabolite profiling with picoPPESI-MS characterised the difference of cellular metabolites under each treatment condition. Heat increased the content of ascorbate, glutathione, and amino acids, particularly cysteine. Contrastingly, nitrogen treatment under heat conditions showed much less cysteine content than just heat, but with higher sugar accumulation, suggesting that cysteine was consumed to form disulphide bonds during osmotic adjustment to sustain PB-II development even under heat conditions, consistent with the microscopic observations. Hence, it is concluded that nitrogen application would reverse the inhibition of protein synthesis, resulting in the normal formation of amyloplasts and PB-II. Our findings also suggest that preservation of PSVs among amyloplasts inadequately formed in the cells accounts for air space formation to cause chalkiness.

## PC10.21 SECONDARY METABOLITE PROFILES, FREE-RADICAL SCAVENGING ACTIVITY AND ANTIMICROBIAL POTENTIAL OF ETHANOL EXTRACTS FROM LEAVES, STEMS, AND ROOTS OF VETIVER GRASS [*CHRYSOPOGON ZIZANIOIDES*(L.) ROBERTY]

WEDNESDAY 5 JULY, 2017 11:00

JULIANA JANET M PUZON (INSTITUTE OF BIOLOGY COLLEGE OF SCIENCE UNIVERSITY OF THE PHILIPPINES DILIMAN, PHILIPPINES), JOSE RAPHAEL D FUDOLIG (INSTITUTE OF BIOLOGY COLLEGE OF SCIENCE UNIVERSITY OF THE PHILIPPINES DILIMAN, PHILIPPINES)

JANETMPUZON@GMAIL.COM

Vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] leaves, stems, and roots were investigated for their phytochemical constituents, as well as antioxidant, toxic, and antimicrobial activities. Phytochemical profiles showed that the leaf had the most diverse classes of secondary metabolites, while the root had the fewest. Quantification of total phenolics and flavonoids confirmed the results of the phytochemical screening. Results of the DPPH assay showed that the leaf extract exhibited 48.78% free-radical scavenging activity (FRSA) relative to gallic acid (control). This is the highest FRSA among the extracts from the vegetative parts, compared to 32.63% and 21.11% FRSA of stem and root extracts, respectively. Moreover, the brine shrimp lethality assay determined that the LC<sub>50</sub> of the root extract (47.57 µg/mL) was not significantly different from the LC<sub>50</sub> of the positive control, indicating high toxicity of the root extract. Leaf and stem extracts had higher LC<sub>50</sub> values (470.68 µg/mL and 380.54 µg/mL, respectively). The disc diffusion assay showed that the root extract exhibited growth inhibition against both *S. aureus* and *E. coli*, with greater extent against *S. aureus*. The stem extract exhibited growth inhibition against *S. aureus*. In conclusion, all the vegetative parts of the Philippine grown vetiver demonstrated various bioactivities, such as antioxidant and antimicrobial activities, including toxicity. Furthermore, locally grown vetiver has unique phytochemical profiles, compared to the profiles of the plant species grown in other countries. The findings of this study point to the potential of vetiver as a promising source of bioactive metabolites which can be developed into pharmaceuticals and drugs.

## PC10.22 A KEY ROLE FOR APOPLASTIC H<sub>2</sub>O<sub>2</sub> IN NORWAY SPRUCE PHENOLIC METABOLISM REVEALED BY TRANSCRIPT AND METABOLITE PROFILING

WEDNESDAY 5 JULY, 2017 11:15

ANNA KÄRKÖNEN (DEPT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI NATURAL RESOURCES INSTITUTE FINLAND (LUKE), FINLAND), TERESA LAITINEN (DEPT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI NATURAL RESOURCES INSTITUTE FINLAND (LUKE), FINLAND), TERESA LAITINEN (DEPARTMENT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI, FINLAND), KRIS MORREEL (GHENT UNIV. DEPT OF PLANT BIOTECHNOLOGY AND BIOINFORMATICS VIB CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), NICOLAS DELHOMME (UPSC DEPT OF FOREST GENETICS AND PLANT PHYSIOLOGY SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), ADRIEN GAUTHIER (DEPARTMENT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI, FINLAND), BASTIAN SCHIFFTHALER (UPSC DEPT OF FOREST GENETICS AND PLANT PHYSIOLOGY SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), KALOIAN NICKOLOV (DEPARTMENT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI DEPT OF BIOLOGY UNIVERSITY OF OULU, FINLAND), GÜNTER BRADER (DEPARTMENT OF BIOSCIENCES UNIVERSITY OF HELSINKI AUSTRIAN INSTITUTE OF TECHNOLOGY GMBH, FINLAND), KEAN JIN LIM (DEPARTMENT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI, FINLAND), TEEMU H TEERI (DEPARTMENT OF AGRICULTURAL SCIENCES UNIVERSITY OF HELSINKI, FINLAND), NATHANIEL R STREET (UMEÅ PLANT SCIENCE CENTRE DEPARTMENT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN), WOUT BOERJAN (GHENT UNIV. DEPT OF PLANT BIOTECHNOLOGY AND BIOINFORMATICS VIB CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM)

ANNA.KARKONEN@LUKE.FI

Apoplastic events such as monolignol oxidation and lignin polymerisation are difficult to study in intact trees. In an extracellular lignin-forming cell suspension culture of Norway spruce (*Picea abies* L. Karst.) used as a model, H<sub>2</sub>O<sub>2</sub> is required for lignin production as its scavenging by potassium iodide represses lignin formation. This observation suggests that in this cell culture peroxidases activate monolignols for lignin polymerisation. In order to investigate the effects of modulation of apoplastic H<sub>2</sub>O<sub>2</sub> levels on phenolic metabolism and lignification, and to identify genes encoding isoenzymes (e.g. oxidative enzymes, transcription factors) specific for lignin biosynthesis, we conducted large-scale phenolic, metabolomic and transcriptomic analyses. Time course analyses coupled to candidate substrate product pair network propagation revealed differential accumulation of low-molecular-weight phenolic compounds, including (glycosylated) oligolignols, (glycosylated) flavonoids and proanthocyanidins, in lignin-forming and H<sub>2</sub>O<sub>2</sub>-scavenging cultures, and supported that in addition to cell wall, oxidative coupling of monolignols occurs also within the cytoplasm. Some dilignol glycoconjugates with reduced structures found in the medium can be formed only by intracellular enzymes suggesting that spruce cells can transport glycosylated dilignols into the apoplast. Scavenging of apoplastic H<sub>2</sub>O<sub>2</sub> resulted in remodulation of the transcriptome, leading to a reduced carbon flux into the shikimate pathway propagating down to monolignol biosynthesis. Aggregated co-expression network

analysis identified candidate enzymes and transcription factors for monolignol oxidation and apoplastic  $H_2O_2$  production as well as potential  $H_2O_2$  receptors. The results indicate that the redox state of the apoplast has a profound influence on cellular metabolism.

### PC10.23 DEVELOPMENT OF THE ON-SITE LIVE CELL METABOLOMICS PERFORMABLE IN CONTROLLED ENVIRONMENT

WEDNESDAY 5 JULY, 2017 11:30

HIROSHI WADA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), HIROSHI NONAMI (EHIME UNIVERSITY, JAPAN), KATSUNORI TANAKA (TSUBUKU CORPORATION LTD., JAPAN), TAIKEN NAKASHIMA (HOKKAIDO UNIVERSITY, JAPAN), YUTO HATAKEYAMA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), HIROSHI NAKANO (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), YAYOI ONDA (EHIME UNIVERSITY, JAPAN), KENZO HIRAOKA (THE UNIVERSITY OF YAMANASHI, JAPAN), MAKOTO HAKATA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN), SATOSHI MORITA (NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION, JAPAN)

HWADA@AFFRC.GO.JP

In this work, a new cell metabolomics was designed to allow on-site determination of cell-specific metabolite profiling in plants growing under controlled environment. The system was composed of pico pressure-probe-electrospray-ionization mass spectrometry (picoPPESI-MS) and two measurement rooms individually attached to walk-in environmentally-controlled growth chambers. The MS itself was placed on the carriage, which was movable between two rooms along the rails laid on the laboratory floor, so that the MS could easily access to each room to be effectively used for the analysis. The combination of the environmental control and picoPPESI-MS allowed us to directly perform on-site metabolites profiling real-time in the target cells without any pretreatments under each set environmental condition, together with the determination of cell water relations parameters (e.g. cell turgor). Adopting light emitting plasma with natural light spectrum in the chambers led to reproduce extreme weather conditions, such as heat conditions in the fields. When this analytical method was used for investigating heat-induced chalkiness in rice plants treated at high temperature (34°C), the system was capable of sampling a picoliter cell sap from the attached tissues with a high spatial-resolution, precisely manipulating at postsampling, and characterizing the metabolites in real time with a high detection sensitivity with a good reproducibility. Additionally, the system could accurately identify differences in the metabolites among treatments. Hence, this method would be a highly robust approach without causing any temperature disturbance during sampling and potentially applicable to a number of cell-specific research, including heat-related damages in rice plants.

### PC10.24 EFFECT OF NUCLEAR MAGNETIC RESONANCE ON THE CIRCADIAN CLOCK AND THE HYPOXIA SIGNALLING PATHWAY

WEDNESDAY 5 JULY, 2017 13:50

REGINA OLIVA (UNIVERSITY OF INNSBRUCK, AUSTRIA), MARGIT EGG (UNIVERSITY OF INNSBRUCK, AUSTRIA)

REGINA.OLIVA@UIBK.AC.AT

The circadian clock and the hypoxia signalling pathway interact bidirectionally, and both are important targets for several diseases, such as osteoarthritis or cancer. Nuclear magnetic resonance (NMR) has been used as therapy for the treatment of human osteoarthritis and osteoporosis, but the biological effects on living systems, such as cells or organisms are still not clearly understood. We therefore analysed the impact of NMR treatment on the zebrafish cell line Z3, which is well characterised in terms of circadian rhythmicity and hypoxic signalling. Both pathways are highly conserved across the animal kingdom and play major roles in the development and progression of osteoarthritis. Our results revealed that the circadian rhythm of cryptochrome1 and period1 showed a phase shift in the NMR group together with a significantly increased gene expression of period1 which clearly demonstrates an effect of NMR on the circadian clock of Z3 cells. Furthermore, protein expression of Hif1, the major regulator of hypoxia signalling pathway, and Peroxiredoxin- $SO_3$ , an antioxidant for tuning the cellular redox status, was as well significantly altered after NMR exposure. In conclusion, NMR leads to a phase shift of the transcriptional clock and, due to the bidirectional interaction of both pathways, also to altered Hif signalling. This study gives further indications of the molecular mechanism of NMR exposure in a cell system.



## PC10.25 CIRCADIAN REGULATION OF PLANT RESPONSES TO HERBICIDES

WEDNESDAY 5 JULY, 2017 14:05

ANTONY N DODD (UNIVERSITY OF BRISTOL, UNITED KINGDOM), FIONA E BELBIN (UNIVERSITY OF BRISTOL, UNITED KINGDOM), GAVIN HALL (SYNGENTA JEALOTT'S HILL INTERNATIONAL RESEARCH CENTRE, UNITED KINGDOM), CARL FORMSTONE (SYNGENTA JEALOTT'S HILL INTERNATIONAL RESEARCH CENTRE, UNITED KINGDOM), KEARA A FRANKLIN (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

@ ANTONY.DODD@BRISTOL.AC.UK

The effectiveness of some herbicides depends on the time of day at which the herbicide is applied. Agriculturally, herbicides are sprayed at high concentrations multiple times per year to maximise weed control. Identifying a particular time of day at which herbicides can be sprayed, to maximise efficacy, could reduce the quantity of chemical used, reduce time and cost to consumers, while minimising potential environmental leaching. The mechanisms underlying the time of day variation in the efficacy of herbicides are unknown. The circadian oscillator regulates a vast number of plant processes, and we hypothesize that circadian regulation has a major role in plant responses to herbicides. By combining the experimental power of *Arabidopsis thaliana* with a broad range of approaches including chlorophyll fluorescence, bioluminescence imaging, RNA sequencing, and developmental phenotyping, the processes underlying time of day variation in herbicide efficacy is being investigated for formulations containing glyphosate, mesotrione, and terbuthylazine. The mechanisms causing these time of day responses are being investigated, including the extent of involvement of the circadian clock and phytohormone signalling. We are exploiting these results from *Arabidopsis* to determine the extent of conservation and homology of responses between *Arabidopsis* and common agricultural weeds.

## PC10.26 CIDER-SEQ: AN UNBIASED LONG-READ ENRICHMENT SEQUENCING APPROACH FOR ACCURATE DEEP SEQUENCING OF CIRCULAR DNA VIRUSES

WEDNESDAY 5 JULY, 2017 14:20

DEVANG MEHTA (INSTITUTE OF MOLECULAR PLANT BIOLOGY ETH ZURICH, SWITZERLAND), MATTHIAS HIRSCH-HOFFMANN (INSTITUTE OF MOLECULAR PLANT BIOLOGY, ETH ZURICH, SWITZERLAND), WILHELM GRUISSEM (INSTITUTE OF MOLECULAR PLANT BIOLOGY ETH ZURICH, SWITZERLAND), HERVÉ VANDERSCHUREN (UNIVERSITY OF LIEGE, BELGIUM)

@ DEVANG@ETHZ.CH

Geminiviruses are circular ssDNA viruses that cause a significant amount of crop damage worldwide, infecting a wide variety of crop plants such as maize, tomato and cassava among others. Geminivirus families are large, diverse and comprise numerous species sharing a high degree of sequence similarity. This presents a unique challenge in sequencing geminivirus populations at depth since conventional short-read NGS methods are unable

to accurately assemble real virus genomes accurately. Further, Sanger-sequencing based methods used in the past are limited since they resort to sequence-based enrichment, oftentimes resulting in biased virus population sequencing. We present a novel SMRT-sequencing based method which can highly enrich circular ssDNA molecules without any sequence bias and provide full length, accurate virus sequences at depth. We have validated our method (christened CIDER-seq for Circular DNA Enrichment) in cassava samples collected from fields in West and East Africa and have resolved the populations of geminiviral isolates present. Since our enrichment method is not sequence dependent, we envision its broader use in virus metagenomics for other circular DNA viruses infecting plants, bacteria and vertebrates.

## PC10.27 ELECTROPHORETIC PROFILE AND HERITABILITY OF PEROXIDASIC ACTIVITIES IN THE TOLERANCE OF THEOBROMA CACAO AGAINST PHYTOPHTHORA MEGAKARYA, THE MOST AGGRESSIVE AGENT OF BLACK POD DISEASE

WEDNESDAY 5 JULY, 2017 14:35

CLAUDE SIMO (UNIVERSITY OF DOUALA, CAMEROON), FRANÇOIS P DJOCGOUÉ (UNIVERSITY OF YAHOUND, CAMEROON), EMILE MINYAKA (UNIVERSITY OF DOUALA, CAMEROON), DENIS N OMOKOLO (UNIVERSITY OF YAHOUND, CAMEROON)

@ SIMOCLAUDE@UNIV-DOUALA.COM

Black pod disease caused by *Phytophthora megakarya* is the main limiting factor in the production of cocoa. Plants exposed to biotic stresses exhibit changes in their physiology and metabolism. The study focused on the heritability and activity of peroxidases (POX) in two hybrid populations F13 (♀SNK13 x ♂T79/467) and F79 (♀T79/467 x ♂SNK13). The results show that more tolerant and more productive hybrid genotypes (F1307, F1314, F7902, F7928) and more tolerant genotypes (F1315, F1313, F7926, F7907) relative to the best parent in the soluble (S) and bound fractions (L) recorded a large amount of POX activity with small areas of necrotic lesion in contrast to less tolerant and productive genotypes (F1324, F1308, F7915 and F7919) which accumulated a small amount of POX activity in large areas of necrotic lesion. A negative and significant correlation ( $P < 0.01$ ) was observed between the development of necrosis and peroxidase activities. The profile of peroxidase isoforms (S) of the mesocarp of infected pods revealed the existence of a specific form (A2) after infection in tolerant genotypes. This isoform is linked to tolerance. The heritability values of POX activity obtained in fractions (S) in the F13 family (♀SNK13 x ♂T79 / 467) in the F79 family (♀T79 / 467 x ♂SNK13) are relatively high (0.69 and 0.64). The existing isoform (A2) in hybrid tolerant genotypes could lead in the creation of more productive and tolerant varieties making it available to farmers.

Key words: *Theobroma cacao*, *Phytophthora megakarya*, black pod disease, heritability, tolerance, peroxidases.

## PC10.28 TO GROW OR NOT TO GROW – TRANSCRIPTIONAL RESPONSES UNDERLYING REDUCED LATERAL ROOT DEVELOPMENT UNDER SALT STRESS CONDITIONS

WEDNESDAY 5 JULY, 2017 14:50

MAGDALENA MARIA JULKOWSKA (KING ABDULLAH UNIVERSITY FOR SCIENCE AND TECHNOLOGY, SAUDI ARABIA), YONG WO (KING ABDULLAH UNIVERSITY FOR SCIENCE AND TECHNOLOGY, SAUDI ARABIA), AYMAN EID (KING ABDULLAH UNIVERSITY FOR SCIENCE AND TECHNOLOGY, SAUDI ARABIA), CHRISTA TESTERINK (UNIVERSITEIT VAN AMSTERDAM, NETHERLANDS), MARK A TESTER (KING ABDULLAH UNIVERSITY FOR SCIENCE AND TECHNOLOGY, SAUDI ARABIA)

@ MAGDALENA.JULKOWSKA@KAUST.EDU.SA

Salt stress rapidly impacts the growth and development of different plant organs to different extents, leading to alterations in plant development and architecture. We hypothesize that those alterations reflect early responses to salt stress and provide significant contributions to salinity tolerance. Previous studies describe that high expression of *Arabidopsis* High Affinity K<sup>+</sup> Transporter (HKT1;1), sequestering sodium from the transpiration stream in root pericycle, results in increased salinity tolerance. Our preliminary results show that pericycle specific overexpression of HKT1 results in reduced lateral root development under saline conditions. Interestingly, this effect seems to be specific to dicot species, as wheat lines with high HKT1 expression do not show reduction in secondary lateral roots. In our study we identified the transcriptional regulation of lateral root development downstream of HKT1 through comparative transcriptomic analysis of two lines with stelar overexpression of HKT1. Among the transcripts that were expressed differentially between HKT1 overexpression lines and their background lines, we found genes involved in cell differentiation, ABA signaling and transport. Further validation of identified candidate genes will enhance our understanding of processes controlling plant development under abiotic stress conditions and the importance of root architecture for salinity tolerance.

## PC10.29 PLANTLINK – A PLANT SCIENCE NETWORK FOR EDUCATION, RESEARCH AND INNOVATION

WEDNESDAY 5 JULY, 2017 15:05

ERIK ALEXANDERSSON (DEPARTMENT OF PLANT PROTECTION SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), TINA D'HERTEFELDT (DEPARTMENT OF BIOLOGY LUND UNIVERSITY, SWEDEN)

@ ERIK.ALEXANDERSSON@SLU.SE

PlantLink is a research network in the area of plant sciences in Southern Sweden formed in 2011 by Lund University and the Swedish University of Agricultural Sciences in Alnarp (SLU Alnarp) with initial financial support from the Skåne Regional Council. PlantLink has about 30 associated principle investigators, but the larger network gathers well above 300 participants, also researchers in private enterprises. Associated to PlantLink, are several research groups in the forefront of plant science. We want to create an internationally leading research environment for plant science within our focus areas. Activities within PlantLink should be attractive to foreign researchers and students, and help young researchers to establish themselves. The long-term goal is to secure skills and knowledge for research leading to a profitable, sustainable and environmentally friendly agriculture.

To achieve our objectives, we have over the first years supported 18 'seed money' projects to promote new collaborations, which has led to major successive grants of around €8 million, and 12 peer-reviewed publications. Furthermore, our PlantLink bioinformatician has supported more than 20 projects leading to more than 15 publications. PlantLink has arranged 28 meetings and workshops, supported eight plant-specific PhD courses, and run three rounds of its industrial mentorship program. PlantLink is active in social media and has a monthly newsletter. We are continuously interacting with other research networks in the Scandinavian countries and the world and sponsors the founding universities membership in the European Plant Science Organisation (EPSO). We participate in public outreach events such as Fascination of Plants Day.

### PC10.30 ASSESSING THE ROLE OF IAA INACTIVATION ON AUXIN HOMEOSTASIS IN PLANTS

WEDNESDAY 5 JULY, 2017 15:45

RUBÉN CASANOVA-SÁEZ (UMEÅ PLANT SCIENCE CENTRE-SLU, SWEDEN), FEDERICA BRUNONI (UMEÅ PLANT SCIENCE CENTRE-UMEÅ UNIVERSITY, SWEDEN), ALEŠ PĚNČÍK (PALACKÝ UNIVERSITY INSTITUTE OF EXPERIMENTAL BOTANY CAS, CZECH REPUBLIC), ONDŘEJ NOVÁK (PALACKÝ UNIVERSITY INSTITUTE OF EXPERIMENTAL BOTANY CAS, CZECH REPUBLIC), MICHAL KARADY (UMEÅ PLANT SCIENCE CENTRE-SLU, SWEDEN), CATHERINE BELLINI (UMEÅ PLANT SCIENCE CENTRE-UMEÅ UNIVERSITY, SWEDEN), KARIN LJUNG (UMEÅ PLANT SCIENCE CENTRE-SLU, SWEDEN)

@ RUBEN.CASANOVA.SAEZ@SLU.SE

Indole-3-acetic acid (IAA), the main auxin in plants, is an important hormone that regulates numerous processes in plant growth and development. IAA-mediated growth depends on the continual formation IAA maxima and minima along plant tissues, which are achieved by transport, biosynthesis, conjugation and degradation mechanisms. Whilst significant progress has been made in understanding auxin transport and biosynthesis, the control of auxin homeostasis by IAA conjugation and degradation remains less well understood.

Here we present the identification of the DIOXYGENASE FOR AUXIN OXIDATION 1 (AtDAO1) enzyme, which catalyzes the major pathway for auxin degradation in *Arabidopsis thaliana*. Loss-of-function mutations in AtDAO1 blocked IAA oxidation and elevated the levels of the auxin conjugates IAA-Asp and IAA-Glu, while IAA levels remained close to wild type. Our data from *in vivo* labelling, IAA metabolite profiling, gene expression analysis and mathematical modelling experiments consistently indicate that IAA conjugation and oxidation pathways redundantly maintain IAA homeostasis in plant tissues.

To understand the importance of IAA inactivation for auxin homeostasis during plant evolution, we are now studying the IAA inactivation pathways in *Picea abies* (Norway spruce). Our preliminary results indicate that oxIAA is a native metabolite in spruce seedlings, and that it accumulates after IAA application. IAA-Asp and IAA-Glu are present in much higher concentrations than oxIAA, suggesting that IAA is preferably inactivated by conjugation rather than by oxidation in spruce. Moreover, we identified putative spruce homologs to AtDAO1 and we are currently investigating which of these genes accounts for IAA oxidation in spruce.

### PC10.31 LIGHT CONTROLS CYTOKININ-RELATED DEVELOPMENT VIA ACTIVITY OF CKI1

WEDNESDAY 5 JULY, 2017 16:00

TEREZA DOBISOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), VENDULA HRDINOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), CANDELA CUESTA (IST AUSTRIA, AUSTRIA), SARKA MICHLICKOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), IVANA URBANKOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), ROMANA HEJATKOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), PETRA ZADNIKOVA (MASARYK UNIVERSITY AND IST AUSTRIA, CZECH REPUBLIC), MARKETA PERNISOVA (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC), EVA BENKOVA (MASARYK UNIVERSITY AND IST AUSTRIA, AUSTRIA), JAN HEJATKO (MASARYK UNIVERSITY CEITEC, CZECH REPUBLIC)

@ DOBISOVA@LABDEERS.COM

Cytokinin responses in plants are mediated via a multistep phosphorelay (MSP) pathway. The constitutively active sensor histidine kinase CYTOKININ INDEPENDENT-1 (CKI1) acts through the MSP to execute its vital regulatory role in the control of female gametophyte and vasculature development. However, the upstream factors controlling CKI1 remain elusive. Using forward genetic approach we identified *hy1-7*, a new mutant allele in *HEME OXYGENASE 1 (HY1)* gene, whose activity is indispensable for spatiotemporally correct *CKI1* expression pattern. *HY1* locus encodes a heme oxygenase involved in biosynthesis of P $\Phi$ B, a chromophore molecule essential for functional light receptors phytochromes (phys). Our analysis confirmed the phytochrome-dependent light regulation of *CKI1* expression. Changes in *CKI1* expression pattern observed in *hy1-7* and phy mutants correlate with misregulation of MSP signaling, changed cytokinin sensitivity and developmental aberrations associated with cytokinin and/or *CKI1* action. Our results show that phytochrome A (phyA) is functioning as both positive and negative regulator of *CKI1* expression and presumably controls CKI1 via light-controlled transcription factors PHYTOCHROME INTERACTING FACTOR 3 (PIF3) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), both of which we have shown to recognize *CKI1* promoter. We also demonstrate novel role of phyA-dependent *CKI1* expression in the hypocotyl elongation and hook development during skotomorphogenesis. Based on these results, we propose the light-dependent regulation of *CKI1* as a plausible mechanistic link underlying the well-known interaction between light- and cytokinin-controlled plant development. Supported by LQ1601, 15-22000S and 13-25280S.

## PC10.32 FLORAL HETEROMORPHY IN PRIMULA: NEW INSIGHTS FOR AN OLD MODEL

WEDNESDAY 5 JULY, 2017 16:15

JONATHAN M COCKER (JOHN INNES CENTRE, UNITED KINGDOM), JINHONG LI (JOHN INNES CENTRE, UNITED KINGDOM), JONATHAN WRIGHT (EARLHAM INSTITUTE, UNITED KINGDOM), MARGARET A WEBSTER (UNIVERSITY OF EAST ANGLIA, UNITED KINGDOM), MARK McMULLAN (EARLHAM INSTITUTE, UNITED KINGDOM), SARAH DYER (NATIONAL INSTITUTE OF AGRICULTURAL BOTANY, UNITED KINGDOM), DAVID SWARBRECK (EARLHAM INSTITUTE, UNITED KINGDOM), MARIO CACCAMO (NATIONAL INSTITUTE OF AGRICULTURAL BOTANY, UNITED KINGDOM), COCK VAN OOSTERHOUT (UNIVERSITY OF EAST ANGLIA, UNITED KINGDOM), PHILIP M GILMARTIN (UNIVERSITY OF EAST ANGLIA, UNITED KINGDOM)

JONATHAN.COCKER@JIC.AC.UK

The genetic and evolutionary basis of floral heteromorphy in *Primula* has been debated for over 150 years. Charles Darwin first demonstrated the significance of the two floral morphs, pin and thrum, with their reciprocal anther and stigma heights, by showing that within-morph crosses result in reduced seed set. This key innovation, which serves to physically promote insect-mediated out-crossing, evolved independently in over 28 angiosperm families.

The *Primula S* locus, which regulates heterostyly and self-incompatibility, is portrayed as a 'supergene', a cluster of tightly-linked genes inherited as an allelic unit. It is predicted that self-fertile homostyle flowers, with anthers and stigma at the same height, arise via rare recombination events between dominant and recessive alleles in heterozygous thrums; such studies contributed to the foundation of modern genetic theory and constitute a model that underpins over 60 years of research into heterostyly.

We show that the *S* locus is hemizygous in thrums, not heterozygous, comprising five thrum-specific genes that are absent from pins; homostyles are therefore a result of mutation, not recombination. Our assembly of the *Primula vulgaris* genome allowed us to identify the *S* locus genes and surrounding genomic regions, estimate the assembly of the supergene at 51.7 MYA, and reveal conserved genetic architecture across the Primulaceae. These findings represent novel insight into the structure and origin of the *Primula S* locus, providing a platform for identification and evolutionary analysis of the genes and downstream pathways that regulate outcrossing in *Primula* and other heterostylous species.

## PC10.33 UNRAVELLING THE FUNCTION OF THE RICE ORTHOLOGUES OF THE F-BOX GENE HAWAIIAN SKIRT (HWS) IN PLANT DEVELOPMENT

WEDNESDAY 5 JULY, 2017 16:30

RITA SARAH BORNA (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JEREMY A ROBERTS (UNIVERSITY OF PLYMOUTH, UNITED KINGDOM), ZINNIA H GONZALEZ-CARRANZA (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM)

ZINNIA.GONZALEZ@NOTTINGHAM.AC.UK

The F-box gene *HAWAIIAN SKIRT* (*HWS*) plays a significant role in overall plant development and timing of floral abscission in *Arabidopsis*<sup>1</sup>. So, the investigation of the role of *HWS* orthologues in rice (*Oryza sativa*) is important for crop improvement.

*ERECTA PANICLE3* (*EP3*)<sup>3</sup> and *Oryza sativa* *HAWAIIAN SKIRT* (*OsHWS*) genes of rice are the functional orthologues of *HWS* from *Arabidopsis* (Gonzalez et al, unpublished data). The loss of function mutant *ep3* creates an erect panicle phenotype in rice<sup>3</sup>. *EP3* also plays a role in regulating stomatal guard cell development<sup>2</sup>.

To further understand the role of these genes in rice development, the present study involves the generation of a series of transgenic lines including reporter promoter fusions, loss of function mutants, downregulation and ectopic expression. *EP3pro::UTR::GFP* and *OsHWSpro::UTR::RFP* lines show co-expression in several organs and tissues. These results will be presented.

<sup>1</sup> Gonzalez-Carranza ZH, Rompa U, Peters JL, Bhatt AM, Wagstaff C, Stead AD and Roberts JA (2007). *HAWAIIAN SKIRT* - an F-box gene that regulates organ fusion and growth in *Arabidopsis*. *Plant Physiology*. 144: 1370-1382

<sup>2</sup> Yu H, Murchie EH, González-Carranza ZH, Pyke KA and Roberts JA (2015). Decreased photosynthesis in the erect panicle 3 (*ep3*) mutant of rice is associated with reduced stomatal conductance and attenuated guard cell development, *J Exp Bot*. 66: 1543-1552. doi:10.1093/jxb/eru525

<sup>3</sup> Piao R, Jiang W, Ham TH, Choi MS, Qiao Y, Chu SH, Park JH, Woo MO, Jin Z, An G, Lee J and Koh HJ (2009) Map-based cloning of the *ERECT PANICLE3* gene in rice. *Theor Appl Genet*. 119: 1497-1506

### PC10.34 PHYTOCHROME-MEDIATED RED:FAR-RED LIGHT SIGNALING IN THE SHOOT CONTROLS ROOT DEVELOPMENT IN ARABIDOPSIS

WEDNESDAY 5 JULY, 2017 16:45

CHIAKAI KANG (PLANT ECOPHYSIOLOGY INSTITUTE OF ENVIRONMENTAL BIOLOGY UTRECHT UNIVERSITY, NETHERLANDS), KASPER VAN GELDEREN (PLANT ECOPHYSIOLOGY INSTITUTE OF ENVIRONMENTAL BIOLOGY UTRECHT UNIVERSITY, NETHERLANDS), RONALD PIERIK (PLANT ECOPHYSIOLOGY INSTITUTE OF ENVIRONMENTAL BIOLOGY UTRECHT UNIVERSITY, NETHERLANDS)

@ C.KANG@UU.NL

Plants use light quality changes to detect competing neighbors. This occurs through detection of Far-Red (FR) light reflected from nearby leaves that lowers the Red:Far-Red (R:FR) ratio and induces the shade avoidance syndrome (SAS). These include shoot elongation to grow towards the light, upward leaf movement early flowering. Although these responses are well studied, very little is known about effects of light quality on root development. To study this, we used a vertical agar plate system where the root system was shielded from light. We show that low R:FR exposure of the shoot suppresses lateral root emergence, and slightly inhibits primary root length. This process is mediated by a mobile signal from the shoot to the root upon low R:FR. Outgrowth of the lateral root primordia is subsequently halted in a PIN3 and LAX3-dependent manner, thus interfering with auxin transport in the cortex cells overlaying the primordia. As a result, lateral root emergence is arrested.

### PC10.7 LEAF PHYSIOLOGY AND THE DISTRIBUTION OF CAM TANK-EPIPHYTE BROMELIADS AT MULTIPLE SPATIAL SCALES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

JAMIE MALES (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), HOWARD GRIFFITHS (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

@ JAMIE\_MALES@HOTMAIL.COM

Trait-mediated divergences in bioclimatic relations could represent an important mode for the evolution of plant diversity, but this effect is sparsely documented. To investigate this topic, we examined the distributions of epiphytic CAM tank-bromeliads of the genus *Aechmea* across the Northern Range of Trinidad (West Indies).

Field surveys were carried out to map species distributions. Structural-functional characterisation of representative plants was undertaken, and species distribution modelling (SDM) performed to determine which environmental factors and species traits are involved in niche segregation. Modelled projections of distributions on Tobago were ground-truthed, and projections of distributions under an aggressive 2070 climate scenario were performed.

Distribution mapping highlighted distinct elevational and latitudinal effects on species presence and abundance, with some evidence for vegetational effects on distributions. Bioclimatic differentiation captured by SDM was associated with contrasting anatomical and physiological traits. Projected distributions under the 2070 scenario suggested extreme vulnerability to upslope shifting of bioclimate envelopes in montane species.

At a regional scale, the distributions of CAM tank-epiphyte bromeliads are strongly influenced by broad environmental gradients acting on species structural and functional traits. Topography generates a complex climatic mosaic that modulates general patterns. At the landscape scale, vegetational composition can be an important factor. Within individual canopies, phenotypic plasticity leads to only weak differentiation in microhabitat preferences. This investigation highlights how interspecific variation in leaf trait complexes is an important and underexplored contributor to bromeliad ecological diversity. Climate change could lead to loss of suitable habitat for more environmentally-specialised species, potentially leading to extinctions.

### PC10.8 STICKY NET-WORK: THE SPATIAL STRUCTURE OF THE SEED MUCILAGE ENVELOPE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

AGNIESZKA KREITSCHITZ (ZOOLOGICAL INSTITUTE: FUNCTIONAL MORPHOLOGY AND BIOMECHANICS KIEL UNIVERSITY, GERMANY), STANISLAV N GORB (ZOOLOGICAL INSTITUTE: FUNCTIONAL MORPHOLOGY AND BIOMECHANICS KIEL UNIVERSITY, GERMANY)

@ AGNIESZKA.KREITSCHITZ@UNI.WROC.PL

Mucilage secreting cells (MSCs) are characteristic element of fruit and seed coat and are able to produce the mucilage envelope after hydration. This phenomenon is an important adaptation to drought habitats in many families of angiosperms. Mucilage envelope is a complex of three main polysaccharides i.e. pectins, hemicelluloses and cellulose and is divided in different plant species into pectic and cellulose mucilage. The first one is characteristic of e.g. *Linaceae*. The second type is widely spread in different plant families like e.g. *Asteraceae*, *Lamiaceae* or *Brassicaceae*. The mucilage envelope is considered as rich in pectins, modified secondary cell wall. Due to the presence of pectins after hydration, the mucilaginous cell wall forms an easily accessible, gel-like envelope. Using Critical Point Drying and SEM we demonstrated the spatial, structural organization of the mucilaginous envelope. It revealed characteristic net-like loose arrangement of the components. Pectic mucilage had a fibrillary, tangled architecture. The second type demonstrated more ordered organization. The cellulose occurred in a form of long, unbranched fibrils forming a scaffold for the rest of components i.e. pectins and hemicelluloses. They were visible as shorter, linear or branched chains spread between fibrils and covering their surface. Our results allowed us to discuss the relationship between structure, morphology and function of the mucilage envelope.

## PC10.9 IS AN AUXIN SIGNALING MODULE REGULATING THE ROOT SYSTEM ARCHITECTURE IN PICEA ABIES (NORWAY SPRUCE) SEEDLINGS?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

FEDERICA BRUNONI (UMEÅ PLANT SCIENCE CENTRE DEPT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN), NICOLAS DELHOMME (UMEÅ PLANT SCIENCE CENTRE DEPT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), KARIN LJUNG (UMEÅ PLANT SCIENCE CENTRE DEPT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), CATHERINE BELLINI (UMEÅ PLANT SCIENCE CENTRE DEPT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN)

@ FEDERICA.BRUNONI@UMU.SE

The root system of terrestrial plants is responsible for anchorage to the substrate as well as for minerals and water supply. The capacity to fulfill these functions is highly dependent on its architecture. Root system architecture (RSA) is determined by root growth, branching through lateral root (LR) formation, and root angle. Overall, the RSA depends on the spatiotemporal regulation of individual LR initiation (LRI) events. A specific subset of auxin/indole-3-acetic acid (Aux/IAA) and auxin response factor (ARF) transcription factors protein were found to be involved in LRF. Aux/IAs and ARFs exist as large, functionally redundant protein families and they are evolutionary conserved in plants. The recent availability of the Norway spruce (*Picea abies*) genome sequence allowed us to carry out a comprehensive genome-wide analysis of these two gene families in this conifer. About 70% of the identified PaAux/IAA and PaARF gene structures are incomplete/partial and, most likely, some of them result from assembly artifacts. We are currently manually reviewing candidate genes to achieve a more accurate gene assembly. In silico expression analysis showed that PaAux/IAA and PaARF genes are expressed in various tissues/organs throughout the adult plant, but different members displayed preference to particular organs. Further RNA-Seq based transcriptome analysis revealed that PaAux/IAA and PaARFs are also expressed in young spruce seedlings. The genetic conservation of the regulatory network components known to control LRI in other species suggests that a similar molecular mechanism might also determine RSA in spruce seedlings. The functional significance of their conservation will be further analyzed.

## PC10.10 COMPARATIVE MOLECULAR ANALYSIS OF GENES UNDERLYING DOMESTICATION TRAITS IN BARLEY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

BEATA I CZAJKOWSKA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), TERRY A BROWN (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ BEATA.CZAJKOWSKA@MANCHESTER.AC.UK

Evidence gathered from living plants and particularly from traits assumed to have been targeted by selection during domestication is one of the principal sources of information on the origin and spread of domesticated plants. A trend towards increasing seed size is one of the easiest phenotypic differences to study in archaeological remains. Seed size is also positively correlated with yield, the ultimate agronomic trait. We investigated the diversity of two genes, HvCKX1 (cytokinin oxidase/dehydrogenase gene) and HvWAK1 (wall associated kinase gene) in an extensive range of geo-referenced wild barley accessions (*Hordeum vulgare ssp. spontaneum*) and historical barley domesticates (*Hordeum vulgare ssp. vulgare*) via Sanger sequencing. HvCKX1 regulates the levels of cytokinins, which are important plant hormones playing a role in growth and development. A lower CKX1 enzyme activity has a positive effect on plant productivity, increasing grain weight and the number of seeds per plant and also increasing root mass. HvWAK1 has a more specific impact on root growth. Our results indicate that HvCKX1 but not HvWAK1 responded to human selection during domestication, implying that early farmers selected for seed size rather than the overall growth vigour of the plant.

## PC10.11 POTATO TUBERIZATION – UNTANGLING THE RELATIONSHIPS WITHIN MULTICOMPONENT SIGNALIZATION NETWORK

WEDNESDAY 5 JULY, 2017 POSTER SESSION

HANA SEVCIKOVA (DEPARTMENT OF EXPERIMENTAL PLANT BIOLOGY FACULTY OF SCIENCES CHARLES UNIVERSITY, CZECH REPUBLIC), PETRA MASKOVA (DEPARTMENT OF EXPERIMENTAL PLANT BIOLOGY FACULTY OF SCIENCES CHARLES UNIVERSITY, CZECH REPUBLIC), LENKA STUPECKA (DEPARTMENT OF EXPERIMENTAL PLANT BIOLOGY FACULTY OF SCIENCES CHARLES UNIVERSITY, CZECH REPUBLIC), DANUSE TARKOWSKA (LABORATORY OF GROWTH REGULATORS PALACKY UNIVERSITY AND INSTITUTE OF EXPERIMENTAL BOTANY ASCR, CZECH REPUBLIC), HELENA LIPAVSKA (DEPARTMENT OF EXPERIMENTAL PLANT BIOLOGY FACULTY OF SCIENCES CHARLES UNIVERSITY, CZECH REPUBLIC)

@ HANA.SEVCIKOVA@NATUR.CUNI.CZ

Potato (*Solanum tuberosum*) is the third most important world crop, henceforth understanding the regulations of tuber onset is crucial from both theoretical and practical points of view. This process is controlled by complex network of external (such as photoperiod, temperature or nitrogen supply) and internal factors. Photosynthesis and related carbohydrate status along with

phytohormone balance belong to the essential factors in its internal regulation. In our work we used potato cv. Lada and its spontaneously tuberizing mutant grown *in vitro* under photoautotrophic conditions to elucidate the responsibility of gibberellins and carbohydrates contents and distribution changes for observed phenotypes. Based on the results and expressions of main tuber-inducing marker genes (such as *StSP6A* and *StBEL5*) along with information about saccharide phloem flow driving their mobility we propose that there are two interconnected pathways, gibberellin- and saccharide-dependent ones, with the power to outcompete each other providing that the signal is extremely strong.

### PC10.12 PLANT RESISTANCE ECOLOGY: INFLUENCE OF PLANT RESISTANCE ON BIOCONTROL OF HERBIVORES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

DANIELA WEBER (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), PAUL EGAN (DEPARTMENT OF PLANT PROTECTION BIOLOGY SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), LARS ERICSON ERICSON (DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE UMEÅ UNIVERSITY, SWEDEN), ANNE MUOLA (DEPARTMENT OF ECOLOGY SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), JOHAN STENBERG (DEPARTMENT OF PLANT PROTECTION BIOLOGY SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN)

@ DANIELA.WEBER@SLU.SE

Biological control of insect pests and intrinsic plant resistance are two fundamental parts of integrated pest management. Yet, altering plant quality traits like resistance can affect performance and abundance of biological control agents. Using woodland strawberry (*Fragaria vesca*) as model system, the aim of this project is to test how and to what extent biological control is affected by variation in intrinsic plant resistance. The high within-species genetic variation of *Fragaria vesca* makes it ideal model system to examine the resistance effects on the Strawberry Leaf beetle (*Galerucella tenella*) as well as to investigate the scope of plant mediated effects on performance of natural enemies. Current results from experiments with the associated parasitoid *Asecodes parviclava* (Hymenoptera: Eulophidae) show that resistant plant genotypes can facilitate successful biological control. The insights gained throughout the project may open up novel opportunities to design future strawberry varieties that support functional tritrophic interactions.

### PC10.13 HIGH LIGHT INDUCES miRNAs EXPRESSION CHANGES IN PHOTOSYNTHETIC TISSUES AND ROOTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ANNA K BARCZAK-BRZYŻEK (WARSAW UNIVERSITY OF LIFE SCIENCES, POLAND), BARBARA KARPINSKA (UNIVERSITY OF LEEDS, UNITED KINGDOM), MARCIN FILIPECKI (WARSAW UNIVERSITY OF LIFE SCIENCES, POLAND)

@ BARCZAK.ANNAK@GMAIL.COM

Plants as sessile organisms are continuously exposed to variable stress conditions. Diurnal and seasonal fluctuations of light intensity and spectra result in episodes of excess energy. Complex response to such dynamic changes requires a coordinated communication at the cellular level but also between leaves and roots. In recent years microRNAs (miRNAs), has been shown to regulate gene expression in a number of plant developmental processes and stress response. Despite its significance the role of miRNAs in light stress signaling and acclimation was not sufficiently studied. To determine if expression of miRNAs is regulated by light both in light-stressed shoots and dark-grown roots we used *Arabidopsis thaliana* plants growing in hydroponics conditions. Physiological response in low light acclimated plants exposed to high light stress was analyzed using chlorophyll fluorescence parameters. Additionally, the expression of several genes known as a markers of redox changes was examined in light-exposed shoots and dark-grown roots. Expression of selected miRNAs were also studied in the time course. Observed changes in miRNAs expression were dynamic and different for both organs. Interestingly, when roots were separated from shoots before high light treatment, light had no effect on expression of selected miRNAs. Our results proved that miRNAs expression is modulated by high light treatment. Stress signal, of unknown nature, is induced in rosettes and travels through the plant to roots to affect miRNAs expression.

### PC10.14 SOUND WAVE AFFECTS THE EXPRESSION OF ETHYLENE BIOSYNTHESIS-RELATED GENES THROUGH CONTROL OF TRANSCRIPTION FACTORS RIN AND HB-1

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MI JEONG JEONG (RURAL DEVELOPMENT ADMINISTRATION (RDA), KOREA (SOUTH)), JOO YEOL KIM (RURAL DEVELOPMENT ADMINISTRATION (RDA), KOREA (SOUTH)), SOO IN LEE (RURAL DEVELOPMENT ADMINISTRATION (RDA), KOREA (SOUTH)), JIN A KIM (RURAL DEVELOPMENT ADMINISTRATION (RDA), KOREA (SOUTH))

@ CENTER1097@KOREA.KR

We previously reported that sound wave treatment (1 kHz) delays fruit ripening in tomato (*Solanum lycopersicum*), affecting the expression of ethylene biosynthesis-related genes encoding 1-aminocyclopropane 1-carboxylic acid (ACC) synthases (ACS) and ACC oxidases (ACO). In this study, we investigated the activity of the transcription factors RIN and HB-1, which function in the ethylene biosynthetic pathway, in response to sound treatment. To investigate whether RIN and HB-1 directly activate the

transcription of ACS and ACO, we performed transcriptional activation analysis in *Arabidopsis thaliana* leaf protoplasts, transiently expressing RIN or HB-1 and using reporter constructs with promoters of the tomato ACS and ACO genes. Activation of the endogenous AtACS and AtACO genes was also measured by qPCR. The RIN- and HB-1-induced expression of these genes decreased, but the HB-1-induced expression of some genes increased after sound treatment. To confirm these results, we performed transient assays in *Nicotiana tabacum*, which produced results similar to those observed in *Arabidopsis*. The major ethylene biosynthesis-related genes harbor a CArG-box as a RIN-binding motif. These findings indicate that RIN and HB-1 affect the expression of ethylene biosynthesis-related genes in response to sound treatment, and they suggest that RIN may regulate the ethylene biosynthesis-related genes by binding to their CArG-boxes.

### PC10.35 OPTIMIZATION OF REGENERATION AND TRANSFORMATION IN *SESAMUM INDICUM* L. CULTIVAR JK-1 FOR STUDYING TISSUE SPECIFIC PROMOTERS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ANIRBAN JYOTI DEBNATH (BOSE INSTITUTE, INDIA)

ANIRBANDEBNATH@YMAIL.COM

For crop improvement of *Sesamum indicum*, heterosis breeding can be an approach in which a male sterile/restorer line of sesame is necessary. Male sterility can be achieved by silencing of anther specific genes. For this approach, study of tapetum specific promoter is a prerequisite phenomenon. Since sesame is an extremely recalcitrant plant, regeneration protocol of this plant was standardised at first for this study. Various combinations of 6-benzylaminopurin, thidiazuron,  $\alpha$ -naphthalinic acetic acid, indole acetic acid, indole butyric acid and gibberellic acid were used to standardise each step of organogenesis, shoot elongation and root induction. Among the different types of tried explants, best regeneration frequency (94.82 $\pm$ 1.34%) was obtained from 4 days old cotyledon. To find an anther specific gene, sesame anther specific cDNA-subtracted library was created and screened thoroughly. One EST having homology with  $\beta$ -1,3 glucanase was isolated. Upstream element of the gene was identified by promoter walking, cloned in pZPY112 vector and was fused with GUS reporter gene. TA29 is a known anther specific gene of *Nicotiana tabacum*. Promoter of TA29 served as control in this study. The promoter region of TA29 was amplified from *Nicotiana* genomic DNA and cloned in pCAMBIA2301 and was fused with GUS reporter gene. *Agrobacterium tumefaciens* A4404 was transformed by both the constructs. Both constructs were used for *Agrobacterium-mediated* transformation of sesame, taking *Nicotiana* as control. Transgenic lines of sesame and *Nicotiana* were raised and confirmed by PCR and southern hybridisation. Anther-specific GUS expression of the both promoters were visualised by sectioning of anther.

### PC10.36 MOLECULAR CYTOGENETIC IDENTIFICATION OF A NOVEL TRITICALE-WHEAT 2R SUBSTITUTION LINE WITH TWO TRANSLOCATIONS (1BL.1RS AND 6BS.6RL) POSSESSING RESISTANCE TO YELLOW RUST AND DROUGHT TOLERANCE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

NAVDEEP SINGH JAMWAL (CSK HIMACHAL PRADESH AGRICULTURAL UNIVERSITY, INDIA), HARINDER KUMAR CHAUDHARY (CSK HIMACHAL PRADESH AGRICULTURAL UNIVERSITY, INDIA), ANILA BADIYAL (CSK HIMACHAL PRADESH AGRICULTURAL UNIVERSITY, INDIA), JS PAT HESLOP HARRISON (UNIVERSITY OF LEICESTER, UNITED KINGDOM), TRUDE SCHWARZACHER (UNIVERSITY OF LEICESTER, UNITED KINGDOM)

NAVDEEPPAU@GMAIL.COM

Wheat (*Triticum aestivum*) is the major staple food crop of North-Western Himalayan region of India. Being under rainfed cultivation in the variable and harsh climate, the crops in this region are inevitably exposed to various abiotic and biotic stresses. The sustainable way to introduce resistance in elite wheat cultivars is through incorporation of desirable agronomic traits from potential wild relatives like rye (*Secale cereale*) and triticale (*xTriticosecale*) through wide hybridization followed by artificial chromosome doubling. Keeping this into account the present investigation was formulated to develop 139 stable wheat-rye recombinants by involving Himalayan rye, five diverse triticale lines and 15 elite wheat cultivars through *Imperata cylindrica*-mediated chromosome elimination approach of doubled haploidy breeding. The molecular cytogenetic tools like Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) revealed one line namely DT126 x VL829 carrying novel translocation (6BS.6RL) as well as substitution (2D.2R) besides 1BL.1RS. *In vitro* studies for the estimation of cultivar tolerance towards drought were carried out in controlled conditions using PEG-6000 where the same line exhibited significant values for shoot length, fresh shoot weight, fresh as well as dry root weight and drought tolerance index demonstrating it as a drought tolerant line. Field evaluation for desirable agronomic traits showed significantly better performance of this line for yield and related traits under consideration. Further the line was found to be highly resistant towards yellow rust in the field. Hence this novel and potential wheat-rye recombinant can be used in future wheat improvement programmes.



### PC10.37 EFFECTS OF INTERACTION BETWEEN RED TO FAR-RED RATIO OF LIGHT AND ATMOSPHERIC HUMIDITY ON EXTENSION GROWTH OF *CUCUMIS SATIVUS* SEEDLINGS

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 TOSHIO SHIBUYA (OSAKA PREFECTURE UNIVERSITY, JAPAN), SAKI KISHIGAMI (OSAKA PREFECTURE UNIVERSITY, JAPAN), RYOSUKE ENDO (OSAKA PREFECTURE UNIVERSITY, JAPAN), RYO MATSUDA (THE UNIVERSITY OF TOKYO, JAPAN)

@ SHIBUYA@ENVI.OSAKAFU-U.AC.JP

Plant responses to red to far-red ratio (R:FR) of light are well known in many species, but interaction with other factors is less well known. Better understanding of the possible interactions help interpret the results obtained under different environmental conditions. In the present study, we investigated effects of the interaction between R:FR and atmospheric humidity on stem elongation and leaf expansion. Cucumber (*Cucumis sativus*) seedlings were grown at a vapor-pressure deficit (VPD) of 0.4 or 3.0 kPa at an air temperature of 28°C, with an illumination R:FR ratio of 1.0 or 10 for 7 days after germination, and determined their growth properties, carbon allocation and water status to identify factors that potentially limit extension growth. The combination of low R:FR and low VPD synergistically stimulated stem elongation. This interaction probably resulted partially from the interaction's effect on cell-wall extensibility and on the driving force (turgor) of individual cells, which respectively respond to changes in the proportion of active phytochrome and evaporative demand. Leaf expansion was also stimulated at low R:FR and low VPD, but unlike stem elongation, low VPD reduced the effect of R:FR on leaf expansion. This difference can be explained by carbon resource competition between stems and leaves. The carbon allocation to leaves decreased under low R:FR and low VPD. This may limit leaf expansion and thereby moderate the variation of leaf expansion.

### PC10.38 THE CROSSABILITY AND CHARACTERISTICS ANALYSIS OF F<sub>1</sub> HYBRID BETWEEN *TRANSGENIC BRASSICA NAPUS* AND *B. RAPA*

📅 WEDNESDAY 5 JULY, 2017 POSTER SESSION

👤 SOO-IN SOHN (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), YOUNG-JU OH (INSTITUTE OF FUTURE ENVIRONMENTAL ECOLOGY CO. LTD., KOREA (SOUTH)), KYEONG-RYEOL LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), HYUN-SUK CHO (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), ANCHEOL CHANG (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), DOH-WON YUN (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), GANG-SEOB LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), SUNG-DUG OH (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH)), YEON-HEE LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES, KOREA (SOUTH))

@ SISOHN@KOREA.KR

A number of studies have been conducted on hybridization between transgenic *Brassica napus* and *B. rapa* or backcross of F<sub>1</sub> hybrid to their parents. Trait changes of interspecific progenies must be analyzed to evaluate hybrid sustainability in nature. In the present study, *B. rapa* and transgenic (*BrAGL20*) *B. napus* were hybridized to verify the early flowering phenomenon of F<sub>1</sub> hybrids, and F<sub>1</sub> hybrid traits were analyzed to predict their impact on sustainability. F<sub>1</sub> hybrids bloomed later than transgenic *B. napus*, but without vernalization, owing to the expression of the *BrAGL20* transgene. The size of F<sub>1</sub> hybrid seeds was intermediate between those of *B. rapa* and transgenic *B. napus*, and ~40% of F<sub>1</sub> pollen exhibited abnormal size and morphology. The form of the F<sub>1</sub> stomata was also intermediate between that of *B. rapa* and transgenic *B. napus*, and the number of stomata was close to the parental mean. Among various fatty acids, the content of erucic acid exhibited the greatest change, owing to the polymorphism of parental *FATTYACIDELONGASE 1* alleles. Furthermore, F<sub>2</sub> hybrids could not be obtained. However, BC<sub>1</sub> progeny were obtained by hand pollination of *B. rapa* with F<sub>1</sub> hybrid pollen, with an outcrossing rate of 50%.

### PC10.39 HOW DO CIRCADIAN RHYTHMS INCREASE PLANT WATER USE EFFICIENCY?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

NORIANE M L SIMON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), HIROSHI KUDOH (KYOTO UNIVERSITY, JAPAN), ALISTAIR M HETHERINGTON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), ANTONY N DODD (UNIVERSITY OF BRISTOL, UNITED KINGDOM)

@ NS1864@BRISTOL.AC.UK

Reduced soil water availability represents a serious threat to modern agriculture, causing significant decreases in crop yield worldwide. Climate change is predicted to exacerbate this situation, with more frequent and longer droughts in future. Therefore, it is a research priority to develop solutions for more sustainable use of water in agriculture. As global agriculture alone represents 70% of human water consumption, one possible solution is to develop plants that lose less, and so use less, water. Circadian rhythms are known to increase plant water use efficiency, but the mechanisms underpinning this phenomenon remain unclear. I am examining the involvement of circadian regulation in the water use efficiency of higher plants, focusing upon the role of the circadian oscillator in stomatal guard cells. I am analysing *Arabidopsis* plants with altered guard cell circadian clocks to better understand the relationship between the circadian oscillator and stomatal aperture. I am also using screening approaches to further investigate the contribution of circadian regulation to plant water use. I have explored the involvement of circadian regulation in stomatal movements in naturally-occurring populations of *Arabidopsis halleri* plants. Overall, this work is providing a deeper understanding of how circadian rhythms optimise plant water use efficiency.

### PC10.40 DISTRIBUTION OF ICE DURING SUBZERO CONDITIONS WITHIN THE HORSETAIL *EQUISETUM HYEMALE* L.

WEDNESDAY 5 JULY, 2017 POSTER SESSION

RENA T SCHOTT (STATE MUSEUM OF NATURAL HISTORY STUTTGART, GERMANY), ANITA ROTH-NEBELSICK (STATE MUSEUM OF NATURAL HISTORY STUTTGART, GERMANY)

@ RENA.SCHOTT@SMNS-BW.DE

During land plant evolution, cold hardiness developed in various groups to allow survival of above ground plant parts under freezing conditions. Freezing avoidance by extracellular ice formation represents one essential component of cold hardiness, and was considered so far mainly in seed plants. Here we studied extracellular freezing in *Equisetum hyemale* var. *robustum*. The stems of this species which thrives at humid sites consist of upright axes subdivided in nodal and intermodal regions containing extensive internal air-filled canals (pith cavity, vallicular and carinal canals). *E. hyemale* shoots can survive up to two frost periods. Our results show that the pith cavity and the vallicular canals provide spaces for extracellular ice crystal growth within the stems. During a frost period, even the substomatal chambers normally filled with air are

filled with ice. During the reversible process of extracellular ice accumulation, the living cells lose considerable amounts of water but are protected from being damaged by internal ice crystal growth. The whole structure of the stem guarantees a short distance between the living cells and spaces for the ice crystal growth, promoting fast dehydration during freezing and rapid rehydration upon thawing.

### PC10.41 GENOMIC SURVEY OF ATP-BINDING CASSETTE (ABC) TRANSPORTERS IN SORGHUM BICOLOR

WEDNESDAY 5 JULY, 2017 POSTER SESSION

CHARLES G OLOGIDI (NIGER DELTA UNIVERSITY, NIGERIA), ODUNAYO J OLAWUYI (UNIVERSITY OF IBADAN, NIGERIA)

@ CHARLES.OLOGIDI@NDU.EDU.NG

ATP-binding cassette (ABC) transporters are the largest members of diverse proteins constituting the most ancient families with representatives in all phyla from prokaryotes to humans. Systematic searches were performed using PSI-Blast program on NCBI to identify sorghum ABC transporters. The identified proteins were subjected to phylogenetic and domain topology analyses and were classified and named according to the Human Genome Organisation (HUGO) system of classification and unified plant nomenclature system. As part of the survey, the subcellular location, presence of signal peptide, and physicochemical parameters were determined. The results from the genomic survey showed that 100 ABC transporters were identified and classified as ABCB, ABCC, ABCD, ABCE, ABCF, and ABCI. Sixty nine proteins encode intrinsic membrane proteins and 28 encode proteins without TMDs. The Sorghum bicolor ABC transporter family consists of 61 full-size molecules, 9 half-size molecules, 3 three-quarter-size molecules, and 26 quarter-size molecules. One of the proteins (GI-992164873) has neither TMD nor NBD. A majority of the proteins are located in the plasma membrane. Signal peptide was observed in SbABCB11, SbABCB34, SbABCC4, SbABCC21, and SbABCI3. The proteins had varying values of physicochemical parameters.

Key words: *Sorghum bicolor*, ABC transporters, transmembrane proteins.

### PC10.42 ACCUMULATION AND SUBCELLULAR LOCALIZATION OF FOREIGN PROTEIN USING TRANSIT PEPTIDES IN TRANSGENIC PLANT TOBACCO

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• SANGHOON MA (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SEO YOUNG PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), MI JIN JEON (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), AH YOUNG KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SE HEE PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), HYUN MIN KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), DAT THANH MAI (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), YOUNG HEE JOUNG (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH))

@ KINGMASALO@NAVER.COM

Oxygen-evolving proteins has transit peptide at their N-terminal region. The oxygen-evolving proteins were transferred into thylakoid after synthesis using the transit peptides. Four different transit peptides of oxygen evolving protein were isolated (TP1~4) from tobacco. The transit peptides were fused to gfp gene (TP:GFP) and the TP fused gfp constructs were transformed into tobacco (*Nicotiana tabacum* cv. Xanthi NC). The TP:GFP over expressed transgenic plant were analyzed by confocal laser scanning microscopy. Fluorescent signals were detected in chloroplast. The transit peptide fused GFP was localized into thylakoid lumen and the transit peptide was removed from GFP after translocation. The TP:GFP transgenic tobacco plants showed higher GFP expression level compared to without transit peptide gfp transgenic plants. The TP fused proteins were transferred into thylakoid lumen and accumulation. It causes increasing the foreign protein level in transgenic plant. Foreign protein targeting and accumulation into chloroplast using transit peptide is very useful method for plant molecular farming.

### PC10.43 ISOLATION AND FUNCTIONAL ANALYSIS OF THE CYTOCHROME P450-27(SLP450-27) GENE FROM TOMATO

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• MI JIN JEON (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SANG HOON MA (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SEO YOUNG PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), AH YOUNG KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SE HEE PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), HYUN MIN KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), DAT THANH MAI (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), YOUNG HEE JOUNG (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH))

@ JMJ8745@NAVER.COM

Cytochrome P450 has a heme cofactor to proteins in all organisms. In plants, cytochrome P450 enzyme is related to the diverse roles such as catabolism of hormones, signaling molecules, and synthesis of pigments, structural macromolecules, defense compounds. Tomato genome has about 400 cytochrome P450 genes. but most of them are not yet revealed their functions. In order to identify the SIP450-27 function, the SIP450-27 gene was isolated from tomato genome using by RT-PCR. The SIP450-27 gene was confirmed that it was CYP736A74 clade. The SIP450-27 was highly expressed in tomato root and flower tissues. To analysis of SIP450-27 function, the gene was cloned into the pCW vector and expressed in E. coli and purification of the enzyme. As a result, polyphenolic compounds (resveratrol and polydatin) were hydroxylated by the SIP450-27. For identify the functions in plants, the SIP450-27 gene cloned into plant expression vector and transformed tobacco (*Nicotiana tabacum* cv. Xanthi-NC) and tomato (*Solanum lycopersicum* cv. Micro-Tom), and the phenotype was observed.

### PC10.44 FUNCTIONAL STUDY OF NADPH-CYTOCHROME P450 REDUCTASE 2 (CaCPR2) GENE ISOLATED FROM HOT PEPPER

WEDNESDAY 5 JULY, 2017 POSTER SESSION

• SEO YOUNG PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SANG HOON MA (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), MI JIN JEON (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), AH YOUNG KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SE HEE PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), HYUN MIN KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), MAI THANH DAT (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), CHUL-HO YUN (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), YOUNG HEE JOUNG\* (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH))

@ SEOYOUNG302@NAVER.COM

Plant NADPH-cytochrome P450 reductase (CPR) exists in the cell membrane that plays important role which transfer the electron to various plant P450s. Two CPR genes (CaCPR1, CaCPR2) were isolated from hot pepper (*Capsicum annuum* L. cv. Bukang). Quantitative PCR

analysis was used for determining CaCPRs mRNA expression levels in various hot pepper tissues. The expression level was gradually increased during fruit ripening. In case of CaCPR2, however, mRNA was constitutively expressed in all tissues and expression level was lower than CaCPR1. CaCPR2 was heterologously expressed in *Escherichia coli* to investigate the enzymatic properties. The enzymatic properties of CaCPR2 were determined by characteristic absorption spectrum and catalytic activities measurement, which were assessed using protein and chemical substrates including P450, cytochrome c, cytochrome b5, MTT, and CTC. These results show that although the CaCPR2 is not a major CPR in most tissues in hot pepper, but it could play important role under the stress condition.

### PC10.45 FUNCTIONAL STUDY OF CYP707A FAMILY GENES (ABA 8'-HYDROXYLASES) FROM HOT PEPPER (*CAPSICUM ANNUUM*)

WEDNESDAY 5 JULY, 2017 POSTER SESSION

HYUN MIN KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), AH YOUNG KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SE HEE PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SANG HOON MA (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SEO YOUNG PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), MI JIN JEON (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), CHUL-HO YUN (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), YOUNG HEE JOUNG (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH))

TBLINJ@NAVER.COM

Cytochrome P450 (P450 or CYP) monooxygenases in plants have roles in the biosynthesis of plant hormones, defense related chemicals, and diverse secondary metabolites. We isolated cDNA for CYP707A family gene (Ca-P12-A1, -A2, -A3 and -A4), which were encoded Abscisic acid 8'-hydroxylase from a cDNA library of hot pepper (*Capsicum annuum* L. cv. Bukang). To understand the roles of the Ca-P12-A1, -A2, -A3 and -A4 genes in hot pepper, the gene expression patterns in various hot pepper tissues were analyzed. The Ca-P12-A3 was highest expressed in all tested tissues. The expression level of Ca-P12-A2 was increased in leaves under stress condition. The Ca-P12-A1 was increased during fruit ripening stage. Although the relative transcript level of Ca-P12-A4 was very low, it was mainly expressed in ovary. The results suggest that these genes play different roles in plant by their specific expression patterns. To identify the catalytic function of the Ca-P12-A1, -A2, -A3 and -A4 genes, these proteins were cloned into *E. coli* expression vector and heterologously produced in *E. coli* system. These recombinant proteins were catalyzed ABA to 8'-hydroxy ABA, phaseic acid, and dihydrophaseic acid. To confirm the function of the Ca-P12-A1, -A2, -A3 and -A4 genes in plant, these genes were cloned into plant expression vector and transformed into tobacco (*Nicotiana tabacum* cv. Xanthi NC). The Ca-P12-A1, -A2, -A3 and -A4 over-expression transgenic tobacco plants showed the wilting phenotype. Furthermore, these transgenic tobacco plants showed that down regulated seed formation and pollen viability compare to non-transgenic tobacco.

### PC10.46 ROOT HYPOXIA INDUCED CHANGES IN LEAVES OF TOMATO AND CUCUMBER SEEDLINGS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ANNA KOŁTON (UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND), MAŁGORZATA CZERNICKA (UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND), KINGA KĘSKA (UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND), ALINA WISZNIĘWSKA (UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND)

AKOLTONWORK@GMAIL.COM

Hypoxia is a state of oxygen deprivation. Hypoxia in root environment occurs frequently during crop production as well as in natural conditions. This phenomenon may cause huge losses in crop yield. Preliminary study was conducted in hydroponics in growth room. Next, two genotypes of tomato and cucumber were cultivated in the greenhouse condition. The root hypoxia treatment was introduced when seedlings had 2-3 leaves. The duration of hypoxia condition was 7 days. Then, plants were cultivated for recovery for 14 days and another 7 days hypoxia treatment were established. During hypoxia stress the production of reactive oxygen species (ROS) was confirmed and the changes in antioxidant system are expected. Peroxidase, catalase and superoxide dismutase activities in seedlings' leaves were differentially affected in tomato and cucumber genotypes as a result of root hypoxia stress. The differences in phenolic compounds accumulation were also observed comparing treated and control plants as well as two genotypes of cucumber or tomato seedlings. Changes in photosynthesis intensity were also observed in dependence on hypoxia treatment and species. Varied reaction for hypoxia in both genotype of each species suggesting different mechanism of tolerance. Results may provide new avenue to understand responses and tolerance in tomato and cucumber plants to hypoxia.

### PC10.47 INCREASING OF TRANSFORMATION EFFICIENCY TO THE PHOSPHINOTHRICIN USING SEVERAL EXPRESSION SYSTEMS IN SOLANACEAE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

AH YOUNG KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SE HEE PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), HYUN MIN KIM (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SANG HOON MA (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), SEO YOUNG PARK (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), MI JIN JEON (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH)), YOUNG HEE JOUNG (CHONNAM NATIONAL UNIVERSITY, KOREA (SOUTH))

KIMAY1243@HANMAIL.NET

The bar gene is used as selection marker for transgenic plants and also agricultural trait for herbicide resistance. We have used the bar gene to select transformed callus and also to develop of herbicide-resistant tomato plants. Some Solanaceae plants such as tomato and tobacco, were sensitively affected by the phosphinothricin and declined transformation efficiency. In addition, these bar overexpressed transgenic plants exhibited low degree of tolerance against

phosphinothricin compare to monocot plants. These reasons, the bar gene is not suitable for selective marker for some Solanaceae especially for tomato plant. We have introduced with two approaches the bar selection system to solve the problems. First was increase bar gene expression level using strong promoter such as enhanced 35S promoter (double 35S), and increase translation efficiency using the modified GUBQ1 promoter (G1-3:to baint) which was isolated from the gladiolus polyubiquitin gene and replacement 5' intron of the promoter with 5' intron of tobacco polyubiquitin gene. The other was targeting the PAT enzyme to chloroplast using the transit peptide of oxygen evolving protein/rubisco small subunit. As a result, the tolerance level against phosphinothricin was correlated to the PAT expression level by different bar expression systems.

### PC10.48 SCREENING FOR INHIBITORS OF INOSITOL PHOSPHORYLCERAMIDE SYNTHASE: A NEW HERBICIDE MODE OF ACTION?

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ELIZABETH PINNEH (UNIVERSITY OF DURHAM, UNITED KINGDOM), PATRICK STEEL (UNIVERSITY OF DURHAM, UNITED KINGDOM), PAUL DENNY (UNIVERSITY OF DURHAM, UNITED KINGDOM), MICHAEL STARK (UNIVERSITY OF DUNDEE, UNITED KINGDOM), STEPHEN LINDELL (BAYER CROP SCIENCE, GERMANY), PETER LUMMEN (BAYER CROP SCIENCE, UNITED KINGDOM)

@ ELIZABETH.PINNEH@DURHAM.AC.UK

There are currently 480 unique cases of herbicide resistant weeds globally, with resistance to two or more herbicides found in 252 species. The rapid increase in multiple resistances in weeds combined with the stagnancy in the identification of a new herbicide mode of action for over three decades, raises a challenge that threatens crop production worldwide. Recent research into sphingolipid synthesis has brought about the identification of a promising new site of action, involving the non-mammalian enzyme inositol phosphorylceramide synthase (IPCS). IPCS catalyses the transfer of phosphoinositol to the C-1 hydroxyl of ceramide thereby generating inositol phosphorylceramide; deletion of this enzyme has been found to be lethal in *Saccharomyces cerevisiae*. The divergence in the sphingolipid biosynthetic pathway in animals and plants allows for the creation of novel herbicides with minimal impact on animals and humans. An 11,000 member library comprising compounds with demonstrable herbicidal activity, and an unknown mode of action, has been screened against IPCS from *Arabidopsis thaliana* and *Oryza sativa*. Following structural clustering (Tanimoto index of 0.7) sets of putative inhibitors associated with apoptosis and membrane disruption were identified. This presentation will describe these studies along with secondary assays aimed at validating these hits as true inhibitors of IPCS, which can be developed for use as herbicides.

### PC10.49 COMBINATORIAL TRANSCRIPTIONAL REGULATION OF THE PLANT DEFENCE RESPONSE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MAURA DI MARTINO (UNIVERSITY OF WARWICK, UNITED KINGDOM), ALEX JONES (UNIVERSITY OF WARWICK, UNITED KINGDOM), EMILY BREEZE (UNIVERSITY OF WARWICK, UNITED KINGDOM), KATHERINE DENBY (UNIVERSITY OF YORK, UNITED KINGDOM)

@ MAURA.DIMARTINO@YORK.AC.UK

Transcriptional reprogramming plays a significant role in the defence of plants against pathogen infection. This defence response involves a complex and highly sophisticated regulatory circuitry in which transcription factors (TFs) play a key role in determining the specificity of the response to different pathogens. We have obtained high-resolution temporal transcriptome data following biotic and abiotic stress treatments in *Arabidopsis*, and have generated regulatory network models of the TF interactions underlying these responses. Such models predict widespread combinatorial transcriptional regulation of many known defense genes and pathways, providing a mechanism for generating complex transcriptional profiles, and thus fine-tuning the plant's response. Combinatorial regulation can also occur by variations in the composition of multi-subunit TFs. NF-Y TFs directly bind CCAAT boxes in target gene promoters as trimers of A, B and C subunits, and can act as positive or negative regulators of transcription. In *Arabidopsis* a multi-gene family encodes each subunit of the complex and many of these subunits change in expression during plant stress responses. A putative trimer (NF-YA2, NF-YB2, NF-YC2) has been identified. We have shown that NF-YA2 expression levels determine accumulation of JA and that the *nf-ya2* mutant displays significantly increased susceptibility to *Botrytis cinerea*. Furthermore, BiFC assays in *N. benthamiana* revealed that NF-YB2 and NF-YC2 are able to heterodimerise in planta, and this interaction was confirmed in *Arabidopsis* expressing tagged NF-YC2. We are now investigating whether this NF-YB2/C2 dimer binds the NF-YA2 subunit, and, if so, the regulatory function of the resulting NF-Y trimer during defence against *B. cinerea*.

## PC10.50 WORLDWIDE GENETIC COMPARISON OF POTATO CYST NEMATODES USING GENOTYPING BY SEQUENCING

WEDNESDAY 5 JULY, 2017 POSTER SESSION

NATHALIE DAUPHINAIS (AGRICULTURE AND AGRI-FOOD CANADA, CANADA), BENJAMIN MIMÉE (AGRICULTURE AND AGRI-FOOD CANADA, CANADA), GUY BÉLAIR (AGRICULTURE AND AGRI-FOOD CANADA, CANADA), VIVIAN BLOK (JAMES HUTTON INSTITUTE, UNITED KINGDOM), ÉRIC GRENIER (INSTITUTE OF GENETIC ENVIRONMENT AND PLANT PROTECTION INRA, FRANCE)

@ NATHALIE.DAUPHINAIS@AGR.GC.CA

The potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are among the most economically important pests of potato and quarantine organisms in many countries. The introduction and potential spread of these species in any part of the world has serious implications for potato production and export. PCN management is primarily achieved through the use of resistant varieties. However, PCN populations differ in virulence against the different resistance sources. It is of paramount importance to be able to identify these variants and to understand the mechanisms leading to the breakdown of plant resistance. To this end, genotyping-by-sequencing has been used to rapidly identify Single Nucleotide Polymorphisms (SNPs) and compare allele frequencies between populations. This method allowed the direct comparison of worldwide populations. For *G. rostochiensis*, our results showed a clear separation of pathotypes Ro1 and Ro2 from Ro3, Ro4 and Ro5. At a local scale, some populations with different pathotypes (Ro1 vs Ro2) but same origin (NY) were very similar, suggesting a recent differentiation event or a high level of passive gene flow between these populations. For *G. pallida*, a major difference was observed between the pathotype Pa1 and Pa2/3. It was also possible to distinguish highly virulent South American populations from European populations. For most of these groups, we identified SNP markers that could be used for diagnostics in management programs. Phylogeographic results also confirmed that both PCN species may share a single common introduction origin into Europe and then to North America.

## PC10.51 INVESTIGATION OF ARSENITE UPTAKE KINETIC IN RICE (ORYZA SATIVA) CULTIVARS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MAHDI YOUSOFINIA (TARBIAT MODARES UNIVERSITY, IRAN), FAEZEH GHANATI (TARBIAT MODARES UNIVERSITY, IRAN), MARKUS J TAMAS (GOTEBORG UNIVERSITY, SWEDEN)

@ M.YOUSOFINIA@MODARES.AC.IR

Arsenic (As) is one of the most poisonous elements in the environment whose inorganic forms such as arsenite are more toxic than organic ones. Arsenic toxicity depends on the speciation and increases with decreasing oxidation states. Due to a reducing environment in rice fields, the level of arsenic uptake and accumulation in this plant is much higher than other cereals.

Hypothetically, As is taken up by via two high- and low-affinity systems at low and high As concentrations, respectively. In the present study As uptake by two Iranian cultivars of rice (Hashemi and Najafi) are compared. These seeds were grown hydroponically in Kamachi medium for twenty days and then high (0-0.05 mM) and high and low affinity uptake (0-25 mM) of As were evaluated. Although kinetics parameters differed considerably between two cultivars, the means of  $R^2$  values showed that As uptake in both cultivars was hyperbolic, suggesting an active transport of As. Uptake rate of As by Hashemi cultivar was higher, regardless the level of supplied substrate. This variety showed higher values of kinetic parameters,  $V_{max}$  and  $K_m$  than Najafi. The higher rate of As (III) uptake may be due to some difference physiological and morphological attributes such as root length or localization and number of transporters. Uptake kinetics characteristics can be considered as an important criterion for selecting varieties with less As uptake in contaminated areas, resulting in lower entrance of As into food chain and minimizing its risk for human health.

## PC10.52 OVEREXPRESSION OF $\beta$ -D-N-ACETYLHEXOSAMINIDASE GENE IN TOMATO RESULTS IN HIGHER FLOWERING AND FRUIT SET WITH REDUCTION IN FRUIT SIZE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

MOHAMMAD IRFAN (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), VINAY KUMAR (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), SUMIT GHOSH (CSIR-CENTRAL INSTITUTE OF MEDICINAL AND AROMATIC PLANTS, INDIA), NIRANJAN CHAKRABORTY (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), SUBHRA CHAKRABORTY (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA), ASIS DATTA (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH, INDIA)

@ IRFANQADRI01@GMAIL.COM

The  $\beta$ -D-N-acetylhexosaminidase ( $\beta$ -Hex) is a cell wall located N-glycan processing enzyme and it breaks the glycosidic bonds between carbohydrates, as well as between carbohydrate and noncarbohydrate. The suppression of gene of this enzyme in tomato was resulted in enhanced shelf life of fruits without affecting plant growth, fruit development and yield. In order to further investigate the role of this enzyme, its overexpression was carried out constitutively in tomato and transgenic plants were raised. The analysis of morphological and agronomic parameters of transgenic plants revealed that the number of flowers and fruit set in overexpressed lines were increased significantly. The size of overexpressed fruits was reduced drastically with no effect on fruit shape. Interestingly, we observed that leaf chlorophyll content and photosynthetic efficiency was decreased in overexpressed lines and the soluble sugar content was also changed in the overexpressed lines. The overexpressed fruits were ripened earlier than control and showed reduced shelf life and susceptibility towards fungal pathogen *Botrytis cinerea*. Further, identification and quantification of significant changes to the fruit proteome was carried out during developmental and ripening stages using high-throughput iTRAQ and high-resolution mass spectrometry. Overall, our data suggests that overexpression of  $\beta$ -Hex results in increased flowering and fruit set with reduction in fruit size and shelf life.

### PC10.53 POTATO SOMACLONES REGENERATED FROM CELLS ADAPTED TO THAXTOMIN A ARE MORE RESISTANT TO COMMON SCAB

WEDNESDAY 5 JULY, 2017 POSTER SESSION

NATHALIE BEAUDOIN (UNIVERSITY OF SHERBROOKE, CANADA),  
IAUHENIA ISAYENKA (UNIVERSITY OF SHERBROOKE, CANADA),  
SAFA LABIDI (UNIVERSITY OF SHERBROOKE, CANADA)

@ NATHALIE.BEAUDOIN@USHERBROOKE.CA

Common scab is a disease which causes scab-like lesions on the surface of potato tubers, thus decreasing their quality and market value. The main causal agent of common scab is the soil actinobacteria *Streptomyces scabies*. During the infection process, *S. scabies* secretes a toxin, thaxtomin A, which is essential for the development of disease symptoms. One of the best strategies to reduce the incidence of the disease is to use cultivars that are more tolerant to the disease. However, little is known on the mechanisms that protect potato tubers from *S. scabies*. Since thaxtomin A is a key factor in the development of the disease, increasing resistance to the toxin may help reduce disease symptoms. We have developed a new approach to progressively adapt potato calli to thaxtomin A. Somaclones were regenerated from thaxtomin A-adapted cells and tested for resistance to common scab. We identified three somaclonal variants that are more resistant to common scab in greenhouse experiments, and this resistance was maintained in field experiments in the next generation. These somaclonal variants are being characterized to identify the molecular and physiological changes associated with a better tolerance to common scab. We will present specific changes in the tuber proteome and the structure of the periderm that may explain the increased resistance to common scab observed in these somaclones.

### PC10.54 SIGNAL ROLES OF REACTIVE CARBONYL SPECIES (RCS) DURING INDUCTION OF RCS DETOXIFICATION ENZYMES AND ANTIOXIDANT DEFENCE SYSTEM IN ARABIDOPSIS THALIANA AND EUTREMA PARVULUM UNDER SALINITY

WEDNESDAY 5 JULY, 2017 POSTER SESSION

TOLGA YALCINKAYA (EGE UNIVERSITY, TURKEY), BARIS UZILDAY (EGE UNIVERSITY, TURKEY), RENGİN ÖZGÜR (EGE UNIVERSITY, TURKEY), ISMAIL TURKAN (EGE UNIVERSITY, TURKEY), ASKIM H SEKMEK (EGE UNIVERSITY, TURKEY)

@ TOLGAYALCINKAYA@HOTMAIL.COM

When plants are exposed to environmental stresses, production of reactive oxygen species (ROS) increase due to imbalance in metabolic processes. Accumulation of ROS damage cellular molecules such as nucleic acids, proteins and lipids. Break-down of lipids in cell membranes by ROS causes production of lipid peroxidation products such as 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE), malondialdehyde (MDA) and acrolein (ACR) which are called as

reactive carbonyl species (RCS). These compounds are detoxified by aldehyde dehydrogenases, aldose/aldehyde reductases and 2-alkenal reductases in plants. Recent studies suggest that RCS and RCS related metabolism take role in salt stress response of plants. However, these findings were obtained from glycophytic plants such as *Arabidopsis thaliana*, *Nicotiana tabacum* and *Oryza sativa*. There are no studies on this topic in halophytic plants which are genetically adapted to salt stress. It is logical to think that in halophytes the roles of RCS might be different from glycophytic plants and RCS might take signalling roles under salinity. Investigating RCS metabolism and its signalling comparatively in glycophytes and halophytes is important for elucidation of these new potential mechanisms that take role in salt stress tolerance. For this purpose, in this work we test if exogenous RCS can induce different defence responses such as RCS detoxification enzymes and antioxidant defence system in *A. thaliana* (glycophyte) and *E. parvulum* (model halophyte).

### PC10.55 PROTEINS FROM GREEN LEAF WASTE IN FOOD STRUCTURES - A STUDY FOCUSED ON LEAF PROTEIN BASED FREEZE-DRIED FOAMS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ANNA-LOVISA NYNÄS (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES DEPARTMENT OF PLANT BREEDING, SWEDEN), WILLIAM NEWSON (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES DEPARTMENT OF PLANT BREEDING, SWEDEN), MAUD LANGTON (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES DEPARTMENT OF MOLECULAR SCIENCES, SWEDEN), EVA JOHANSSON (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES DEPARTMENT OF PLANT BREEDING, SWEDEN)

@ ANNA-LOVISA.NYNAS@SLU.SE

In the production of many vegetables a large part of the crop, mainly green leaves, is considered as waste and left in the field. Finding ways to utilize the leaves will increase sustainability of the primary production and add value to the crops. Fresh green leaves are composed of 1-8% w/w protein, and approximately 50% of the protein is ribulose-1,5-bisphosphate carboxylase/oxygenase, also known as RuBisCO. RuBisCO has promising functional properties relevant in food structures. The structures in food determine its texture, which is an important aspect of how food is perceived when consumed. The properties of RuBisCO as a key element in several food structures, such as gels and wet foams, have been studied before providing information on RuBisCO's potential high performance, but there is no knowledge of how the protein behaves when the structures have been frozen and subsequently freeze-dried. We propose a possible way to produce RuBisCO protein isolates from the leaves of different crops, such as carrot, beet root, broccoli and cabbage, through a process including thermal coagulation of the green fraction and pH-shift precipitation of the white fraction. We aim to increase the understanding of the functional properties of RuBisCO in food structures by investigating freeze-dried foams based on the green leaf proteins from different plants.

## PC10.56 RE-WIRING PLANT REGULATORY NETWORKS TO ENHANCE STRESS TOLERANCE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

IULIA GHERMAN (UNIVERSITY OF WARWICK, UNITED KINGDOM), MATHIAS FOO (UNIVERSITY OF WARWICK, UNITED KINGDOM), DAVID L WILD (UNIVERSITY OF WARWICK, UNITED KINGDOM), KATHERINE J DENBY (UNIVERSITY OF YORK, UNITED KINGDOM), DECLAN G BATES (UNIVERSITY OF WARWICK, UNITED KINGDOM)

@ I.GHERMAN@WARWICK.AC.UK

By using synthetic biology and control engineering to inform experimental design, we aim to engineer Arabidopsis plants that are more resilient to stress.

Abiotic (such as drought, high light, senescence) and biotic (such as *Botrytis cinerea*, *Pseudomonas syringae* infection) stresses result in large-scale transcriptional reprogramming in plants. We have elucidated models of the complex regulatory networks underlying this reprogramming from time series transcriptome data of Arabidopsis genes which are specific to each individual stress. Using a combination of simulations and Bayesian statistics, with knockout mutant transcriptome and yeast one-hybrid data, we have ensured these transcription factor networks are as accurate as possible. These models have been used to predict key regulators and reporter genes for Arabidopsis defence responses for biotic stresses, as well as to predict the network re-wirings that will enhance the defence response.

Re-wiring involves changing the regulatory regions of transcription factors, influencing their expression levels and having a knock-on effect on the rest of the network. In order to validate the conclusions of our simulations, the hypotheses from our computer modelling are being tested in a transient protoplast systems with target genes re-wired with promoters predicted to have the desired effect. Reporter genes track the strength of the Arabidopsis defence response in comparison to the control, after induction with chitin or flg-22. A few rounds of the design-build-test cycle will increase our confidence in the computer modelling and chosen re-wirings, allowing us to engineer stable transformants in Arabidopsis plants.

## PC10.57 NICOSULFURON RESISTANCE IN *IXOPHORUS UNISETUS* (J. PRESL) SCHLTDL. BY POINT MUTATION IN THE ALS (ACETOLACTATE SYNTHASE) GENE

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ALEJANDRO DOMÍNGUEZ-LÓPEZ (COLEGIO DE POSTGRADUADOS, MEXICO), VÍCTOR CONDE-MARTÍNEZ (COLEGIO DE POSTGRADUADOS, MEXICO), EBANDRO USCANGA-MORTERA (COLEGIO DE POSTGRADUADOS, MEXICO), JESÚS R TORRES-GARCÍA (CENTRO DE INVESTIGACIÓN Y ESTUDIOS AVANZADOS DEL IPN, MEXICO), OBDULIA L SEGURA-LEÓN (COLEGIO DE POSTGRADUADOS, MEXICO)

@ DOMINGUEZ.JESUS@COLPOS.MX

As a weed species, *Ixophorus unisetus* (J. Presl) Schtdl. (zacatepillo) affects the maize crop in Jalisco, Mexico. Nicosulfuron is an ALS

inhibiting selective herbicide for this crop. In Mexico, populations of this grass weed have been found that cannot be controlled by the recommended dose. Resistance to ALS inhibitors is most commonly caused by point mutations in the ALS gene. The objective of this study was to detect nicosulfuron resistance-associated point mutations in the ALS gene in *I. unisetus* by using restriction enzymes. Random samples of the weed were collected from two maize producing locations, namely La Barca and Ameca, Jalisco. Genomic DNA was extracted from 30 plants from each location. PCR was performed using primers targeting the positions 197 and 574 in the ALS gene, which have previously been reported as target-site mutations. The amplicons were digested with the restriction enzymes Nla-IV and Bst-XI by the dCAPS method, which detects the changes that confer resistance to ALS inhibitors. Amplified fragments of the expected size (100 bp) and intensity were obtained from all the plants. Resistance to nicosulfuron of *I. unisetus* from La Barca was confirmed by a characteristic polymorphic profile obtained by digesting the amplicon targeting ALS-197 with Nla-IV in 60% of the plants. However, in the plants from Ameca, this polymorphic profile was absent. It can be concluded that resistance of *I. unisetus* to nicosulfuron is caused by mutations in the target site.

## PC10.58 SOLUTE ACCUMULATION AND PROTEIN EXPRESSION IN MAIZE (*ZEA MAYS* L.) PLANTS UNDER WATER DEFICIT

WEDNESDAY 5 JULY, 2017 POSTER SESSION

VÍCTOR CONDE-MARTÍNEZ (COLEGIO DE POSTGRADUADOS, MEXICO), SABINA VELÁZQUEZ-MÁRQUEZ (CENTRO DE INVESTIGACIÓN Y ESTUDIOS AVANZADOS DEL IPN, MEXICO), CARLOS TREJO (COLEGIO DE POSTGRADUADOS, MEXICO), ADRIANA DELGADO-ALVARADO (COLEGIO DE POSTGRADUADOS, MEXICO), EBANDRO USCANGA-MORTERA (COLEGIO DE POSTGRADUADOS, MEXICO)

@ VCONDE@COLPOS.MX

Water deficit has a negative impact on plant growth, biomass and seed production. Maize genotypes tolerant to water deficit can have some response mechanisms which allow them to adapt and survive under stress conditions. Water deficit reduces the relative water content, osmotic potential and solute accumulation, and causes changes in protein expression. The objective was to analyse a number of physiological and biochemical traits in two maize varieties: VS-22 (tolerant) and AMCCG-2 (susceptible) subjected to water stress. Samples were taken 49 days after emergence (DAE) from plants under two treatments: control (well-watered) and water-deficit (50% of soil water availability). Water deficit in VS-22 showed a RWC of 80% and an osmotic potential ( $Y_o$ ) of -2.0 MPa, compared with the control plants VS-22 which had a RWC of 98% and  $Y_o$  of -0.64 MPa. Water stress in the AMCCG-2 genotype showed a 70% RWC and a  $Y_o$  of -1.52 MPa. An increase in the accumulation of proline, glucose, fructose and sucrose was detected, and the trehalose disaccharide was also evidenced. The effect of water deficit produced a reduction in the RuBPCase protein expression in both genotypes, with AMCCG-2 being more affected. Water stress showed a positive correlation between water relations traits and solute accumulation. The lowest decrease in RWC and  $Y_o$  in VS-22 indicated a better osmotic adjustment compared with AMCCG-2.



## PC10.59 FUNCTIONAL CHARACTERIZATION OF ZEA MAYS XIPOTL FAMILY GENES

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ESTEFANY S SÁNCHEZ MARTÍNEZ (CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS DEL IPN LANGEBIO, MEXICO), RUBÉN RELLÁN ÁLVAREZ (CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS DEL IPN LANGEBIO, MEXICO), RUAIRIDH J H SAWERS (CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS DEL IPN LANGEBIO, MEXICO)

@ ESTEFANY.SANCHEZ@CINVESTAV.MX

The Phosphatidylcholine (PtCho) is the most abundant phospholipid in eukaryotic cell membranes, its biosynthesis diverges within the Kennedy pathway or the triple methylation of phosphoethanolamine by the enzyme phosphoethanolamine methyltransferase (PEAMT). In *Arabidopsis thaliana*, an insertional mutant for PEAMT (*xipotl*, *xpl1*) presented short root phenotype coincident with the observed in phosphorous deficiency. So far, 3 genes are reported for the methylation steps. However, in *Zea mays*, 4 genes are predicted for PEAMT function and show unique tissue specific expression patterns in early stages of root development despite having the same biochemical function. To clarify the activity of *Zm xpl* family genes, we performed heterologous complementation of the *Arabidopsis* insertional mutants *xpl1* and *xpl1,2,3*, using *Zm xpl* CDS family genes driven by *Arabidopsis* PEAMT promoters to study if the maize genes can perform all three methylation steps. Besides, we identified 'loss of function' insertional mutants in maize with the transposon system Uniform Mut to understand their role in the plant. In addition, we are studying the spatial patterns of *Zm xpl* genes expression at early stages of root development on WT and *xpl* mutants. Taken together, the results will give us an insight into the functional role *Zm xpl* family genes in the biosynthesis of PtCho.

## PC10.60 THE TIME IS RIPE: SUGAR AND HORMONE SIGNALS IN WHEAT GRAIN GERMINATION

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ROSS DENNIS (CSIRO ANU, AUSTRALIA), JEAN PHILIPPE-RAL (CSIRO, AUSTRALIA), ROBERT FURBANK (ANU, AUSTRALIA)

@ ROSS.DENNIS@CSIRO.AU

Pre-harvest Sprouting (PHS), which is when mature wheat grains germinate on the plant prior to harvest, is a major problem for wheat farmers. It can occur when there is rain or very high levels of humidity prior to harvest. PHS results in a downgrading of the quality of the grain to feed, and represents a loss of income for the farmer. The current model of cereal germination consists of: (1) hormone signals breaking dormancy and initiating germination; (2) the release of  $\alpha$ -amylases and proteases into the endosperm; (3) the degradation of starch by amylases releasing sugars to fuel early plant growth. High amylase expression is thus strongly associated with germination; however, some plants accumulate high levels of amylase in mature grain without germination. The aim of this study is to investigate the effects of  $\alpha$ -amylase expression and sugar signalling on germination and dormancy in wheat. We generated transgenic wheat with amylase expression in the endosperm prior to grain maturity, allowing uncoupling of amylase activity from grain dormancy. This amylase activity was shown to be sufficient to remove dormancy and initiate germination. However, the germination process itself was delayed. This work highlights as yet unexplored interactions of sugar and hormone signalling during germination and dormancy. In the long term this project aims to define differences between dormancy and germination in an important cereal crop leading to new solutions to prevent pre-harvest sprouting.

### PC10.61 TEMPERATURE SENSING AND SIGNALLING IN *ARABIDOPSIS* METABOLISM

WEDNESDAY 5 JULY, 2017 POSTER SESSION

HELENA HERRMANN (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GILES N JOHNSON (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), JEAN-MARC SCHWARTZ (THE UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

HELENA.HERRMANN@POSTGRAD.MANCHESTER.AC.UK

The allocation of transient carbon assimilates such as sugars, starch, malate and fumarate shifts in *Arabidopsis thaliana* as the plant is subjected to different stresses. Using both modelling and wet lab techniques we explore how the tightly regulated interplay of carbon assimilates provides the conditions necessary for photosynthetic acclimation. We aim to understand both the metabolic changes which signal an acclimation response as well as the different metabolic states required for acclimation. We have identified cytosolic fumarate accumulation as necessary requirement for acclimation to both cold and warm temperatures.

### PC10.62 NEXT GENERATION SEQUENCING OF AFRICA YAM BEAN ACCESSIONS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

ADEMOLA AINA (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

ATAINA@UCDAVIS.EDU

African yam bean (*Sphenostylis sternocarpa* Hochst. ex. A. Rich Harms), is the most economically important tuberous legume of tropical Africa. African yam bean (AYB) is a crop with numerous potential for food and nutrition security considering its nutritional profile. However, the crop is neglected and underexploited. To date, there are no improved varieties of this crop and genetic information on its diversity is scanty. The AYB is indigenous to Africa and is a climate resilient legume which helps in soil reclamation through nitrogen fixation. To efficiently utilize AYB genetic resources in crop breeding programs, there is a need to understand the crop's diversity. A total of 93 AYB accessions obtained from the International Institute of Tropical Agriculture in Nigeria were used for this study. Genomic DNA was extracted using the Qiagen extraction kit, DNA library preparation was done using the double digest GBS protocol followed by single end sequencing using the Illumina HiSeq 3000 platform. Using the Tassel's UNEAK Pipeline, 43061 (Restriction site associated DNA sequencing) RAD Tags were identified with 43061 SNPs (Tassel UNEAK pipeline only identified RAD Tag pairs differentiated by one SNP), 3722 SNPs called in at least 83 individuals (90%) used for admixture analysis for  $K=2$ ,  $K=3$  and  $K=4$ . The data generated from the RAD-Seq GBS are being subjected to further analysis to develop a high density genetic map.

### PC10.63 PHYLOGENOMICS AND SYSTEMS BIOLOGY APPROACHES REVEALS CONSERVED ADAPTIVE PROCESSES IN ATACAMA DESERT PLANTS

WEDNESDAY 5 JULY, 2017 POSTER SESSION

RODRIGO A GUTIÉRREZ (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), SOLEDAD UNDURRAGA (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), DANIELA SOTO (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), KRANTHI VARALA (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), GIL ESHEL (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), ERIKA VIVIANA ARAUS (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), RICARDO NILO (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), FRANCISCA DÍAZ (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), GABRIELA CARRASCO (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE), GLORIA CORUZZI (PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, CHILE)

RGUTIERREZ@BIO.PUC.CL

The Atacama Desert in Chile is the oldest and driest desert on Earth. Despite being one of the harshest environments in the world, plants have colonized and adapted to its extreme abiotic conditions. In order to identify genes underlying adaptive traits of these 'extremophile' plants, we used a systems biology and phylogenomic methodology. Our approach identifies the evolutionary divergence of 'extremophile' Atacama plants by comparing their sequences to publicly available phylogenetically-related ('sister') species, which are not adapted to Atacama's extreme conditions. We identified, collected and sequenced the transcriptome of 32 plant species within an altitudinal transect that spans the limits for life in Atacama. All these species are seed plants, with a preponderance of Angiosperms. An overall set of 70 species (32 from Atacama, 32 sisters and 6 model plants) was processed, generating nearly 1.7 million predicted proteins and over 70 thousand orthologue families. The resulting phylogenomic tree displayed twenty nodes that account for the divergence of extremophile Atacama plants from their non-Atacama sister species. We identified 9,591 genes that provide support to twenty independent origins of environmental adaptation. We identified a subset of 1,379 genes that gave recurring support (>18 independent origins). This data set was enriched ( $p$ -value < 0.05) in processes related to response to stress, response to radiation, embryo development, photosynthesis, nitrogen compound metabolic processes, among others. This data was used for the identification of key genes involved in the adaptation to marginal soils. Functional characterization of key candidate genes is ongoing in plant model systems.