

**ABSTRACT BOOK**

**SEB BRIGHTON 2016**

4-7 July, 2016  
BRIGHTON CENTRE, UK

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# PLANT BIOLOGY ABSTRACTS









# P1 FROM SOURCE TO SINK: RESOURCE PARTITIONING IN PLANTS

**ORGANISED BY:** MISS ANGELA WHITE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), PROF COLIN OSBORNE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), DR JENNIFER CUNNIFF (CABI, UNITED STATES) & PROF STEPHEN LONG (UNIVERSITY OF ILLINOIS, UNITED STATES)

## P1.1 IMPROVING LEAF CARBON ASSIMILATION TO INCREASE PLANT YIELD

WEDNESDAY 6 JULY, 2016 09:00

CHRISTINE A RAINES (UNIVERSITY OF ESSEX, UNITED KINGDOM), ANDREW SIMKIN (UNIVERSITY OF ESSEX, UNITED KINGDOM), PATRICIA LOPEZ (UNIVERSITY OF ESSEX, UNITED KINGDOM), KENNY BROWN (UNIVERSITY OF ESSEX, UNITED KINGDOM), STUART FISK (UNIVERSITY OF ESSEX, UNITED KINGDOM), TRACY LAWSON (UNIVERSITY OF ESSEX, UNITED KINGDOM)

@ RAINC@ESSEX.AC.UK

The primary determinant of crop yield is the cumulative rate of photosynthesis over the growing season which is the result of the crop's ability to capture light, the efficiency by which this light is converted to biomass and how much biomass is converted into the usable product. Traditional breeding and agronomic approaches have maximised light capture and conversion of biomass to end products and therefore, in order to increase yield, the efficiency of energy conversion will have to be improved. Evidence has now accumulated demonstrating that transgenic manipulation of the  $C_3$  cycle can increase the efficiency of photosynthesis and yield. We have used the knowledge gained from empirical and in silico modelling to produce plants with altered combinations of proteins and enzymes to enhance photosynthetic  $CO_2$  assimilation. Our approaches to the production and the results from analysis of these plants will be presented.

## P1.2 THE ROLE OF ATMOSPHERIC $CO_2$ AS A LIMITING FACTOR IN THE ORIGINS OF AGRICULTURE

WEDNESDAY 6 JULY, 2016 09:40

JENNIFER CUNNIFF (CABI, UNITED KINGDOM), GLYNIS JONES (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), MICHAEL CHARLES (UNIVERSITY OF OXFORD, UNITED KINGDOM), COLIN P OSBORNE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

@ J.CUNNIFF@CABI.ORG

Limitation of plant productivity by the low level of atmospheric  $CO_2$  experienced during the last glacial period is hypothesised to have been an important constraint on the origins of agriculture. To test this hypothesis, four wild progenitors of  $C_3$  (einkorn wheat and barley) and  $C_4$  crops (foxtail and broomcorn millets) were grown at glacial (180 ppm) and post-glacial (270 ppm)  $CO_2$ ; grain yield, and

the morphological and physiological components contributing to these yield changes were measured. The  $C_3$  species showed a significant increase in grain yield of ~50% with the increase in  $CO_2$ , which matched the stimulation of photosynthesis, suggesting that increases in photosynthesis are directly translated into yield at sub-ambient levels of  $CO_2$ . Increased yield was controlled by a high rate of tillering, and increases in seed number and size. The  $C_4$  species showed smaller, but significant, increases in grain yield of 10-15%, arising from larger seed numbers and sizes. Photosynthesis was enhanced by  $CO_2$  in only one  $C_4$  species suggesting that an indirect mechanism mediated by plant hydraulics could also be playing a role in the yield increase. Interestingly, the  $C_4$  species at glacial  $CO_2$  showed some evidence that photosynthetic capacity was upregulated to enhance carbon capture. Development under glacial  $CO_2$  also impacted negatively on the subsequent germination and viability of seeds. These results suggest that productivity of both  $C_3$  and  $C_4$  crop progenitors was limited by the atmospheric conditions of the last glacial period, with important implications for the origins of agriculture.

## P1.3 HOW DOES PHOTOSYNTHETIC EFFICIENCY INFLUENCE GROWTH AND ALLOCATION? INSIGHTS FROM THE EVOLUTION OF $C_4$ PHOTOSYNTHESIS

WEDNESDAY 6 JULY, 2016 09:55

COLIN P OSBORNE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), MARJORIE LUNDGREN (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), REBECCA ATKINSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), EMILY MOCKFORD (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), CHRIS BENNETT (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), PASCAL-ANTOINE CHRISTIN (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), BETH SPRIGGS (YALE UNIVERSITY, UNITED STATES), KEN THOMPSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), ROB FRECKLETON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), MARK REES (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

@ C.P.OSBORNE@SHEFFIELD.AC.UK

Photosynthesis has become an important target in crop improvement programmes, but the success of this approach will depend on how higher photosynthetic efficiency influences yield. Some insights into this problem have been gained by studying the evolution of photosynthesis in nature.  $C_4$  photosynthesis has evolved repeatedly to improve the efficiency of carbon fixation compared with the ancestral  $C_3$  photosynthetic type in hot, open environments. This replicated evolutionary experiment offers exciting opportunities for investigating how improved

photosynthetic efficiency changes productivity and allocation within a particular genetic background. We used a large-scale phenotyping approach to tackle this issue, comparing growth of 382  $C_4$  and  $C_3$  grass species under controlled environmental conditions, whilst accounting for the ecological and phylogenetic diversity among species. As expected, we found greater photosynthetic efficiencies in  $C_4$  than  $C_3$  species under high temperature and light. Growth was also correspondingly higher in  $C_4$  than  $C_3$  species. However, in small plants this effect arose largely from the construction of 35% cheaper leaves, with less mass per unit area and lower tissue density in  $C_4$  species. The differences in leaf efficiency and construction enabled  $C_4$  species to allocate >50% more biomass to roots than their  $C_3$  relatives. A potential sink feedback was also identified, with growth slowing more rapidly with increasing size, and final size five-times smaller in  $C_3$  than  $C_4$  species. Overall, these results demonstrate the importance of leaf allocation for rapid growth, the potential for greater photosynthetic efficiency to facilitate root growth, and the significance of size-mediated feedbacks for productivity.

### P1.10 DISRUPTION OF THE SUGAR TRANSPORTERS AT SWEET11 AND AT SWEET12 AFFECTS VASCULAR DEVELOPMENT AND FREEZING TOLERANCE IN ARABIDOPSIS

WEDNESDAY 6 JULY, 2016 11:00

CATHERINE BELLINI (UMEÅ PLANT SCIENCE CENTRE DEPARTMENT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN), ROZENN LE HIR (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH, FRANCE), LARA SPINNER (UMEÅ PLANT SCIENCE CENTRE DEPARTMENT OF PLANT PHYSIOLOGY UMEÅ UNIVERSITY, SWEDEN), PATRICK A. W. KLEMENS (UNIVERSITÄT KAISERSLAUTERN PFLANZENPHYSIOLOGIE, GERMANY), DIPANKAR CHAKRABORTI (UMEÅ PLANT SCIENCE CENTRE DEPARTMENT OF FOREST GENETICS AND PLANT PHYSIOLOGY SLU, SWEDEN), FEDRICA DE MARCO (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH, FRANCE), FRANÇOISE VILAINE (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH, FRANCE), RÉMI LEMOINE (UNITÉ MIXTE DE RECHERCHE 7267 ECOLOGIE ET BIOLOGIE DES INTERACTIONS UNIVERSITÉ DE POITIERS CNRS, FRANCE), EVELYNE TÉOULÉ (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH 78026 VERSAILLES, FRANCE), GRÉGORY MOUILLE (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH 78026 VERSAILLES, FRANCE), EKKEHARD H. NEUHAUS (UNIVERSITÄT KAISERSLAUTERN PFLANZENPHYSIOLOGIE, GERMANY), SYLVIE DINANT (INSTITUT JEAN-PIERRE BOURGIN UMR1318 INRA-AGROPARISTECH 78026 VERSAILLES, FRANCE)

@ CATHERINE.BELLINI@UMU.SE

In higher plants, soluble sugars are mainly present as sucrose, glucose, and fructose, and sugar allocation is based on both long-distance source-to-sink transport, intercellular and intracellular transport between the different organelles. The plant SWEET (for SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTERS) family of sugar transporters is a recently identified protein family of sugar uniporters. Here we report that AtSWEET11 and AtSWEET12 are expressed in the phloem and xylem parenchyma cells of the flower stem of Arabidopsis and that they can transport glucose and fructose in addition

to sucrose. Arabidopsis sweet11-1 sweet12-1 double mutant shows increased freezing tolerance due to sugar accumulation. This phenotypic effect was only reported for the double mutants sweet11-1 sweet12-1 suggesting a functional redundancy of AtSWEET11 and AtSWEET12 genes. Nevertheless in our growth conditions we also observed subtle differences in the single sweet11-1 and sweet12-1 mutants compared to the wild type. They show a slightly reduced growth, which is emphasized in the double mutant, but also a defect in the vascular bundles development associated to a modification of the chemical composition of xylem cell wall. Our data support the hypothesis that AtSWEET11 and AtSWEET12 are important for the export of sugars required for cell wall formation during vascular development.

### P1.11 WHEAT TIN MUTANTS WITH LARGER SINKS CAN HAVE LOWER PHOTOSYNTHETIC EFFICIENCY

WEDNESDAY 6 JULY, 2016 11:30

GONZALO M ESTAVILLO (CSIRO AGRICULTURE, AUSTRALIA), DAVID R COLEMAN (UNIVERSITY OF SYDNEY, AUSTRALIA), HUGO ALONSO-CANTABRANA (RSB ANU, AUSTRALIA), ANTHONY CONDON (CSIRO AGRICULTURE, AUSTRALIA), ANDREW MERCHANT (UNIVERSITY OF SYDNEY, AUSTRALIA), SUSANNE VON CAEMMERER (RSB ANU, AUSTRALIA), WOLFGANG SPIELMEYER (CSIRO AGRICULTURE, AUSTRALIA)

@ GONZALO.ESTAVILLO@CSIRO.AU

Carbon (C) source-sink balance modulates plant yield and optimising this balance could result in more C allocated to harvestable organs. We investigated C partitioning in near isogenic lines (NILs) of wheat (*Triticum aestivum*) containing different alleles of the tiller inhibition (*tin*) gene. The phenotype severity varied with *tin* allele, resulting in contrasting source and sink capacities. All *tin* mutants had fewer tillers than wild-type but thicker leaves with more N and delayed senescence, thicker stems that elongated faster and larger spikes. We investigated how C assimilation by leaves responded to these changes in source and sink. We found that the penultimate leaves of *tin* NILs displayed increased C assimilation rates (A) in proportion to thicker leaves and higher leaf N concentrations. In contrast, flag leaves of the *tin* lines were less efficient at fixing C per unit leaf N because A was the same as in the wild-type despite increased leaf N and Rubisco amount and greater Rubisco activity. Only penultimate leaves presented higher leaf sucrose concentration, in accord with higher A. Thus, it appears that the higher sink strength of a growing, thicker stem imparted higher photosynthetic demands on the penultimate leaf of the *tin* NILs but the same did not occur between flag leaves and larger spikes, where C demand, rather than assimilation, may be limiting. The observed spatial and temporal changes suggest that a whole plant approach may be needed to investigate source-sink relationships in crops.

## P1.12 CONCEPTS AND APPROACHES TO IMPROVE SOURCE-TO-SINK CARBON ALLOCATION

WEDNESDAY 6 JULY, 2016 13:50

UWE SONNEWALD (UNIVERSITY OF ERLANGEN-NÜRNBERG, GERMANY)

@ UWE.SONNEWALD@FAU.DE

Allocation of photoassimilates to harvestable plant organs is the most important determinant of crop yield. This process is affected by environmental and endogenous factors. In several crop plants temperature and day length significantly determine the switch between vegetative and generative growth. In potato for instance, elevated temperatures promote shoot growth and at the same time inhibits tuber-induction, leading to a reduced tuber yield. Similarly, biotic stress often alters source-to-sink relations to support growth of the pathogen. Source-to-sink interactions are not static but change during development. In young growing plants the rate of photosynthesis often exceeds sink demand. Thus photoassimilates accumulate in leaves and reduce photosynthetic efficiency. After storage sink induction, this relation shifts and photoassimilate supply by source tissues can get limiting. Over the last decades, many factors influencing source-to-sink relations have been deciphered and this knowledge has been used to design transgenic plants with improved biomass production. First-generation transgenic plants aimed at either tackling source, transport or sink limitations. Source manipulation concentrated on improved efficiency of photosynthesis, sucrose biosynthesis or sugar signaling. Transport processes were altered by tissue-specific manipulation of sucrose hydrolysis and active transport. Sink strength was targeted by improved energy, sucrose and starch metabolism. In all cases, increases in plant biomass production have been reported, indicating that bottlenecks limiting plant productivity could be overcome. To further boost productivity, second-generation transgenic plants, simultaneously altered in source and sink tissues, were designed. Concepts and approaches to improve source-to-sink carbon allocation will be discussed.

## P1.13 CARBON AND NITROGEN SOURCE-SINK INTERACTIONS IN ANNUAL AND PERENNIAL BARLEY

WEDNESDAY 6 JULY, 2016 14:30

ANGELA C WHITE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), ALISTAIR ROGERS (BROOKHAVEN NATIONAL LABORATORY, UNITED STATES), MARK REES (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), COLIN P OSBORNE (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

@ AVENETIANMORNING@GMAIL.COM

Increasing crop yield is a critical research target for future food security, and advancing our understanding of the factors contributing to plant growth is vital if yields are to be improved effectively. Source:sink interactions are a vital determinant of plant growth, but the relative effects of sources and sinks in controlling growth are not fully understood. Here, two congeneric barley species with different growth habits were grown along a CO<sub>2</sub> gradient and along a nitrogen gradient in controlled environment

chambers in order to establish the relative limitations imposed on growth by carbon and nitrogen sources and sinks. Resource uptake, metabolite concentrations, allocation and growth were measured. We found that fast growing annual barley is both carbon and nitrogen sink limited, whilst slow growing perennial barley is carbon source limited and nitrogen sink limited, during vegetative development. This indicates that barley crops are unable to invest excess nitrogen into growth and storage during the vegetative stage, and that optimising the growth and yield of barley for future higher CO<sub>2</sub> environments may be more challenging than was previously anticipated.

## P1.14 IS VARIATION IN APPARENT SINK-STRENGTH WITHIN GERMPLASM OF OUR MAJOR FOOD CROPS SUFFICIENT TO ALLOW FULL REALIZATION OF INCREASED PHOTOSYNTHESIS?

WEDNESDAY 6 JULY, 2016 14:45

STEPHEN P LONG (UNIVERSITY OF ILLINOIS, UNITED STATES), ELIZABETH E AINSWORTH (UNIVERSITY OF ILLINOIS, UNITED STATES), WILL HAY (UNIVERSITY OF ILLINOIS, UNITED STATES), VENKAT SRINIVASAN (UNIVERSITY OF ILLINOIS, UNITED STATES), NIKHIL JAIKUMAR (UNIVERSITY OF ILLINOIS, UNITED STATES)

@ SLONG@ILLINOIS.EDU

Sink limitations were once considered the major barrier to increased yield of our major food crops through attempts to increase photosynthetic capacity. Today, both open-air elevation of CO<sub>2</sub> using FACE (Free-Air CO<sub>2</sub> Enrichment) technologies that increase photosynthesis and transgenic manipulations to increase photosynthesis show that increasing leaf photosynthesis in crops can substantially increase yields. However, yield increases are commonly less than the measured increases in crop photosynthesis. This indicates some degree of sink limitation or shared control. Physiological and developmental limitations will be examined from experimental observation and mechanistic modeling of resource partitioning in field crops, which show that this lower-than-expected increase is not simply sink limitation. Is there sufficient variation within existing crop germplasm to overcome such limitations? It will be shown from FACE that this appears to be the case. Specifically, this will be addressed by reference to: 1) Our own field studies and literature meta-analyses of genotypic variation in soybean and rice responses of photosynthesis and seed yield within FACE; 2) Field trials of plants transgenically altered to increase photosynthesis and the extent to which this translates to increased yield in soybean, sugarcane and into tobacco as a model system.



## P1.19 IS REPRODUCTIVE DEVELOPMENT FAILURE UNDER WATER OR HEAT STRESS DUE TO C SHORTAGE?

WEDNESDAY 6 JULY, 2016 15:45

BERTRAND MULLER (INRA, FRANCE)

@ BERTRAND.MULLER@SUPAGRO.INRA.FR

Flower or grain abortion causes large yield loss under water deficit or heat stress. In maize under water deficit, it has been proposed to be due to a disruption of sugar supply to ovaries. We have tested this hypothesis via a precise spatio-temporal analysis of ovary and silk development along the ear. The first molecular events associated with water deficit occurred in silks rather than in ovaries, and involved genes affecting expansive growth rather than sugar metabolism. Our analysis also suggests that the cause of ovary abortion is the growth arrest of silks 2-3 days after first silk emergence. In grapevine, we have hypothesized that elevated temperatures negatively impact reproductive development through alteration of carbohydrate reserves. We have tested this hypothesis using microvine, a natural, gibberellic acid insensitive and dwarf mutant of grapevine that shows continuous reproductive development. Combining two contrasted years in terms of plant vigor and shoot carbohydrates status, we found that elevated temperature or a low carbohydrate status alone were not able to trigger reproductive failure (inflorescence drop). Rather, it always occurred in pace with sudden changes in supply / demand balance due to temperature shift or a boost in sugar demand by ripening berries in oldest nodes. Both examples show that the availability of carbohydrates can have contrasting effect on reproductive development depending on the context and recall that it must be interpreted as being possibly either cause or consequence of changes in the demand for carbon by sink tissues.

## P1.20 MODIFICATION OF STARCH METABOLISM IN TRANSGENIC ARABIDOPSIS THALIANA LEADS TO INCREASED PLANT BIOMASS AND OILSEED PRODUCTION

WEDNESDAY 6 JULY, 2016 16:15

MICHAEL J. EMES (UNIVERSITY OF GUELPH, CANADA), FUSHAN LIU (UNIVERSITY OF GUELPH, CANADA), QIANRU ZHAO (UNIVERSITY OF GUELPH, CANADA), NOEL MANO (UNIVERSITY OF GUELPH, CANADA), ZAHEER AHMED (UNIVERSITY OF GUELPH, CANADA), FELIX NITSCHKE (HOSPITAL FOR SICK CHILDREN TORONTO, CANADA), MARTIN STEUP (UNIVERSITY OF GUELPH, CANADA), IAN J. TETLOW (UNIVERSITY OF GUELPH, CANADA)

@ MEMES@UOGUELPH.CA

Carbon is provided from vegetative source leaves to support the growth and development of reproductive tissues. As a consequence of modifying starch biosynthesis and structure in the leaf, we found substantial and unexpected increases in vegetative biomass and oilseed production in the model plant *Arabidopsis thaliana*. Using *Arabidopsis* in which both endogenous isoforms of branching enzyme (SBE) were absent, we complemented the null-mutant by

expressing either one of the endosperm-specific maize (*Zea mays*) branching isozymes, ZmSBEI or ZmSBEIb. Each of the maize-derived SBEs, separately, restored starch biosynthesis to a higher level compared with wild-type, and transgenic lines possessed residual leaf starch at the end of the 8h dark period, with ZmSBEI lines containing substantially more than other lines. Morphology and structure of starch granules was also altered in transformants. Photosynthesis was higher in rosette leaves of transgenic SBE lines at early and late stages of development compared to wild-type. Altered starch metabolism in the transformants is associated with enhanced biomass formation, and a remarkable 3-4 fold increase in flowers and silique number per plant. Oil content and seed number per silique is essentially unchanged, though seeds from transgenic lines were slightly smaller. Total seed production per plant increased from ca 11,000 in wild-type to ca 50,000 in some ZmSBEI lines. The overall effect of introduction of cereal endosperm SBE isozymes represents a potentially useful strategy to increase biomass and oilseed production in related crops.

## P1.21 HARNESSING ARABIDOPSIS AND THE FRAMEWORK MODEL TO UNDERSTAND GROWTH

THURSDAY 7 JULY, 2016 09:00

ANDREW MILLAR (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), YIN HOON CHEW (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), DANIEL D. SEATON (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ARGYRIS ZARDILIS (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ALLY HUME (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), URIEL URQUIZA (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), ROBERT MUETZELFELDT (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

@ ANDREW.MILLAR@ED.AC.UK

The 24-hour circadian clock controls biological processes from the sleep-wake cycle to the cell cycle. Breeders have selected clock-associated gene variants in crop species (barley, wheat, tomato). Our ultimate aim is to understand both this artificial selection and the natural selection of the clock genes in the model plant species, *Arabidopsis thaliana*, where the clock controls the expression of 30% of all genes, and physiological processes ranging from elongation growth and biomass to flowering time.

The rich data of the *Arabidopsis* community has allowed us and our partners in the BBSRC ROBuST and EU FP7 TiMet projects to link ODE clock models to growth processes between germination and flowering, including the crucial, nightly utilisation of starch carbon stores (e.g. Seaton et al. *Interface* 2014; Seaton et al. *Mol Syst Biol* 2015). We also combined models from three further biological research areas into the *Arabidopsis* Framework Model (FM), which predicts biomass quantitatively in reference conditions (Chew et al. *PNAS* 2014). A key feature of this model is the simple but effective source-sink balance model from Greenlab (Christophe et al. *Functional Plant Biology*, 2008). We have now used the Framework Model, together with metabolic, molecular and whole-plant data, to understand quantitatively the pleiotropic phenotypes of a 'slow' clock mutant. We are also refactoring the clock model in absolute units (e.g. protein copies per cell). I will discuss the challenges for research, infrastructure and community organisation in extending and applying this proof-of-concept model in a distributed, community-led fashion.

## P1.22 TREHALOSE 6-PHOSPHATE IS A MAJOR REGULATOR OF SOURCE-SINK INTERACTIONS FOR SUCROSE ALLOCATION FOR CROP YIELD POTENTIAL AND YIELD RESILIENCE

THURSDAY 7 JULY, 2016 09:40

MATTHEW PAUL (ROTHAMSTED RESEARCH, UNITED KINGDOM), CARA GRIFFITHS (ROTHAMSTED RESEARCH, UNITED KINGDOM), YIQUN GENG (OXFORD UNIVERSITY, UNITED KINGDOM), RAM SAGAR (OXFORD UNIVERSITY, UNITED KINGDOM), BEN G DAVIS (OXFORD UNIVERSITY, UNITED KINGDOM)

@ MATTHEW.PAUL@ROTHAMSTED.AC.UK

Trehalose 6-phosphate (T6P) is a powerful sugar signal linking sucrose supply with growth and development underpinning source-sink interactions. Whilst biochemical pathways themselves such as sucrose synthesis are highly optimised by natural selection, the allocation of sucrose in crops is highly variable and is not currently optimised for yield. Evidence suggests that T6P in interaction with the feast/famine protein kinase, SnRK1, is a major regulatory hub for sucrose allocation and use regulating numerous genes. Modification of T6P levels in crops is showing unprecedented success for an intrinsic plant process. This success depends on an understanding of fundamental science and regulation of the whole plant and hence source-sink interactions and careful targeting of T6P to tissue and developmental stage. Low T6P as a starvation signal improved sucrose import into developing maize kernels and field yield with and without drought (Nuccio et al. 2015 Nature Biotechnology doi:10.1038/nbt.3277) and improved anaerobic growth and mobilisation of reserves under starvation enabling direct seeding of rice (Kretzschmar et al. 2015 Nature Plants doi: 10.1038/NPLANTS.2015.124). In other cases it is beneficial to increase T6P to promote biosynthetic processes such as starch synthesis and vegetative cell wall synthesis to stimulate growth recovery after drought, and gene priming for recovery from cold. In collaboration with Oxford University we have pioneered a chemical intervention approach using caged T6P which deliver pulses of T6P to crops which can increase both yield potential and crop resilience in an unprecedented manner. These compounds are also enabling new fundamental knowledge about T6P signalling.

## P1.23 COMPARATIVE CALCULATION OF ASSIMILATE PARTITIONING IN WINTER WHEAT USING THE LINTUL, SUCROS AND GECROS GROWTH MODELS

THURSDAY 7 JULY, 2016 09:55

MORITZ KUPISCH (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

Contemporary crop growth models are commonly source driven and calculate plant sink strengths based on empirical approaches which work well under optimum growing conditions but often fail when applied under water stress conditions. The mathematical characterization of plant carbon metabolism must be improved to overcome this problem. Three source driven crop growth models, LINTUL, SUCROS and GECROS, with contrasting assimilation and assimilate partitioning algorithms were compared against each other to understand the underlying causes using data from wheat experiments conducted in West Germany. Partitioning in LINTUL and SUCROS is calculated with fixed tabular functions depending on the phenological development of the crop. GECROS, in contrast, applies the functional balance theory assuming maximization of relative carbon gain. Applying these approaches resulted into different computed values of growth, LAI dynamics and yield when plant available water was not limiting. All models failed under severe stress. Simulation of assimilation per unit leaf area was correct under any water supply conditions, however, indicating that there is a need for incorporating quantitative routines for characterizing water stress effects on sink dynamics in crop growth models.

## P1.24 METABOLIC SIGNALS REGULATING RESOURCE ALLOCATION DURING SENESCENCE

THURSDAY 7 JULY, 2016 10:10

ASTRID WINGLER (UNIVERSITY COLLEGE CORK, IRELAND), SAYED JAFFAR ABBAS (UNIVERSITY COLLEGE LONDON, UNITED KINGDOM)

@ ASTRID.WINGLER@UCC.IE

In annual plants, leaf senescence is required for the recycling of nutrients, such as nitrogen, from the old leaves to the seeds. We have shown that sugars serve as signals for high carbon availability during senescence and that the signalling molecule trehalose-6-phosphate (T6P) reflects increased sugar levels in old leaves. Plants with increased T6P content through constitutive expression of the T6P synthase gene (*otsA*) showed increased anthocyanin content, whereas plants with reduced T6P through expression of the T6P phosphatase gene (*otsB*) showed delayed senescence. To differentiate between a direct involvement of T6P in senescence regulation and altered senescence as a consequence of earlier developmental changes, we created transgenic Arabidopsis plants with developmentally targeted changes in T6P metabolism under control of the highly senescence-specific *SAG12* and the seed-specific *OLE1* promoters. Surprisingly, transgenic lines expressing the Arabidopsis T6P synthase gene *TPS1* under control of the *OLE1*



promoter showed delayed leaf senescence, suggesting that T6P formation in the seeds may exert a sink effect for carbon, e.g. by stimulating the synthesis of storage lipids. Results for seed yield and composition will be presented.

## P1.25 NIGHT IN THE ARABIDOPSIS PLANT

THURSDAY 7 JULY, 2016 10:55

ALISON SMITH (JOHN INNES CENTRE)

ALISON.SMITH@JIC.AC.UK

Plants can only photosynthesis and hence produces sugars for growth during the day. Continued production of sugars for metabolism during the night requires mechanisms that sequester a fraction of photosynthate during the day, then make it available for sugar synthesis at night. In Arabidopsis, these mechanisms involve starch synthesis in the light, and conversion of starch to sugars in the dark. Poorly-understood processes regulate starch synthesis and degradation to bring about precise control of sugar availability over the day-night cycle. They include control of the partitioning of photosynthate into starch according to day length, matching of the rate of starch degradation at night to the anticipated time of dawn, and a gradual transition between starch synthesis and degradation at twilight. These processes involve the circadian clock, suites of enzymes that phosphorylate and dephosphorylate the surface of starch granules, protein complexes that define the size, shape and number of starch granules in chloroplasts, and proteins that influence the organisation of the starch granule matrix.

The mechanisms that control sugar availability in leaves over the day-night cycle appear to be coupled with mechanisms in roots that use information from cytosolic sucrose-to-hexose ratios to match root growth and development to the sugar supply from the leaves.

## P1.26 TREHALOSE-6-PHOSPHATE AND SUCROSE – A TALE OF TWO SUGARS

THURSDAY 7 JULY, 2016 11:25

JOHN E LUNN (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

LUNN@MPIMP-GOLM.MPG.DE

In the last 15 years, trehalose 6-phosphate (Tre6P) has been recognised as an essential signal metabolite in plants, with influence on growth and development that rivals any other signalling molecule, including the major phytohormones. Tre6P is an intermediate in the synthesis of trehalose, a very minor disaccharide sugar in plants. Mutants with altered Tre6P levels show severe defects in germination, leaf growth, stomatal regulation, flowering, shoot architecture and embryogenesis. Tre6P closely tracks diurnal and externally imposed fluctuations in the levels of sucrose, which is a major product of photosynthesis and the most common transport sugar in plants, and the main source of carbon and energy for growing sink organs. We propose that Tre6P functions as both a signal and negative feedback regulator of sucrose levels, helping to maintain intracellular sucrose concentrations within

an optimal range. This function can be compared with the insulin-glucagon system for regulating blood glucose levels in animals. In leaves, Tre6P regulates photoassimilate partitioning to sucrose during the day and the remobilization of transitory starch reserves to sucrose at night, linking both of these to demand for sucrose from sink organs. In meristems and other growing tissues, Tre6P signals the availability of sucrose for growth, influencing developmental decisions and the fate of imported sucrose. The intertwined relationship between sucrose and Tre6P is captured in the sucrose-Tre6P nexus concept. This model helps us to understand how Tre6P exerts such a profound influence on plant growth and development, and provides a framework for engineering Tre6P metabolism for crop improvement.

## P1.27 PHOTOSYNTHATE PARTITIONING PATTERN AS A KEY COMPONENT DETERMINING BIOMASS AND YIELD POTENTIAL

THURSDAY 7 JULY, 2016 13:50

XINGUANG ZHU (PARTNER INSTITUTE FOR COMPUTATIONAL BIOLOGY, CHINA), TIANGEN CHANG (PICB, CHINA)

ZHUXINGUANG@PICB.AC.CN

Photosynthesis generates photosynthate which ultimately determines the yield potential of crops. However, theoretical analysis and experimental evidence suggest that photosynthate partitioning patterns have a major impacts on the biomass accumulation and achievable yield as well. However, currently we are still lack of a mechanistic understanding of the photosynthate partitioning process and hence no mechanistic model of photosynthate partitioning model has been developed. In this presentation, we will introduce our current effort of developing a fully mechanistic model of photosynthate partitioning. The current status of the modules describing the leaf, root, grain filling and photosynthate transport between different organs will be presented. The demand for experimental data collection for model parameterization and validation will be discussed.

## P1.28 ALLOCATION AND TRANSLOCATION OF RESOURCES IN SORGHUM UNDER DIFFERENT WATER STATUS ENVIRONMENTS

THURSDAY 7 JULY, 2016 14:30

JEREMIAH T SAMAILA (HARPER ADAMS UNIVERSITY, UNITED KINGDOM), PETER S KETTLEWELL (HARPER ADAMS UNIVERSITY, UNITED KINGDOM), IVAN G GROVE (HARPER ADAMS UNIVERSITY, UNITED KINGDOM), MITCH CROOK (HARPER ADAMS UNIVERSITY, UNITED KINGDOM)

JSAMAILA@HARPER-ADAMS.AC.UK

Sorghum is the fifth most cultivated cereal crop in the world and Nigeria is the world's third largest producer where sorghum is the third most widely cultivated crop. Sorghum is recognised as a remarkably drought tolerant crop with the capacity to thrive in hot and dry climates. Apart from being grown as a major staple food crop

in the semi-arid tropics, it is also grown in large quantities in China, USA, and Mexico, consequently its production spans through different geographical locations under different environments. In addition, while it is grown in the semi-arid tropics mainly as food for human consumption, it is grown in China and USA mainly for its non-food uses as sources of energy, fibre and forage. Investigating sorghum growth, resource allocation and translocation is an essential step towards harnessing the crop's potential for food and non-food uses. A glasshouse experiment investigated the allocation of dry matter in sorghum under different environmental conditions involving drought and antitranspirant treatment. Sorghum grown in well-watered pots in a glasshouse was later subjected to drought and/or antitranspirant treatment at forty-five days after emergence for a thirty-day period and later reverted to well-watered conditions for another thirty days. Data were taken on grain yield, harvest index and total biomass, during and after the treatment period and analysed using factorial ANOVA. Results will be discussed and interpreted in the light of the crop's potentials for non-food use and implications for its current food uses for Nigeria.

### P1.29 'C<sub>4</sub>' METABOLISM IN THE VASCULATURE: RELATIONSHIPS BETWEEN PEP CARBOXYKINASE ACTIVITY AND XYLEM NITROGEN RECYCLING AND HYDRAULIC CONDUCTIVITY IN RICE LEAVES

THURSDAY 7 JULY, 2016 14:45

RICHARD C LEEGOOD (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), KAREN J BAILEY (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

R.LEEGOOD@SHEFFIELD.AC.UK

Measurements of amino acids in the guttation fluid and in the xylem exudates of cut leaves from intact plants provide evidence of the remarkable efficiency with which these compounds are reabsorbed from the xylem sap. This could be achieved by mechanisms involving intercellular transport and/or metabolism. Developmental changes in transcripts and protein showed that transcripts for PEP carboxykinase (PEPCK) increased from the base to the leaf tip, and were markedly increased by supplying asparagine. Supplying amino acids also increased the amounts of protein of PEPCK and, to a lesser extent, of PPDCK. PEPCK is present in the hydathodes, stomata and vascular parenchyma of rice leaves. Evidence for the role of PEPCK was obtained by using an activation-tagged rice line that had an increase in PEPCK activity and by using 3-mercaptopicolinic acid, a specific inhibitor of PEPCK, to show that activation of PEPCK resulted in a decrease in N in the guttation fluid and xylem sap and that inhibition of PEPCK resulted in an increase in N in the guttation fluid and xylem sap. Furthermore, increasing PEPCK decreased the volume of xylem sap or guttation fluid, whereas decreasing PEPCK increased the volume of xylem sap or guttation fluid. These findings suggest that (a) both metabolism and transport are involved in xylem recycling and (b) excess N is the signal involved in modulating xylem hydraulic conductivity, whether in the roots or shoots. Water relations, nitrogen recycling and vascular metabolism and transport are thus intimately linked.

### P1.30 NEW APPROACH IDENTIFIES HORMONES AS A NEW PHYSIOLOGICAL TRAIT FOR SPIKE FERTILITY RESILIENCE TO DROUGHT STRESS

THURSDAY 7 JULY, 2016 15:00

ARNAULD A. THIRY (LANCASTER UNIVERSITY, UNITED KINGDOM), WILLIAM J. DAVIES (LANCASTER UNIVERSITY, UNITED KINGDOM)

A.THIRY@LANCASTER.AC.UK

Global warming and the increased food demand by a growing world population increase the need to speed up the selection of crop genotypes resistant to and productive under abiotic stress. However, selection for tolerance to a stress environment is not entirely clear and oscillates between the concept of yield stability and high production under stress and no-stress environments. In this work, a new method for evaluation of genotypes in terms of resilience to face the stress and production capacity is suggested. The method quantifies the components of a new index related to yield under abiotic stress. This method offers a simple and easy visualisation and identification of resilient and productive genotypes according to their grain yield. Nevertheless, wheat yield is a complex trait as it can be affected differently between genotypes according to the spike development. Consequently, genotypes with similar yield under the same stress can have different susceptibility by modifying differently their yield components. Consistency of the indices between years and stresses and the simplicity in identifying contrasting genotypes in terms of yield and yield components offer the possibility of identifying new traits that can be associated to one or another tolerance mechanism, enabling a better understanding of the stress tolerance physiology. Here, the indices have been applied to identify a relationship between hormones and drought stress resilience on wheat at key phenological stages. The results suggest that hormones could be used as a new physiological trait to identify genotypes with high resilience in terms of spike fertility.

### P1.31 GENERAL PATTERNS IN BIOMASS ALLOCATION AND ALLOMETRY AMONG HIGHER PLANTS

THURSDAY 7 JULY, 2016 15:45

HENDRIK POORTER (FORSCHUNGSZENTRUM JÜLICH, GERMANY)

H.POORTER@FZ-JUELICH.DE

It is well-known that plants may adjust the distribution of biomass over leaves, stems and roots depending on environmental conditions. It is also clear that size is an important factor as well. However, good quantitative insights are lacking. In this talk I analyse biomass allocation patterns to leaves, stems and roots of herbs and woody species. A database was compiled with ~11.000 records of leaf, stem and root biomass for ~1200 species. First, I'll derive general dose-response curves that describe the relationship between biomass allocation and the 12 most important a-biotic environmental factors and compare them with the changes in leaf, stem and root morphology. Second, I'll focus on allometric relationships between the various organs and test to what extent they comply with models like that for Metabolic Scaling Theory,



where the slope of the log-log relationship between leaf and root biomass is expected to have a value of  $\frac{3}{4}$ . Third, I analyse how leaf, stem and root mass fractions change as a function of total plant size. This offers a great opportunity to test to what extent there are systematic differences in allocation patterns related to phylogeny (e.g. Gymnosperms vs. Angiosperms) and functional group (e.g. deciduous vs. evergreens).

### P1.32 ELEVATED CO<sub>2</sub>, DROUGHT AND NUTRIENT SUPPLY AFFECT GROWTH, RESOURCE PARTITIONING AND NUTRITIONAL QUALITY OF CASSAVA AND TARO

THURSDAY 7 JULY, 2016 16:15

ROS GLEADOW (MONASH UNIVERSITY, AUSTRALIA), STEVEN CRIMP (CSIRO, AUSTRALIA), BRUCE WEBBER (CSIRO, AUSTRALIA)

ROS.GLEADOW@MONASH.EDU

Over one billion people eat cassava every day (primarily the tuberous roots), and taro (especially the underground corm) is a dietary staple in the Pacific and Asia. Below ground storage organs are an important component of global food security but are likely to respond differently to climate change relative to other crops. There is a trade-off the allocation of resources to growth, storage organ and plant toxins, affecting crop yield and nutritional value. For example, cassava stores toxic cyanogenic glycosides that, if not properly processed, may cause cyanide intoxication, permanent paralysis and even death. Drought stressed plants are more cyanogenic, but the effects of temperature, nutrient availability and atmospheric CO<sub>2</sub> concentration are less well understood. Taro also contains cyanogenic glycosides, but not in toxic concentrations. Taro does, however, contain crystals of calcium oxalate that may cause irritation. We grew cassava and taro at four different levels of CO<sub>2</sub> (400, 500, 700 and 900 ppm) under well-watered conditions and adequate nutrients. Plants were monitored weekly and destructively harvested at the time of tuber initiation (in cassava) and at 6-8 months of age. Measurements of photosynthesis, growth, water use efficiency and nutritional quality showed that nutritional status, growth rate and crop yield changes with plant age and with CO<sub>2</sub> treatment. This information will be incorporated into agronomic models of cassava and taro to guide future production and climate change adaptation in the Pacific Islands.

### P1.4 POST-TRANSLATIONAL REGULATION OF MAIZE STARCH SYNTHASE IIA, A KEY ENZYME OF STARCH BIOSYNTHESIS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

FATEMEH (SAHAR) MEHRPOOYAN (UNIVERSITY OF GUELPH, CANADA), IAN J. TETLOW (UNIVERSITY OF GUELPH, CANADA), MICHAEL J. EMES (UNIVERSITY OF GUELPH, CANADA)

FMEHRPOO@UOGUELPH.CA

Starch is the most abundant storage carbohydrate in plants, providing 70% of human caloric intake and has many industrial applications. Starch biosynthesis involves the coordination of starch synthases (SSs), starch branching enzymes (SBEs) and debranching enzymes. Starch biosynthetic enzymes function through formation of multi-enzyme complexes, and protein phosphorylation plays a crucial role in this enzyme complex formation in cereals. In maize, the isozyme SSIIa, forms the core of a heteromeric protein complex with SBEIIb and SSI, and is responsible for the localization of this complex between the plastid stroma and starch granule. The activity of this particular protein complex is crucial for normal starch biosynthesis in maize. Recent evidence suggests that SSIIa is post-translationally regulated by protein phosphorylation in amyloplasts of maize endosperm. Protein phosphorylation has a significant effect on the electrophoretic mobility of SSIIa, when analysed by western blots following non-denaturing PAGE. Multiple bands of SSIIa were identified, suggestive of major conformational changes and/or association with other proteins. Results will be presented on the effect of phosphorylation on the catalytic activity of endogenous and recombinant forms of SSIIa as well as on formation of complexes with other enzymes of starch biosynthesis. The sites of SSIIa phosphorylation have been investigated by site-directed mutagenesis and data on the amino acid residues involved will be discussed. The present study provides new insights into our understanding of the signal transduction system regulating amylopectin biosynthesis in plants. This work is of strategic importance and has the potential to identify novel genes for crop improvement.

### P1.5 ENVIRONMENTAL REGULATION OF STOMATAL DEVELOPMENT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JORDAN C BROWN (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), STUART CASSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM)

JBROWN9@SHEFFIELD.AC.UK

Stomata are the small pores on the surface of the leaf. Stomatal number and pore aperture influence the amount of water lost through transpiration and have a direct impact on plant water use efficiency (WUE; carbon gain per unit of water loss). Understanding plant water use and performance would enable its manipulation to produce strains with the water use and performance desired for various terrains, reducing the current 70-80% global fresh water consumption for irrigation.

Environmental signals such as light and CO<sub>2</sub> impact on stomatal development resulting in increases or decreases in stomatal number

on developing leaves. Although individual signal responses are increasingly well characterised, what remains unclear is how these signals interact to affect stomatal development. In this project, we are examining how light and CO<sub>2</sub> signals interact to influence the stomatal development pathway, and how this then affects plant WUE. Work in our lab has identified that the key light receptor, phytochrome B (phyB), is responsible for white light regulation of stomatal development. Gas exchange analysis of phyB mutants shows that phyB regulates plant WUE in a CO<sub>2</sub> dependent manner.

## P1.6 EXPLOITING MAGIC ARABIDOPSIS TO IDENTIFY GENETIC CONTROLS OF STOMATAL DEVELOPMENT BY ATMOSPHERIC CO<sub>2</sub>

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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

@ HEGSEWELL1@SHEFFIELD.AC.UK

Rising atmospheric CO<sub>2</sub> directly affects development of stomata on the leaf surfaces of many plant species, typically leading to lower stomatal densities (SD) in elevated CO<sub>2</sub>. This reduction in stomata can be an advantage to the plant by reducing water loss and promoting increased water use efficiency (carbon gain per unit of water loss per unit time). However, although this developmental response to CO<sub>2</sub> is widely recognized, little is known about the genes controlling the effects of elevated CO<sub>2</sub>.

In this project, I am exploiting Multiparent Advanced Generational Inter-Cross (MAGIC) Arabidopsis, which are a series of recombinant inbred lines, to identify Quantitative Trait Loci (QTL) implicated in the CO<sub>2</sub> response of stomata. This will identify regions of the genome likely to contain genes in the SD CO<sub>2</sub> response pathway. Identified suggested genes will be tested through growing knockouts in elevated and ambient CO<sub>2</sub> and examining the resulting phenotypes.

By screening several hundred MAGIC lines I have shown phenotypes with a wide range of SD responses to elevated CO<sub>2</sub>, some of which are more extreme than the parental lines from which they are derived. This highlights the complicated nature of how development is affected by elevated CO<sub>2</sub> and suggests that the genes interact synergistically to produce epistasis.

## P1.7 STARCH SYNTHASE 2 POST-TRANSLATIONAL REGULATION AND STARCH BIOSYNTHESIS IN LEAVES OF *ARABIDOPSIS THALIANA*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JENELLE A PATTERSON (UNIVERSITY OF GUELPH, CANADA), QIANRU ZHAO (UNIVERSITY OF GUELPH, CANADA), IAN J TETLOW (UNIVERSITY OF GUELPH, CANADA), MICHAEL J EMES (UNIVERSITY OF GUELPH, CANADA)

@ JPATTE01@UOGUELPH.CA

Starch is the main source of caloric intake for human consumption and is also used for a wide array of non-food applications. Starch is produced in plant plastids for either long-term storage or transiently in photosynthetic tissues during the light-period to be degraded at night to support cellular respiration. Both types of starch are synthesized by the coordinated actions of three main enzyme classes: starch synthases (SS), branching enzymes (SBE) and debranching enzymes (DBE), with each class consisting of several isoforms. In cereal endosperm, the SSII isoform forms the core of a heteromeric protein complex with SBEII and SSI, and is responsible for localization between the plastid stroma and starch granule. Consequently, mutations in SSII result in a marked starch phenotype. The goal of this study is to determine the role and regulation of SS2 in transient starch biosynthesis using the model system, *Arabidopsis thaliana*. Bioinformatic analysis indicates that the SS2 N-terminal region (NRT) is highly variable compared to known orthologous SS2 sequences. This NRT is predicted to be intrinsically disordered, a conformation state associated with both protein phosphorylation and protein-protein interactions. In vitro, SS2 can be phosphorylated at multiple sites solely within this NTR. SS2 forms a phosphorylation-dependent complex with SBE2.2, but the precise role of SS2 phosphorylation is still unknown. Work in this study aims to characterize the role of NTR modifications on SS2 regulation both in vitro, and in vivo by complementing ss2-*Arabidopsis* with modified SS2 sequences.

## P1.8 REGULATION OF STARCH BRANCHING ENZYMES IN ARABIDOPSIS BY POST-TRANSLATIONAL MODIFICATION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

GREG J MACNEILL (UNIVERSITY OF GUELPH, CANADA), QIANRU ZHAO (UNIVERSITY OF GUELPH, CANADA), IAN J TETLOW (UNIVERSITY OF GUELPH, CANADA), MICHAEL J EMES (UNIVERSITY OF GUELPH, CANADA)

@ MACNEILL@UOGUELPH.CA

Starch is a water insoluble, semi-crystalline granule comprised of two glucose homopolymers, amylose and amylopectin. It is an important carbon store in higher plants, and a major part of the human diet. Starch is synthesised in plastids from ADP-glucose through the cooperative activity of starch synthases, starch branching enzymes (SBE), and starch debranching enzymes. Two branching enzyme isoforms, SBE2.1 and SBE2.2, are expressed in leaves of *Arabidopsis*. Using [<sup>32</sup>P]-ATP, recombinant forms of SBE were shown to be phosphorylated by soluble chloroplast extracts.



SBE2.2 accounts for most of the measurable activity in Arabidopsis leaves and is demonstrated here to be phosphorylated on Ser290 and Ser301. SBEs have been shown to form multi-enzyme complexes with other starch biosynthetic enzymes. A putative protein-protein interaction domain, conserved across all class II SBEs, was identified. Site-directed mutagenesis is being used to alter this site as well as phosphorylation sites of SBE2.2 to determine their importance in regulating the formation of complexes with other enzymes of starch biosynthesis, as well as effects on catalysis of branching. The in vitro effect of phosphorylation site and interaction domain mutations on enzyme activity and protein-protein interactions will be presented. Effects on plant growth and starch production in vivo will be discussed based on functional complementation of Arabidopsis mutants devoid of SBE activity. In planta protein-protein interactions are being investigated using bimolecular fluorescence complementation (BiFC) in a transient tobacco expression system and data will be presented on the effects of specific modifications the interaction of SBEs with other enzymes.

### P1.9 SPRUCE SEEDLING SHOOT AND ROOT GROWTH AFTER DEHARDENING. EFFECTS OF REDUCED GREEN TISSUE ON BASAL SHOOT PARTS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

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

A.V.VOLLSNES@IBV.UIO.NO

We have studied the root and shoot dynamics of spruce seedling growth after dehardening. During winter, parts of the shoot may be damaged while the root system is hidden and protected in the soil. The root may still suffer from the loss of needles, since we expect the needles on the lower stem to be the main sources for fuelling root growth. Needles at increasing heights on the lower stem have been removed or injured by heat treatments to stop them from producing photosynthates and the effects on root growth have been studied. As the new shoots emerging from the buds on the shoot part are competing sinks, there may be a threshold value for the distance from the root system to the nearest green needles. At too long distances, the root may lose the competition against the shoot for photosynthates. Shoot growth initially requires only water for expansion of pre-produced needles hidden in the buds during winter. A mal-functioning root may therefore be hard to discover when only examining the shoot after the plant has started to grow. We describe the observed root and shoot interactions after manipulating the photosynthesising needles on the lower stem.

### P1.15 DOES ARABIDOPSIS THALIANA TREHALOSE-PHOSPHATE SYNTHASE 1 (ATTPS1) HAVE OTHER FUNCTIONS IN ADDITION TO TRE6P SYNTHESIS?

WEDNESDAY 6 JULY, 2016 POSTER SESSION

MARTIN A LAUXMANN (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), MARK STITT (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY), JOHN E LUNN (MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY, GERMANY)

LAUXMANN@MPIMP-GOLM.MPG.DE

Trehalose 6-phosphate (Tre6P) is a sugar-signalling metabolite that coordinates plant growth and development with carbon status. Tre6P is proposed to be both a signal and feedback regulator of sucrose levels, helping plants to maintain sucrose levels within a range that is optimal for survival and growth. Tre6P is synthesized by Tre6P synthase (TPS) and dephosphorylated to trehalose by Tre6P phosphatase (TPP). To deepen our understanding of TPS1 in signalling, we have investigated the subcellular localisation and searched for protein interactors of trehalose-phosphate synthase 1 (AtTPS1), the main isoform involved in Tre6P synthesis. We found that AtTPS1-GFP is not only located in the cytosol, as previously reported, but also in the nucleus and the plasmalemma. Moreover, spinning-disc confocal microscopy showed that AtTPS1-GFP blinks dynamically at the plasmalemma and integrates into cytoplasmic particles which co-align with cortical microtubules and behave differently depending on the sucrose status. By means of blue native PAGE, AtTPS1 was found to migrate in two gel fractions corresponding to native protein complexes of ~550 kDa and ~700 kDa both in carbon-starved seedlings, which have low Tre6P levels, and in seedlings resupplied with sucrose for three hours, which have high Tre6P levels. Co-immunoprecipitation assays revealed that AtTPS1 protein interacts with multiple proteins involved in metabolic, signalling and growth-related processes. These results suggest that AtTPS1 might have other functions, via protein-protein interactions, in addition to synthesis of Tre6P.

## P1.16 DECIPHERING THE GENETICS BASIS OF PLANT-PLANT INTERACTIONS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

GINA A GARZON-MARTINEZ (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), JAY BIERNASKIE (UNIVERSITY OF OXFORD, UNITED KINGDOM), FIONA CORKE (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), JOHN H DOONAN (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), ANYELA CAMARGO-RODRIGUEZ (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM)

@ GIG7@ABER.AC.UK

Plant competition for resources, such as light, water or nutrients, is a complex dynamic process that has implications for the formation and diversity of plant communities and ecosystems as well as impacting on agricultural practice for optimizing yield. The processes that affect plant-plant interactions are poorly understood at both the genotypic and phenotypic/physiological levels. For example, traits involved in reproductive fitness can be differentially affected by intraspecific and interspecific interactions. A decrease in a genotype's fecundity is defined as competition whereas an increase can be defined as co-operation. Some studies have described the phenotypic outcome of kin and non-kin interactions in *Arabidopsis thaliana* but, even in this model system, the genetic basis remains poorly understood. The aim of this study is to assess the extent of natural variation in the response of plants to neighbours and thereby identify genomic regions associated with changes in life history traits under competitive/cooperative interactions. High-throughput phenotyping approaches will be used to quantify traits related to growth, survival and reproductive success and undertake a genome wide association study (GWAS) to link the phenotype and genotype. It is expected that the results of this study help us to dissect the genetic basis of adaptation to plant density, which could have an impact in the development of plant breeding strategies to improve crop yield.

## P1.17 CARBON STARVATION AND SATIATION DURING THE LEAF ONTOGENY

WEDNESDAY 6 JULY, 2016 POSTER SESSION

BERTRAND MULLER (INRA-MONTPPELLIER, FRANCE), FLORENT PANTIN (INRA-MONTPPELLIER, FRANCE), YVES GIBON (INRA-BORDEAUX, FRANCE), MARIA PIQUES (MPI-GOLM, GERMANY), THIERRY SIMONNEAU (INRA-MONTPPELLIER, FRANCE)

@ BERTRAND.MULLER@SUPAGRO.INRA.FR

In previous studies, we found that the control of leaf expansion switches from a metabolic to a hydraulic origin during ontogeny. We aimed at evaluating the synchronicity between this switch and (i) the source-sink transition of the leaf and (ii) the occurrence of carbon starvation/satiation as seen through molecular markers. We used the model plant *Arabidopsis thaliana* cultivated under well-watered or under water stress conditions that we suspected to affect both C availability and biophysical constraints. The *pgm* starchless mutant was also included as a control for C starvation at night. Measurements of growth, photosynthesis and respiration on a 24-h basis allowed to locate the sink-to-source transition at 46% of final leaf area in leaves of Col-0 under well-watered conditions, significantly later than the switch from metabolic to hydraulic control. Both water stress and the *pgm* mutation delayed this transition. Using the day/night metabolite turnover, we could locate differentially a day and a night sink-to-source transition which occurred as soon as 18% of final area in the daytime but shifted at 66% in the night time. Despite a decreased photosynthesis, promotion of carbon satiation under water stress was confirmed by most molecular markers. We conclude that leaf growth normally operates at source limitation in the young leaves as well as during the night, while a sink, biophysical limitation establishes gradually in the daytime.



### P1.18 HIGH TEMPERATURES REDUCE YIELD THROUGH ALTERED CARBON ASSIMILATION AND RESPIRATION IN THE C<sub>4</sub> GRASS *MISCANTHUS*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

RICHARD J WEBSTER (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), GIOVANNI SCALICI (UNICT, ITALY)

@ RCW@ABER.AC.UK

*Miscanthus x giganteus*, a result of a cross between *M. sinensis* and *M. sacchariflorus*, is unique among C<sub>4</sub> species in its remarkable ability to maintain high photosynthetic productivity at low temperatures. However there is anecdotal evidence to suggest that at high temperatures yield in *M. x giganteus* is reduced. In order to investigate this, *M. x giganteus*, *M. sinensis* and *M. floridulus* plants were grown and measured at 30°C or 22°C. Measurements of photosynthesis, combined CO<sub>2</sub> assimilation and modulated chlorophyll fluorescence were measured along with biomass partitioning and stomatal responses. The high temperature treatment decreased the height of *M. x giganteus* (~43%), above ground DM yield (~66%), below ground DM yield (~26%), and the response of carbon assimilation to absorbed irradiance was reduced by (~14%) and dark respiration increased significantly. Whereas responses in *M. sinensis* and *M. floridulus* were less severe.

### P1.33 EXPRESSION AND STRUCTURAL ANALYSIS OF STARCH METABOLISM GENES IN PLANT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

UNG-HAN YOON (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), GANG-SEOB LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), TAE-HO KIM (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), HYEON-SO JI (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), JAE CHEOL JEONG (KOREA RESEARCH INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY, KOREA (SOUTH)), SUNG-WON YOON (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), YOUNG-JOO SEOL (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), CHANG-KUK KIM (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), JONG-YEOL LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), TAE-HO LEE (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH)), JANG-HO HAHN (NATIONAL INSTITUTE OF AGRICULTURAL SCIENCE, KOREA (SOUTH))

@ UHYOON@KOREA.KR

Starch metabolism gene expression research in plants is important especially when studying starch component and plant productivity. In this study, we carried out gene expression analysis and structural analysis of starch metabolism genes in plant. For rice starch metabolism gene structure and expression analysis; 51,383 EST's from immature and germinating seeds of *Ilpumbyeo* were sequenced. Thirty one full length cDNA clones were isolated and were found to be homologous to starch synthesis genes. A search was conducted on starch metabolism genes from which 156 genes were selected in TIGR rice annotation DB. For phylogenetic analysis, nucleotide and amino acid sequences were aligned for 34 kinds of plants with AGPase genes, 8 kinds of plants with starch branching enzyme genes, 11 kinds of plants with waxy genes and 8 kinds of plants with starch synthase genes by MEGA 6 software. In order to understand the functional role of starch synthesis and degradation genes, the expression profiling of these genes in rice were analysed using 135K microarray. As a result, high levels of gene expressions for AGPase, starch synthase, starch branching enzyme, and pullulanase were observed in immature seeds. In germinating seedlings an amylase gene, Os08g047380 which is involved in starch degradation was expressed strongly. Finally it was revealed that these genes were expressed in a tissue specific manner. Further work focuses on the development of over expression and knock-out lines to better understand the functions of these genes in starch metabolism.

# P2 HORMONE RECEPTORS: STRUCTURES, COMPLEXES AND BIOSENSORS

**ORGANISED BY:** ORGANISED BY: PROF RICHARD NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM) & DR STEFAN KEPINSKI (UNIVERSITY OF LEEDS, UNITED KINGDOM)

**SPONSORED BY:** THE JOURNAL OF EXPERIMENTAL BOTANY

## P2.1 SEEING AND MODELING SELF-ORGANIZATION DYNAMICS AT THE SHOOT APEX

📅 MONDAY 4 JULY, 2016 ⌚ 11:00

👤 TEVA VERNOUX (ÉCOLE NORMALE SUPÉRIEURE DE LYON)

@ TEVA.VERNOUX@ENS-LYON.FR

The initiation of plant aerial organs at the shoot apex follows precise spatio-temporal patterns that control the shoot primary architecture, the phyllotaxis. A combination of modeling and experiments have shown that hormone-based inhibitory fields are central to the dynamics of morphogenesis at the shoot apex, making it a system of choice to understand the self-organization driven by lateral inhibitions. We have developed biosensors for plant hormones that have allowed the first visualization of inhibitory fields. We have also uncovered a second-type of hormone-based fields that controls specifically the timing of organ initiation at the shoot apex and further shown that this timing is particularly prone to noise. We will discuss these mechanisms and show that a stochastic model of phyllotaxis allows understanding the function of hormone-based fields and how phyllotaxis is influenced by biological noise.

## P2.2 EVOLUTION AND ORIGIN OF ARABIDOPSIS THALIANA HISTIDINE-CONTAINING PHOSPHOTRANSMITTERS – REVELATION FROM MODULAR APPROACH

📅 MONDAY 4 JULY, 2016 ⌚ 11:40

👤 SIARHEI A. DABRAVOLSKI (MASARYK UNIVERSITY, CZECH REPUBLIC), RESHMA NIBHANI (UNIVERSITY OF HAIFA, ISRAEL), ZAKHARIA M. FRENKEL (ORT BRAUDE COLLEGE UNIVERSITY OF HAIFA, ISRAEL), EDWARD N. TRIFONOV (UNIVERSITY OF HAIFA, ISRAEL), JAN HEJÁTKO (MASARYK UNIVERSITY, CZECH REPUBLIC)

@ SERGEDOBROWOLSKI@GMAIL.COM

Multistep phosphorelay (MSP) is a key signaling pathway mediating response to various stimuli in Prokaryota and Eukaryota. The histidine-containing phosphotransfer (HPt) proteins differentiate MSP from its ancestral version, the two-component signaling.

Here we use the recently introduced modular search in elucidating evolutionary origin of HPt proteins and thus MSP signaling in *Arabidopsis thaliana*. We identified two more HPt proteins in *Arabidopsis*, hereinafter designated AHP7 and AHP8, and more than 250 HPt proteins in various taxa. We defined modular

structure of HPt proteins reflecting their evolutionary complexity with the central module being completely conserved. Based on the detailed phylogenetic analysis we propose that HPt proteins appeared as a part of multi-domain proteins in Proteobacteria that spread via horizontal gene transfer into other taxa.

Surprisingly, our findings imply that *Arabidopsis* HPt proteins do not originate from Cyanobacteria, the endosymbionts establishing green eukaryotic lineage, but the green sulfur bacteria of genus *Chlorobi*.

## P2.3 THE EVOLUTION OF THE PERCEPTION OF THE PHYTOHORMONE CYTOKININ

📅 MONDAY 4 JULY, 2016 ⌚ 11:55

👤 ALEXANDER HEYL (ADELPHI UNIVERSITY, UNITED STATES)

@ AHEYL@ADELPHI.EDU

Cytokinins are adenine derivatives and as such they are found in every organism. However, only plants use them as signaling molecules. Plants transduce the cytokinin signal via a variant of the two-component signaling system. Although this signaling system is very common in bacteria, it is unique to plants among higher eukaryotes. The ubiquitous distribution of the signaling molecule and the wide spread use of the type of signaling system, combined with the fact that only plants use cytokinin and the two-component system as a signaling circuit, make the cytokinin signal transduction system an ideal model for the analysis of the evolution of signaling systems in general. Cytokinin is detected by a hybrid-histidine kinase receptor, and the signal is transduced by a multi-step phospho-relay system of histidine phospho-transfer proteins and different classes of response regulators. To shed light on the origin and evolution of the members of this signaling system, a comprehensive domain-based phylogenetic study across the relevant kingdoms was conducted. Surprisingly, we identified and subsequently experimentally characterized a novel subfamily of cytokinin receptors. These and further results will be discussed in the context of evolution of signal perception.

## P2.4 EVIDENCE FOR NOVEL CO-RECEPTOR FUNCTIONS IN AUXIN PERCEPTION

MONDAY 4 JULY, 2016

12:25

SIGURD RAMANS HARBOROUGH (UNIVERSITY OF LEEDS, UNITED KINGDOM), ARNOU KALVERDA (UNIVERSITY OF LEEDS, UNITED KINGDOM), GARY THOMPSON (UNIVERSITY OF LEEDS, UNITED KINGDOM), MARTIN KIEFFER (UNIVERSITY OF LEEDS, UNITED KINGDOM), MUSSA QUARESHY (UNIVERSITY OF WARWICK, UNITED KINGDOM), VESELINA UZUNOVA (UNIVERSITY OF WARWICK, UNITED KINGDOM), NATHAN KIDLEY (SYNGENTA, UNITED KINGDOM), JOHN PAUL EVANS (SYNGENTA, UNITED KINGDOM), TIM HAWKES (SYNGENTA, UNITED KINGDOM), IAIN MANFIELD (UNIVERSITY OF LEEDS, UNITED KINGDOM), RICHARD NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM), STEFAN KEPINSKI (UNIVERSITY OF LEEDS, UNITED KINGDOM)

@ BSSRH@LEEDS.AC.UK

Auxin signalling involves the ubiquitin-mediated degradation of Aux/IAA transcriptional repressor proteins. This is dependent on the interaction between a TIR1/AFB auxin co-receptor protein, an auxin molecule, and the Aux/IAA protein. Here we characterise the structure of an Aux/IAA protein AXR3 domains I and II, focussing in particular on the conformations adopted within the region of the Aux/IAA protein known as the degron before and during its interaction with the auxin molecule and TIR1. We use a range of biophysical techniques including Nuclear Magnetic Resonance (NMR) Spectroscopy, Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR). Our work provides the first evidence that a large component of an Aux/IAA's structure is intrinsically disordered with tendencies to form secondary structure around the degron. We discuss the possible regulatory significance of apparent conformation shifts within the degron core acts as a molecular switch to control the Aux/IAA's intrinsic disorder and how this disorder may relate to its hypothesized function as a co-receptor for auxin.

## P2.6 IMAGING PHYTOHORMONES DURING DEVELOPMENT AND ENVIRONMENTAL RESPONSES USING FRET BIOSENSORS

MONDAY 4 JULY, 2016

13:55

ALEXANDER M JONES (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), WOLF FROMMER (CARNEGIE PLANT BIOLOGY, UNITED STATES), ANNALISA RIZZA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), VIVIANE LANQUAR (CARNEGIE PLANT BIOLOGY, UNITED STATES)

@ ALEXANDER.JONES@SLCU.CAM.AC.UK

Plants use phytohormones – a suite of mobile small molecules – as potent regulators that coordinate and adjust development to match their environmental conditions. For example, accumulation of gibberellins (GA) is integral to numerous plant growth processes such as germination, tissue enlargement, and fruit set as well as key developmental transitions such as photomorphogenesis and flowering. It is no surprise, then, that accumulation of GA is tightly regulated in a cell-type and temporally specific

manner by a series of biosynthetic, modification, catabolic and transport proteins. However, we currently lack methods for high spatiotemporal resolution measurement of GA in living tissues, and this limitation hampers analysis of cell-specific GA accumulations. Using an accelerated biosensor engineering platform, a genetically-encoded, ratiometric fluorescent biosensors for the high-resolution measurement of GA in living tissues has been developed. Fluorescence imaging of the Gibberellin Perception Sensor (GPS) in the nuclei of Arabidopsis hypocotyls undergoing photomorphogenesis allows comparison of the timing of GA accumulation vs cell expansion. In addition to tracking endogenous GA accumulations, treatment of GPS plants with exogenous GA reveals that GA accumulation is patterned across tissues and varies with the type of GA applied. Potential mechanisms governing these cellular GA patterns and dynamics will be discussed. In the future, GPS can be used to address fundamental questions regarding how multiple signals integrate to control the hormone patterns and dynamics that, in turn, influence plant developmental and environmental responses.

## P2.7 UNDERSTANDING AUXIN PERCEPTION AND SELECTIVITY

MONDAY 4 JULY, 2016

14:25

MUSSA QUARESHY (UNIVERSITY OF WARWICK, UNITED KINGDOM), RICHARD NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM)

@ MUSSAQUARESHY@GMAIL.COM

Auxin (Indole-3-acetic acid) can be considered one of the most important hormones in plant development as it coordinates plant responses through transcriptional regulation. Auxin binds Transport Inhibitor Response 1 (TIR1) of which there are 5 other homologues; Auxin Signalling F-Box (AFB1-5). TIR1 and AFB5 are the most distantly related in terms of sequence homology and are studied in this work.

Currently 25 molecules are marketed as synthetic auxins and there is still a drive to discover new auxin-like molecules, in particular from an agrochemical perspective to overcome weed-based resistance and reduce field dose. Interestingly, there is as yet no definitive descriptor that defines an auxin chemically and until recently there has been no compound that has complete selectivity for one of the individual receptor proteins.

We will present our work using purified TIR1 and AFB5 and compound screening by SPR, in-silico docking, kinetic parameterization and 3D QSAR modelling to identify and synthesize potential novel auxins or anti-auxins. This has yielded a novel class of auxin molecule, which has also shown selectivity between some of the auxin receptors. It is a potential scaffold for receptor-specific auxins and thus a new generation of herbicides. We also present our work on auxin-molecular field descriptors as a tool in the search for rationally designed novel auxins.



## P2.8 MULTISTEP PHOSPHORELAY – SIGNAL INTEGRATION, SPECIFICITY AND ACTIVATION

📅 MONDAY 4 JULY, 2016 ⌚ 14:50

👤 JAN HEJÁTKO (CEITEC-CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY MASARYK UNIVERSITY, CZECH REPUBLIC)

@ JAN.HEJATKO@CEITEC.MUNI.CZ

In our recent work, we demonstrate that the multistep phosphorelay (MSP) pathway in *Arabidopsis* comprises light, ethylene and cytokinin signaling. However, the mechanisms allowing integration of multiple signals, while maintaining the specificity of individual inputs in the MSP signaling remained elusive. Similarly, the mechanisms underlying activation of the phosphorelay-initiating sensor histidine kinases are unknown.

We determined the structure of AHP2 acting downstream of sensor kinase CKI1. Using molecular dynamics simulations complemented by NMR measurements we show that the relative orientation of the interaction partners, controlled by a small number of AHP residues, underlies molecular recognition in CKI1 signaling, providing evidence for a unique mechanism determining MSP specificity in Eukaryotes.

We studied three forms of the receiver domain of CKI1 (CKI1RD): free protein, magnesium-bound form, and a stable analog of the active, phosphorylated receiver domain. While crystal structures of all three CKI1RD forms are identical, NMR data revealed dramatic alteration of molecular dynamics in a loop close to the CKI1RD phosphorylation site. It documents that dynamics of the receiver domain is a key factor in the activation of CKI1-mediated signaling. We propose a model in which the magnesium binding and phosphorylation activates CKI1RD via shift in the pre-existing equilibrium of conformations in favor of the active state.

Supported by P305-11-0756, 13-25280S and LQ1601.

## P2.11 PLANT MEMBRANE RECEPTOR ACTIVATION BY SHAPE-COMPLEMENTARY CO-RECEPTOR KINASES

📅 MONDAY 4 JULY, 2016 ⌚ 16:10

👤 MICHAEL HOTHORN (UNIVERSITY OF GENEVA, SWITZERLAND)

@ MICHAEL.HOTHORN@UNIGE.CH

Plants have evolved unique membrane receptor kinases which control plant growth, development and interactions with other organisms. These receptors harbor leucine-rich repeat (LRR) ectodomains, which can sense rather different small molecule, peptide and protein ligands. I will compare the LRR receptor kinases BRI1 (which senses a growth-promoting steroid hormone) and HAESA (which senses an abscission-controlling peptide hormone) in mechanistic detail. I will present structural, biochemical and genetic evidence that the co-receptor kinase SERK1 contributes to specific ligand recognition and to receptor activation in both BRI1 and HAESA. Finally, I will discuss how formation of a different receptor-co-receptor signaling complexes at the plasma membrane can trigger specific signaling outputs in the cytoplasm.

## P2.12 INVESTIGATION OF CYTOKININ RECEPTOR AHK4 SPECIFICITY USING HIGH-THROUGHPUT SCREENING TECHNOLOGY

📅 TUESDAY 5 JULY, 2016 POSTER SESSION

👤 PAVEL MAZURA (MENDEL UNIVERSITY IN BRNO, CZECH REPUBLIC), PAVEL KLIME (MENDEL UNIVERSITY IN BRNO, CZECH REPUBLIC), DUŠAN TUREK (MENDEL UNIVERSITY IN BRNO, CZECH REPUBLIC), LUKÁ SPÍCHAL (PALACKÝ UNIVERSITY INSTITUTE OF EXPERIMENTAL BOTANY ASCR CENTRE OF THE REGION HANÁ CZ, CZECH REPUBLIC), BRETISLAV BRZOBHAT (MENDEL UNIVERSITY IN BRNO, CZECH REPUBLIC)

@ MAZURA@SCI.MUNI.CZ

Histidine kinase AHK4 is one of the plant cytokinin receptors with an important role in hormone signal transduction. The cytokinin receptor AHK4, originally from *Arabidopsis thaliana*, is expressed in *E. coli* heterologous expression system to study an interaction between the receptor and a library of natural and synthetic ligands. The signal generated by ligand interacting with the receptor is transduced in *E. coli* and a reporter enzyme  $\beta$ -galactosidase is expressed. To monitor  $\beta$ -galactosidase expression levels the substrate 4-methylumbelliferyl  $\beta$ -D-galactopyranoside is used for fluorescence intensity measurements. The automation and miniaturization of this method enables interaction studies of AHK4 receptor with potential ligands. Structural diversity of ligands tested in the study reveals interesting insights into molecular basis of interaction between receptor AHK4 and its ligands.

## P2.13 UNCOVERING THE ROLE OF DISORDERED PROTEINS IN PLANT HORMONE SIGNALLING

📅 MONDAY 4 JULY, 2016 ⌚ 16:55

👤 ERIK H. A. RIKKERINK (PLANT AND FOOD RESEARCH, NEW ZEALAND), BIN XUE (DEPARTMENT OF CELL BIOLOGY COLLEGE OF FINE ARTS AND SCIENCES UNIVERSITY OF SOUTH FLORIDA TAMPA F, UNITED STATES), A. KEITH DUNKER (CENTER FOR COMPUTATIONAL BIOLOGY AND BIOINFORMATICS INDIANA UNIVERSITY SCHOOL OF MEDICINE, UNITED STATES), VLADIMIR N. UVERSKY (DEPARTMENT OF MOLECULAR MEDICINE MORSANI COLLEGE OF MEDICINE UNIVERSITY OF SOUTH FLORIDA, UNITED STATES), XIAOLIN SUN (PLANT AND FOOD RESEARCH, NEW ZEALAND)

@ ERIK.RIKKERINK@PLANTANDFOOD.CO.NZ

The unusual flexibility and versatility of disordered protein domains has led to proteins containing these domains occupying central controlling positions in many biological processes. Originally uncovered in mammalian systems, the role of disorder in plant signalling has become a subject of more recent interest. We have discovered that DELLA proteins contain disordered domains coupled with a conserved (and ordered) GRAS domain and suggest a coordinated approach by both of these domains is required for DELLA proteins to play their key roles in Gibberellin signaling processes. Our interest in this coordinated approach has led to a more general interest in the role of disorder in plant hormone signalling pathways.

In this presentation we briefly introduce the topic of protein disorder, describe our DELLA/GRAS protein research and the specific role that disorder has played in understanding the possible mode of action of GRAS proteins. We then describe our more recent analysis of disorder in members of the Ethylene Responsive Factor (ERF) family and another example in the literature of a disordered protein playing a key role in hormone signalling. We end by highlighting the peculiar properties of disordered protein regions that make them such an excellent platform for integrating diverse cues into a coherent signalling response. These properties fulfill the requirements that flow from the complex intersecting network of hormone signals that sessile plants deploy to coordinate their response to a constantly changing environment of biotic and abiotic challenges.

## P2.14 STRIGOLACTONE PERCEPTION BY DAD2 AND THE ENVIRONMENTAL CONTROL OF BRANCHING

TUESDAY 5 JULY, 2016 10:30

KIM SNOWDEN (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND), REVEL DRUMMOND (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND), CYRIL HAMIAUX (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND), ZHIWEI LUO (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND), HUI WEN LEE (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND), BART JANSSEN (THE NEW ZEALAND INSTITUTE FOR PLANT FOOD RESEARCH, NEW ZEALAND)

KIMBERLEY.SNOWDEN@PLANTANDFOOD.CO.NZ

The strigolactone (SL) hormone signalling system of plants controls the number of branches produced and responds to nutrient status and light. We have identified a key regulator in the SL pathway, the DAD2 gene from petunia. The crystal structure of DAD2 reveals an  $\alpha/\beta$  hydrolase fold containing a canonical catalytic triad with a large internal cavity capable of accommodating strigolactones. DAD2 can interact with the F-box protein PhMAX2A in a strigolactone dependent manner at nanomolar concentrations. DAD2 is destabilised in the presence of strigolactone, potentially indicating conformational change in the presence of the hormone. These observations suggest that DAD2 acts to bind the mobile strigolactone signal and then interacts with PhMAX2A to initiate an SCF-mediated signal transduction pathway. DAD2 also has slow enzymatic activity, hydrolysing its ligand. Mutation of the catalytic triad abolishes catalytic activity and the ability of DAD2 to interact with PhMAX2A. The hydrolysis products of DAD2 can neither stimulate the protein-protein interaction nor modulate branching, suggesting that DAD2 is the strigolactone receptor. DAD2 is postulated to act at a position in the network controlling axillary bud outgrowth that integrates nutrient availability and light quality. We investigated the relative importance of these two factors by simultaneously altering both light and nutrient conditions (red:far-red ratio and phosphate availability). An analysis of gene expression of SL pathway genes showed a co-ordinated response to phosphate and light and that the regulation of the SL receptor plays an important role in the response of plants to the environment.

## P2.15 TOMOGRAPHIC DOCKING SUGGESTS THE MECHANISM OF AUXIN RECEPTOR TIR1 SELECTIVITY

TUESDAY 5 JULY, 2016 11:10

VESELINA V UZUNOVA (UNIVERSITY OF WARWICK, UNITED KINGDOM), MUSSA QUARESHY (UNIVERSITY OF WARWICK, UNITED KINGDOM), CHARO I DEL GENIO (UNIVERSITY OF WARWICK, UNITED KINGDOM), RICHARD M NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM)

V.UZUNOVA@WARWICK.AC.UK

We study the binding of plant hormone IAA on its receptor TIR1 using a tomographic approach to docking. Our results suggest that the selectivity of the mechanism is related to a series of constraints that potential ligands encounter on their way from the surface of the protein to their final position at the bottom of a deep binding pocket. The tomographic docking results help develop specific hypotheses about ligand binding selectivity, distinguishing binders from non-binders, and suggest that binding is a two-step mechanism, consisting of engagement with a niche in the back wall of the pocket and interaction with a molecular filter which allows or precludes passage to the docking platform. Analyzing the interactions within the binding pocket at different depths, our new method helps in the identification of critical residues that constitute preferred future study targets and in the quest for safe and effective herbicides. Also, it has the potential to extend the utility of docking from ligand searches to the study of selectivity processes.

## P2.16 CHEMICAL BIOLOGY OF STRIGOLACTONE PERCEPTION IN THE PARASITIC PLANT STRIGA HERMONTICA

TUESDAY 5 JULY, 2016 11:25

PETER MCCOURT (UNIVERSITY OF TORONTO, CANADA)

PETER.MCCOURT@UTORONTO.CA

*Striga* spp. (Witchweed) is an obligate parasitic plant that attaches to host roots to deplete them of nutrients. In Sub-Saharan Africa, the most destructive *Striga* species, *S. hermonthica*, parasitizes major food crops affecting two-thirds of the arable land and over 100 million people. One potential weakness in the *Striga* infection process is the way it senses the presence of a host crop. *Striga* only germinates in the presence of the plant hormone, strigolactone, which exudes from a host root. Hence small molecules that perturb strigolactone signalling may be useful tools to disrupt the *Striga* lifecycle.

Fortunately, Strigolactones also have roles in model plant genetic systems and this has allowed researchers to frame a mechanistic understanding of how these small molecules function in higher plants. In this presentation I will talk about our present understanding of the molecular nature of strigolactone perception and how using *Arabidopsis* has moved forward our understanding of the roles of strigolactones in *S. hermonthica* germination. I will also focus on chemical biology approaches and the development of chemical probes to identify small molecules that may perturb *Striga* germination.

## P2.17 EVALUATING THE TARGET SELECTIVITY OF TWO NEW AUXIN HERBICIDES

TUESDAY 5 JULY, 2016 12:05

JUSTYNA PRUSINSKA (UNIVERSITY OF WARWICK, UNITED KINGDOM), MUSSA QUARESHY (UNIVERSITY OF WARWICK, UNITED KINGDOM), VESELINA UZUNOVA (UNIVERSITY OF WARWICK, UNITED KINGDOM), JARED BELL (DOW AGROSCIENCES LLC, UNITES STATES), PAUL SCHMITZER (DOW AGROSCIENCES LLC, UNITES STATES), RICHARD NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM)

@ J.M.PRUSINSKA@WARWICK.AC.UK

Auxins are the basis of one of the most successful classes of herbicide with a commercial history dating back 50 years. The family of auxinic herbicides is broadly-based, is variously selective, has a number of chemical scaffolds and incidences of tolerance have not been widespread or pervasive. Hence there is an interest in novel, commercially relevant actives. The target site for auxins, the auxin receptor known as TIR1, was identified in 2005 and its crystal structure was solved in 2007. Following these discoveries, we are expressing and purifying AtTIR1 and other members of the TIR1 family as the basis of compound activity screens, especially AtAFB5. We are also using structure-led strategies to describe in detail the binding sites and the mechanism of selectivity for auxins. Details of our assays will be presented along with a mode-of-action analysis of new arylpicolinates from Dow AgroSciences. The results will demonstrate our progress towards novel chemistries and the structural basis of selectivity.

## P2.18 A STRUCTURE-ACTIVITY PROFILE FOR THE AUXIN UPTAKE CARRIER AUX1

TUESDAY 5 JULY, 2016 12:20

RICHARD NAPIER (UNIVERSITY OF WARWICK, UNITED KINGDOM), PETR HOSEK (INSTITUTE FOR EXPERIMENTAL BOTANY, CZECH REPUBLIC), MARTIN KUBES (INSTITUTE FOR EXPERIMENTAL BOTANY, CZECH REPUBLIC), ASHUTOSH TRIPATHI (INSTITUTE OF BIOTECHNOLOGY ALAHABAD, INDIA), KLARA HOYEROVA (INSTITUTE FOR EXPERIMENTAL BOTANY PRAGUE, CZECH REPUBLIC)

@ RICHARD.NAPIER@WARWICK.AC.UK

Auxins are the basis of one of the most successful classes of herbicide with a commercial history dating back 50 years. The family of auxinic herbicides is broadly-based, is variously selective, has a number of chemical scaffolds and incidences of tolerance have not been widespread or pervasive. Hence there is an interest in what contributes to the modes of action and elements of selectivity of different actives. The principal route for cellular auxin uptake is by the activity of the proton-coupled auxin uptake carrier AUX1. Models have shown that a factor referred to as diffusion is also important, but selectivity in uptake is conferred by the AUX1 protein. Using both root growth bioassays with aux1 mutants and accumulation assays using tobacco BY-2 cell cultures we have mapped the structure-activity relationship of AUX1. We have

developed mathematical models for accumulation of 2,4-D and competitive inhibition of 2,4-D uptake, yielding both accurate IC50 values and insights into the relative importance of AUX1-mediated uptake vs diffusion. We have used a wide panel of auxins and analogues to give a new cheminformatic map of AUX1 substrate selectivity. Amongst the results we will show that major families of auxinic herbicides are not carried by AUX1 and reflect on how this affects our understanding of auxin selectivity as herbicides.

## P2.5 HORMONAL INTERACTIONS IN ROOT RESPONSES TO MECHANICAL IMPEDANCE

TUESDAY 5 JULY, 2016 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. A commonly encountered stress in soils is mechanical impedance, which becomes an increasing problem in drying soils as 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 mechanical impedance. The work described here aims to investigate the molecular mechanisms governing the relationship between polar auxin transport and ethylene signalling as well as other signalling pathways in response to mechanical impedance, and the link to root growth and development.



## P2.9 MECHANISMS OF PLANT GROWTH RESPONSES TO POTASSIUM

📅 TUESDAY 5 JULY, 2016 POSTER SESSION

👤 FLORA M HETHERINGTON (DURHAM UNIVERSITY, UNITED KINGDOM), JEN F TOPPING (DURHAM UNIVERISTY, UNITED KINGDOM), KEITH LINDSEY (DURHAM UNIVERISTY, UNITED KINGDOM)

@ F.M.HETHERINGTON@DURHAM.AC.UK

The potassium ion ( $K^+$ ) is essential for plant growth and development. It is essential for enzyme activity, stomatal movement, photosynthesis, protein synthesis and transport of sugars, water and nutrients.  $K^+$  deficient plants exhibit symptoms typified by chlorosis and necrosis of leaves, as well as an overall reduction in the growth of the above-ground parts of the plant, and these translate to greatly reduced yields. Given the importance of  $K^+$  deficiency in agriculture, it is important to understand the mechanisms by which  $K^+$  is taken into the plant and how the architecture of the roots affects the ability of the crop to forage for  $K^+$  in the soil. Changes in root architecture in response to soil  $K^+$  starvation are not well characterized but are believed to be controlled, in part, by combinations of hormones. Synergistic or antagonistic interactions between different combinations of hormones such as auxin and ethylene are key in coordinating processes such as lateral root formation, main root growth, and root hair development, and therefore are key in defining the root system architecture. The work described here aims to better understand the relationship between  $K^+$  availability, hormone signalling pathways and root architecture.

## P2.10 THE DYNAMICS OF AUXIN RESPONSE FACTORS (ARFS) DURING GRAIN DEVELOPMENT IN *BRACHYPODIUM*

📅 TUESDAY 5 JULY, 2016 POSTER SESSION

👤 SOFIA KOURMPETLI (CRANFIELD UNIVERSITY, UNITED KINGDOM), SYABIRA YUSOFF (UNIVERSITY OF LEICESTER, UNITED KINGDOM), SINEAD DREA (UNIVERSITY OF LEICESTER, UNITED KINGDOM)

@ S.KOURMPETLI@CRANFIELD.AC.UK

Auxin plays a key role in many aspects of plant growth and development, from cell differentiation and organ patterning to cell elongation and responses to light and other environmental stimuli. Even though it is found in every part of the plant, its effect on different organs is not yet clear.

Transcriptional changes among families of auxin-responsive genes have been profiled during different stages of grain development in rice, highlighting the distinct patterns of ARF, GH3, Aux/IAA and SAUR family genes and indicating their role in different aspects of grain development, maturation and germination (Jain *et al.*, 2006).

We are undertaking a similar approach to shed light on the role of auxin response factors during grain development in *Brachypodium distachyon*, as a sister to the core pooids that include wheat, barley and rye. We have identified the ARF orthologues of *Brachypodium* and using RNAseq, combined with RT-PCR/qPCR and mRNA *in situ* hybridisation analyses, we are evaluating their temporal and spatial expression levels across grain development, from pre-anthesis ovaries to mature grain and germination stages. We hope that our results will provide a detailed expression map of auxin responsive factor genes during *Brachypodium* grain development.

# P3 SEED BIOLOGY: FROM LABORATORY TO FIELD

**ORGANISED BY:** DR GEORGE BASSEL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM) & DR STEVEN PENFIELD (JOHN INNES CENTRE, UNITED KINGDOM)

## P3.1 AUXIN-MEDIATED CONTROL OF EARLY SEED DEVELOPMENT

📅 WEDNESDAY 6 JULY, 2016 ⌚ 09:00

👤 CLAUDIA KÖHLER (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), DUARTE FIGUEIREDO (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), RITA BATISTA (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN), PAWEŁ ROSZAK (SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, SWEDEN)

@ CLAUDIA.KOHLER@SLU.SE

Seed development in flowering plants is initiated by double fertilization, whereby one of the paternal sperm cells fertilizes the female egg cell, giving rise to the embryo, and a second sperm cell fertilizes the central cell, which originates the endosperm. The endosperm is a triploid nutritive tissue supporting embryo development. Surrounding the two fertilization products is the seed coat, which derives from the ovule integuments and is of purely maternal origin. The successful establishment of a seed requires the developmental coordination of these three genetically-distinct structures. Seed development in most plant species is tightly coupled to fertilization, but this requirement can be bypassed in mutants for components of the Fertilization Independent Seed Polycomb Repressive Complex2 (FIS-PRC2), which maintains gene repression by modifying chromatin structure. Lack of FIS-PRC2 function results in the formation of autonomous seeds that develop in the absence of fertilization. I will discuss data showing that the FIS-PRC2 represses the expression of auxin biosynthesis genes in the female gametophyte and that the paternal-specific expression of these genes drives endosperm development after fertilization. Auxin production is both necessary and sufficient to initiate endosperm development in *Arabidopsis* and also a trigger for seed coat initiation.

## P3.2 REACTIVATION OF MITOCHONDRIAL BIOENERGETICS AND DYNAMICS DURING GERMINATION OF *ARABIDOPSIS THALIANA*

📅 WEDNESDAY 6 JULY, 2016 ⌚ 09:40

👤 DAVID C LOGAN (IRHS AGROCAMPUS-OUEST INRA UNIVERSITÉ D'ANGERS SFR 4207 QUASAV., FRANCE), GAËL PASZKIEWICZ (IRHS AGROCAMPUS-OUEST INRA UNIVERSITÉ D'ANGERS SFR 4207 QUASAV., FRANCE), JOSÉ M GUALBERTO (IBMP CNRS UNITÉ PROPRE DE RECHERCHE 2357 UNIVERSITÉ DE STRASBOURG, FRANCE), ABDEL BENAMAR (AGROCAMPUS-OUEST INRA UNIVERSITÉ D'ANGERS SFR 4207 QUASAV, FRANCE), DAVID MACHEREL (IRHS AGROCAMPUS-OUEST INRA UNIVERSITÉ D'ANGERS SFR 4207 QUASAV., FRANCE)

@ DAVID.LOGAN@UNIV-ANGERS.FR

Mitochondrial dynamics underpins their function and maintenance in plants. For example, due to the uneven distribution of mtDNA within physically discrete mitochondria, mitochondrial fusion and fission are required to ensure mixing of the mtDNA pool and to likely facilitate mutation repair and the identification of dysfunctional mitochondria. Mitochondria are inactive in the dry seed but must re-activate rapidly upon imbibition in order to provide ATP for germination. This reactivation requires not only a reactivation of mitochondrial metabolism, but also of mitochondrial dynamics. Using a bioimaging approach, we investigated reactivation of mitochondrial bioenergetics and dynamics using *Arabidopsis thaliana* as a model. Bioenergetic reactivation, visualised by the presence of a membrane potential, is almost immediate upon re-hydration. However, reactivation of mitochondrial dynamics only occurs after transfer of seed to optimal conditions. The re-activation of mitochondrial bioenergetics and dynamics is followed by a dramatic re-organisation of the chondriome involving transient massive fusion to form a perinuclear cage followed by division. These data will be presented alongside results testing our hypothesis that the change in mitochondrial dynamics is associated with the activation of mitochondrial quality control mechanisms to repair mtDNA damage incurred during maturation drying and imbibition.

### P3.12 THE CLONING OF DELAY OF GERMINATION 6, A PROMOTER OF SEED GERMINATION

WEDNESDAY 6 JULY, 2016 11:00

LEONIE BENTSINK (WAGENINGEN UNIVERSITY, NETHERLANDS), HANZI HE (WAGENINGEN UNIVERSITY, NETHERLANDS), KERSTIN GÜHL (WAGENINGEN UNIVERSITY, NETHERLANDS), MARIEKE VAN BOLDEREN (WAGENINGEN UNIVERSITY, NETHERLANDS), SJORS VAN DER HORST (WAGENINGEN UNIVERSITY, NETHERLANDS), BAS DEKKERS (WAGENINGEN UNIVERSITY, NETHERLANDS)

@ LEONIE.BENTSINK@WUR.NL

Quantitative trait loci (QTL) mapping for seed dormancy in *Arabidopsis thaliana* has revealed eleven *DELAY OF GERMINATION (DOG)* loci. The cloning of strongest of these QTL, *DOG1*, has shown us how important the identification of natural variation can be. *DOG1* is a key regulator of seed dormancy and has shown to be conserved in other species as well. Here we present the cloning of another dormancy QTL that confers a high level of dormancy, *DOG6*. Near isogenic lines containing an introgression fragment of the Cape Verde Island, Shakdara or Kashmir accession at the position of the *DOG6* locus in a Landsberg *erecta (Ler)* genetic background all are more dormant than *Ler* itself. Fine-mapping, complementation cloning and mutant analyses revealed that in contrast to *DOG1*, *DOG6* is a promoter of seed germination. *DOG6* knock-out mutant seeds are more dormant than wild type seeds. This finding illustrates the power of analysing natural genetic variation for the identification of important life history traits. The full characterisation of the *DOG6* gene, including its localization and the proposed mechanism of how the *DOG6* protein promotes germination will be presented.

### P3.13 CIS-12-OXO-PHYTODIENOIC ACID: A NOVEL LIGHT-DEPENDENT REGULATOR OF SEED GERMINATION IN *ARABIDOPSIS THALIANA*

WEDNESDAY 6 JULY, 2016 11:30

THIAGO BARROS-GALVÃO (UNIVERSITY OF YORK, UNITED KINGDOM), FABIAN E VAISTIJ (UNIVERSITY OF YORK, UNITED KINGDOM), IAN A GRAHAM (UNIVERSITY OF YORK, UNITED KINGDOM)

@ TBG501@YORK.AC.UK

Light-dependent seed germination is induced by gibberellic acid (GA) and inhibited by abscisic acid (ABA). This widely accepted view of GA/ABA control of germination does not however explain the fact that seeds deficient in ABA biosynthesis still germinate poorly under far-red (FR) light conditions. We have discovered that this text-book understanding of germination control is missing a key player: cis-12-oxo-phytodienoic acid (OPDA). We found that OPDA, a precursor of the wound related phytohormone jasmonic acid, accumulates in imbibed seeds under FR conditions and represses germination in an ABA-independent manner, and it does so through the same phytochrome signal transduction pathway that is involved in modulating GA and ABA levels. Our findings therefore add a new player to the repertoire of phytohormones involved in controlling light-dependent germination.

### P3.14 PARENTAL CONTROL OF SEED DORMANCY IN *ARABIDOPSIS THALIANA*

WEDNESDAY 6 JULY, 2016 13:50

LUIS LOPEZ-MOLINA (UNIVERSITY OF GENEVA, SWITZERLAND), URSZULA PISKUREWICZ (UNIVERSITY OF GENEVA, SWITZERLAND), MAYUMI IWASAKI (UNIVERSITY OF GENEVA, SWITZERLAND)

@ LUIS.LOPEZMOLINA@UNIGE.CH

Mature seeds maintain the plant embryo in a metabolically inert and highly resistant state. When newly produced, seeds are dormant, i.e. they will not germinate when exposed to otherwise favorable germination conditions. Dormancy allows the plant to maintain its embryonic and highly protected state while preventing germination out of season. Dry seeds eventually lose dormancy, a process referred as dry after-ripening, i.e. they acquire the capacity to germinate when exposed to favorable germination conditions. Dormancy levels stored in a seed can be defined as the amount of dry after-ripening time required by the seed to lose its dormancy. Dormancy levels are strongly influenced by the climatic conditions experienced by the mother plant. In particular, *Arabidopsis* plants exposed to cold temperatures produce markedly more dormant seeds. Interestingly, this response is maternally controlled. However, the general nature and extent of maternal control of seed dormancy, beyond cold-imposed dormancy, remains poorly characterized. In *Arabidopsis thaliana*, the endosperm is essential to repress dormant seed germination. Indeed, upon dormant seed imbibition the endosperm continuously synthesizes and releases the growth repressive hormone abscisic acid (ABA) towards the embryo, thus blocking its growth over time. In fully after-ripened seeds, the endosperm ceases to release sufficient ABA upon imbibition, thus allowing undelayed germination to take place. We will describe our current work aimed at understanding the nature of the mechanisms operating in the *Arabidopsis* endosperm that could underlie maternal inheritance of seed dormancy levels. In particular, recent findings on epigenetic and genomic imprinting mechanisms will be presented.

### P3.15 LAB TESTING & MODELLING TO UNDERSTAND GERMINATION AND FIELD ESTABLISHMENT OF BIOENERGY CROP *MISCANTHUS*

WEDNESDAY 6 JULY, 2016 14:30

DANNY AWTY-CARROLL (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), CHRIS ASHMAN (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), MICHAL MOS (TERRAVESTA LTD, UNITED KINGDOM), JOHN CLIFTON-BROWN (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM), PAUL ROBSON (ABERYSTWYTH UNIVERSITY, UNITED KINGDOM)

@ DGC1@ABER.AC.UK

*Miscanthus* is a perennial C4 biomass crop that is mostly rhizome propagated. This propagation method is costly and difficult to upscale. To improve the uptake of *Miscanthus* and the speed to upscale alternative propagation methods are required. Direct field sowing is one possible method; however, existing *Miscanthus sinensis* seed has been previously demonstrated not to germinate with high frequency in the UK climate (Clifton-Brown et al., 2011).



This study aims to model *Miscanthus* germination and early survival in order to predict the best inputs and protocol for UK direct sowing. Modelling utilised a version of SIMPLE (SIMulation of PLant Emergence) (Dürr et al., 2001), this prediction can be applied to existing *Miscanthus* data and compared to field trials. In order to parameterise the model, the germination rates of a range of new hybrids were tested at a variety of field temperatures. Seed germination in dark and light treatments and hypocotyl elongation were tested in the lab to understand the effect of sowing depth. Biodegradable films were tested for improvements in field germination conditions. Field data on a range of new genotypes direct sown with and without 'mulch film' showed an increase in temperature under film and the resulting 'cold' germination was used to produce a new *Miscanthus* sowing range. The combination of field data and model predictions from lab parametrization increased the accuracy of the model. Novel seed treatment effects will be modelled based on lab testing of seed germination, allowing improved prediction of *Miscanthus* propagation success by direct sowing.

### P3.16 A CELLULAR BASIS FOR DECISION-MAKING IN ARABIDOPSIS SEEDS

WEDNESDAY 6 JULY, 2016 14:45

GEORGE W BASSEL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), ALEC T TOPHAM (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), IAIN JOHNSTON (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

@ GBASSEL@GMAIL.COM

The decision to terminate dormancy in *Arabidopsis* is mediated through the antagonistic interactions between the hormones gibberellic acid (GA) and abscisic acid (ABA). The cellular sites where these hormones act within dormant seeds remains unknown. Using 3D digital single cell analysis, we have localized GA and ABA synthesis, signaling and response reporters within individual cells of the dormant embryo. Here, we found the cellular response sites of these hormones to be spatially distinct, while the cellular sites of synthesis for these are overlapping. This indicates that the GA/ABA antagonism in seeds acts non-cell autonomously through the intracellular movement of hormones across the cellular network. To better understand the role of this spatial separation of hormone response and synthesis, we developed a mathematical model capturing hormone metabolic interactions across different cell types, and how these constitute a developmental fateswitch. Results of this model indicate an advantage to decision-making is conferred through the spatial separation of signaling centres.

### P3.17 PUSHING NATURE IN THE RIGHT DIRECTION: SEED PHYSIOLOGICAL RESEARCH IN A SEED COMPANY

WEDNESDAY 6 JULY, 2016 15:45

FRANK LANFERMEIJER (SYNGENTA SEEDS BV, NETHERLANDS), RENÉ BENJAMINS (SYNGENTA SEEDS BV, NETHERLANDS), MARTIN BUIS (SYNGENTA SEEDS BV, NETHERLANDS), BARBARA WESTLAND (SYNGENTA SEEDS BV, NETHERLANDS), TONKO BRUGGINK (SYNGENTA SEEDS BV, NETHERLANDS)

@ FRANK.LANFERMEIJER@SYNGENTA.COM

Seeds contain the promises of the seed companies. They need to guarantee the seed companies that their customers harvest the high quality crop they have set their sights on and obtain high yields. Hence, seed products need to be of high quality. However, the requirements a seed company demands from seeds are in conflict with the natural requirements (nature versus agriculture). Consequently, challenges arise to adjust natural seed and seed biology to meet the commercial requirements. Moreover, crop species are not wild plants and have gone through genetic bottlenecks (breeding). We discuss the requirements, which seed companies have for their seed products and relate those to seed biology. Next to this, the challenges seed companies face in order to produce large volumes of high quality seeds will be discussed. Finally, seed biological research in a seed company will be described.

### P3.18 MOLECULAR MECHANISMS OF COMBINED PRIMING AND AGING TREATMENTS IN SUGAR BEET SEEDS

WEDNESDAY 6 JULY, 2016 16:15

MICHAEL M IGNATZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), JULIANE MEINHARD (KWS SAAT AG, GERMANY), ANNETTE BÜTTNER-MAINIK (AGROSCOPE, SWITZERLAND), FRIDTJOF WELTMEIER (KWS SAAT AG, GERMANY), ILSE KRANNER (UNIVERSITY OF INNSBRUCK, AUSTRIA), LOUISE COLVILLE (KEW'S MILLENIUM SEED BANK, UNITED KINGDOM), VERONIKA TURECKOVA (PALACKÝ UNIVERSITY, CZECH REPUBLIC), MIROSLAV STRNAD (PALACKÝ UNIVERSITY, CZECH REPUBLIC), UWE FISCHER (KWS SAAT AG, GERMANY), GERHARD LEUBNER-METZGER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

@ MICHAEL.IGNATZ@RHUL.AC.UK

Priming is an important process to improve sugar beet seed quality. It leads to accelerated and uniform germination but also to enhanced aging during seed storage, especially in suboptimal storage conditions which cause a drastic reduction in longevity and germination. We designed differing aging models for the three factors temperature, relative air humidity and duration, which were used for comparative population-based modelling of seed aging of primed versus unprimed seeds delivering storage conditions with defined stress responses. A detailed analysis of the transcriptome, metabolites and important hormones was made to investigate the molecular mechanisms of sugar beet seed aging for those conditions. While ABA inhibits sugar beet germination, 1-aminocyclopropane-1-carboxylic acid (ACC), the biosynthetic precursor of ethylene, is known to promote it. Additionally to the measured hormone levels,

we analyzed the transcript levels of enzymes that are important in the hormonal pathways. Differentially regulated genes of the sugar beet seed transcriptome for redox systems were supported by biochemical quantification of the relevant reduced and oxidised metabolites. The redox couple glutathione/glutathione-disulfide (GSH/GSSG) is known to be the main antioxidative system of the dry seed and an aging marker. These and other metabolites as well as the transcripts of related enzymes were quantified and connected to the germination kinetics to show the role for the antioxidant system in alleviating seed damage in some combined priming and aging conditions. ([www.seedbiology.eu](http://www.seedbiology.eu))

### P3.19 TYPIFYING THE BIOMECHANICAL PROPERTIES OF WHEAT GRAINS RELEVANT TO THE MILLING PROCESS

WEDNESDAY 6 JULY, 2016 16:30

JAMES E HOURSTON (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MICHAEL M IGNATZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MARTIN REITH (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), GERHARD LEUBNER-METZGER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), TINA LH STEINBRECHER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

@ JAMES.HOURSTON@RHUL.AC.UK

The milling of plant starches and grains has been carried out since around 28,000 BC and from this time, ceaseless innovation has today brought us to a high level of technological sophistication. We have progressed to a point where, due to the scale of modern mills, fine tuning and context specific adjustments to milling machinery can result in significant gains in energy saving and flour quality. In this study we present novel techniques for measuring the biomechanical properties of wheat grains, with direct relevance to the milling and pearling processes. Using two cultivars; Runal and Cambrena, examples of 'hard' and 'soft' grain types, we can test the effectiveness of our techniques and explore their contrasting biomechanical properties. We present data on the shear forces required to shear starch from 'hard' and 'soft' wheat grains as well as the force required to shear individual bran layers. We also demonstrate a method for assessing the effects of hormone and enzyme treatments associated with both germination processes and the conditioning of grain prior to milling by testing layers of the wheat bran. Specifically, we focus on the living aleurone and dead, testa containing, bran layers, and following industry relevant pre-treatments, apply puncture force testing to these layers to assess changes in mechanical resistance. We believe that these new techniques can provide an industrially relevant tool which can aid in the optimisation of milling, with wider applications to the biomechanical characterisation of seeds from other plant species.

### P3.20 THE ROLE OF GIBBERELLIN IN WHEAT GRAIN DEVELOPMENT

WEDNESDAY 6 JULY, 2016 16:45

AAKRITI WANCHOO-KOHLI (ROTHAMSTED RESEARCH, UNITED KINGDOM), SIMON VAUGHAN (ROTHAMSTED RESEARCH, UNITED KINGDOM), MICHAEL HOLDSWORTH (THE UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), ANDY PHILLIPS (ROTHAMSTED RESEARCH, UNITED KINGDOM), PETER HEDDEN (ROTHAMSTED RESEARCH, UNITED KINGDOM)

@ AAKRITI.WANCHOO-KOHLI@ROTHAMSTED.AC.UK

The growth hormone gibberellin (GA) controls many aspects of plant development and in wheat is known to influence grain size and flour quality. Germinating wheat embryos produce GA inducing the production of  $\alpha$ -amylase by the aleurone causing hydrolysis of starch in the endosperm. Under certain environmental conditions premature induction of  $\alpha$ -amylase results in degraded starch and poor quality flour. However, while GA is proposed to have a negative effect on flour quality, it is required for early grain development. As these effects are separated temporally and spatially in the grain, it may be possible to improve grain yield and quality by manipulating GA levels in specific tissues at different developmental stages. We hypothesise that increasing GA content in early stages of development may promote grain size without negatively impacting flour quality, while reducing GA content late in development, or conferring insensitivity to GA in specific tissues may improve flour quality, without affecting grain size. In order to test this and obtain a better understanding of the role of GA in grain development, constructs were designed to alter GA metabolism or signalling in the seed-coat, endosperm, embryo or aleurone of developing wheat grains. The tissue and temporal specificity of each promoter was confirmed by co-transformation with GFP reporter constructs. Grain yield and quality in the transgenic lines were measured in terms of number, weight, size, shape,  $\alpha$ -amylase activity, protein content, grain hardness and moisture. Transcript levels of the transgenes were also measured using qRT-PCR to determine linkages between genotype and phenotype.

### P3.21 A DEFENCE-RELATED INTER-COMPARTMENTAL SIGNALLING PATHWAY REGULATES EMBRYONIC CUTICLE INTEGRITY IN ARABIDOPSIS

📅 THURSDAY 7 JULY, 2016 ⌚ 09:00

👤 GWYNETH INGRAM (ÉCOLE NORMALE SUPÉRIEURE DE LYON, FRANCE), LYSIANE BROCARD (CNRS UNIVERSITY OF BORDEAUX BORDEAUX IMAGING CENTER, FRANCE), VINCENT DUPLAN (CNRS INRA LABORATOIRE DES INTERACTIONS PLANTES-MICROORGANISMES, FRANCE), JÉRÔME JOUBÉS (UNIVERSITÉ DE BORDEAUX CNRS LABORATOIRE DE BIOGÈNE MEMBRANAIRE, FRANCE), LUDIVINE TACONNAT (INSTITUT OF PLANT SCIENCES PARIS-SACLAY (IPS2), FRANCE), STEPHANIE PASCAL (UNIVERSITÉ DE BORDEAUX CNRS LABORATOIRE DE BIOGÈNE MEMBRANAIRE, FRANCE), ROBERTA GALLETI (UNIVERSITÉ LYON 1CNRS INRA ÉCOLE NORMALE SUPÉRIEURE DE LYON, FRANCE), STEVEN MOUSSU (UNIVERSITÉ LYON 1CNRS INRA ÉCOLE NORMALE SUPÉRIEURE DE LYON, FRANCE), FRÉDÉRIC DOMERGUE (UNIVERSITÉ DE BORDEAUX CNRS LABORATOIRE DE BIOGÈNE MEMBRANAIRE, FRANCE), SUSANA RIVAS (CNRS INRA LABORATOIRE DES INTERACTIONS PLANTES-MICROORGANISMES, FRANCE)

@ GWYNETH.INGRAM@ENS-LYON.FR

The embryonic cuticle is necessary for normal seed development and seedling establishment in Arabidopsis. Although mutants with defective embryonic cuticles have been identified, neither the deposition of cuticle material, nor its regulation, has been described during embryogenesis. Here we show that cuticle deposition initiates *de novo* in patches on globular embryos, and that successful patch coalescence to form a continuous cuticle requires inter-compartmental signalling involving a secreted protease, two receptor kinases expressed in the embryonic protoderm, and a defence-related MPK protein. Transcriptome analysis shows that this pathway regulates defence-related gene-expression in seeds. Consistent with these findings we show that the receptor kinases implicated in embryonic cuticle formation play a hitherto unsuspected role in plant defence against bacterial pathogens. We propose that a defence-associated signalling pathway has been hijacked in some angiosperm seeds through the recruitment of endosperm specific components to ensure the loss of apoplastic continuity between the developing embryo and endosperm via an 'auto-immune' type interaction.

### P3.22 SEED PRODUCTION ENVIRONMENT EFFECTS ON SEED VIGOUR AND MITIGATION VIA POST-HARVEST SEED TREATMENTS

📅 THURSDAY 7 JULY, 2016 ⌚ 09:40

👤 ISABELLE CAMU (JOHN INNES CENTRE, UNITED KINGDOM), FERNANDO GOFFMAN (ENZA ZADEN BV, NETHERLANDS), STEVEN PENFIELD (JOHN INNES CENTRE, UNITED KINGDOM)

@ ISABELLE.CAMU@JIC.AC.UK

Seed germination vigour is an important factor in crop yield. Seedling vigour is defined as the sum of those seed properties which determine the level of activity and performance during germination and seedling emergence. A poor seed lot can be improved by post-harvest treatment such as steeping as used in the seed industry, but the mechanism is unknown. The aim of this study is to understand the mechanism of steeping in order to improve seed vigour and seed germination. We set seeds at different temperatures and showed that this produces variation in tomato seed vigour that can be improved by steeping. Using LC-MS and ICP, we characterised compounds that leach from seeds during steeping, and show that some of these are putative germination inhibitors. By adding these compounds to the water during steeping we are testing which of these may play a role in the effectiveness of the steeping process.

### P3.23 ACTIVE PROTEINS IN DEAD ORGANS ENCLOSING EMBRYOS

📅 THURSDAY 7 JULY, 2016 ⌚ 09:55

👤 BUZI RAVIV (FAAB BEN GURION UNIVERSITY, ISRAEL), GILA GRANOT (FAAB BEN GURION UNIVERSITY, ISRAEL), GODWIN JAMES (FAAB BEN GURION UNIVERSITY, ISRAEL), OMER FRENKEL (ARO, ISRAEL), GIDEON GRAFI (FAAB BEN GURION UNIVERSITY, ISRAEL)

@ BUZIRAVIV@GMAIL.COM

Seed development culminates in programmed cell death and hardening of organs enclosing the embryo providing essentially a physical shield for embryo protection during storage in the soil. We wanted to address the possibility that these organs store and release upon hydration active proteins and other substances that may assist seed germination and seedling establishment. Seeds and seed coats of various Brassicaceae and Fabaceae species and hardened parts of dispersal units of wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) and of *Avena sterilis* L. were hydrated, substances released were collected and subjected to proteomic analysis, in-gel activity assays and microbial growth regulating activities. We found that organs enclosing the embryo in dicots and monocots function as a storage that releases upon hydration multiple stress-associated proteins among them functional hydrolases including nucleases, proteases and chitinases. The Brassicaceae seed mucilage is not required for storage and release of hydrolases following hydration. Substances released from seeds and seed coats showed microbial growth controlling activities. Our data suggest that storage in essentially dead organs enclosing embryos and release upon hydration to the "seedsphere" of hydrolases and other substances is a general theme in plants and might have evolved to increase survival rate of germinating seeds.



### P3.24 MATERNAL CONTROL OF SEED DEVELOPMENT MEDIATED BY THE FLAVONOID BIOSYNTHESIS PATHWAY

THURSDAY 7 JULY, 2016 10:10

MAHA ALJABRI (BATH UNIVERSITY, UNITED KINGDOM)

MAHWIII@HOTMAIL.COM

Many plants exhibit post-zygotic barriers to hybridization, in both interploidy crosses within species and interspecific crosses between related species. For example, crosses between diploid (2x) and tetraploid (4x) plants cause a triploid block where severe endosperm under- or over-proliferation kills the developing triploid embryo. While most ecotypes of the model species *Arabidopsis thaliana* tolerate 2x X 4x crosses to produce large viable seed, one ecotype, Columbia (Col-0), exhibits a triploid block when the paternal parent is tetraploid. Recently, loss of function mutants in the flavonoid biosynthesis pathway (FBP) that operate in the seed coat have been identified as powerful maternal suppressors of the paternal Col4x-mediated triploid block. The present hypothesis is that a maternal messenger responsible for regulating the correct timing of endosperm cellularisation is attenuated or blocked by a functional FBP; hence mutations in the FBP promote cellularisation and reduce seed lethality by removing the signalling block. We are attempting to identify the cellularisation factor, understand the role of the FBP in its regulation and to explore further the effect that different ecotypes have on the severity of the triploid block and the underlying mechanism behind this effect.

### P3.25 A ROLE FOR VP1/FUS3/LEC2 SUBFAMILY OF B3 TRANSCRIPTION FACTORS IN THE CONTROL OF SEED MATURATION AND GERMINATION

THURSDAY 7 JULY, 2016 10:55

PILAR CARBONERO (CENTRO BIOTECNOLOGÍA GENÓMICA PLANTAS (CBGP) UNIVERSIDAD POLITÉCNICA DE MADRID, SPAIN), RAQUEL IGLESIAS-FERNÁNDEZ (CBGP-UNIVERSIDAD POLITÉCNICA DE MADRID, SPAIN), JESÚS VICENTE-CARBAJOSA (CBGP-UNIVERSIDAD POLITÉCNICA DE MADRID, SPAIN)

P.CARBONERO@UPM.ES

The accumulation of storage compounds in the barley seeds and its hydrolysis upon germination are highly regulated at the transcriptional level. Although several transcription factors (TFs) of the MYB-R2R3 (GAMYB), DOF (BPBF, SAD, HvDOF19/17) and bZIP (BLZ2, BLZ1), have been described in both processes, members of the VP1/FUS3/LEC2 subfamily of B3 TFs, modify their transcriptional activities and control the precise mechanism switching from maturation to germination programs. Thus, *HvVP1* transcripts accumulate in the early stages of both the developing seeds and the embryo and aleurone of germinating seeds, decreasing drastically after 24h of imbibition. *HvVP1* interacts with *HvGAMYB* and *BPBF*, master activators in both phases of seed development, decreasing their DNA binding affinity to their corresponding cis-motives in *Hor2* and *Amy6.4* gene promoters, and their transcriptional activation of these genes. The *HvFUS3* that complements the loss of function

mutant *fus3* in *Arabidopsis thaliana*, restoring the transcription from the albumin encoding gene *At2S3* and normal accumulation of anthocyanins in the seeds, participates in the transcriptional activation of the endosperm specific genes *Hor2* and *Itr1*, by binding to RY-boxes in their promoters interacting with the OPAQUE bZIP factor BLZ2 and this interaction is essential for full trans-activation of *Hor2* gene. The LEC2 TFs in cereals have not been fully characterized although two such genes have been annotated in the barley genome. This subfamily of B3 TFs originated in one gene in *Chlamydomonas reinhardtii* evolutionary expanding through mosses, ferns, gymnosperms and angiosperms by subsequent neo sub-functionalization and pseudogenization processes.

### P3.26 MOTHER-OF-FT-AND-TFL1 IS A KEY REPRESSOR OF SEED GERMINATION UNDER FAR-RED LIGHT IN *ARABIDOPSIS THALIANA*

THURSDAY 7 JULY, 2016 11:25

FABIÁN E. VAISTIJ (CENTRE FOR NOVEL AGRICULTURAL PRODUCTS, UNIVERSITY OF YORK, UNITED KINGDOM), THIAGO BARROS-GALVÃO (CENTRE FOR NOVEL AGRICULTURAL PRODUCTS, UNIVERSITY OF YORK, UNITED KINGDOM), ALISON D. GILDAY (CENTRE FOR NOVEL AGRICULTURAL PRODUCTS, UNIVERSITY OF YORK, UNITED KINGDOM), IAN A. GRAHAM (CENTRE FOR NOVEL AGRICULTURAL PRODUCTS, UNIVERSITY OF YORK, UNITED KINGDOM)

FABIAN.VAISTIJ@YORK.AC.UK

Primary dormancy is induced during seed development and prevents germination of mature seeds under favourable conditions. Dormancy prevents sprouting before seeds are released from the fruit and allows time for seed dispersal. Once dormancy is lost through after-ripening seeds still require favourable environmental conditions such as temperature, humidity and quality of light to germinate. It is well established that exposure to certain environmental conditions during seed development and germination impacts on the production of the phytohormones gibberellic acid (GA) and abscisic acid (ABA), which promote and repress germination, respectively. We have shown recently that 12-oxophytodienoic acid (OPDA), a precursor of the phytohormone jasmonic acid, also plays a key role in repressing germination. Phytochromes perceived (R) and far-red (FR) light to control germination of non-dormant seeds by regulating phytohormones accumulation. While R light promotes germination, FR light represses it. We have shown previously that MOTHER-OF-FT-AND-TFL1 (MFT), a member of the PEBP-family, is a promoter of dormancy in *Arabidopsis thaliana*. Expression of MFT is directly repressed during seed development by the SPATULA (SPT) bHLH-type transcription factor. Now, we have established that, in non-dormant seeds, (i) FR light represses SPT expression, which in turn allows MFT to be expressed, and (ii) MFT is required to repress germination. We will present data showing the effect of MFT on phytohormone levels and a model integrating our latest results on repression of germination under FR light.

### P3.27 SEED DORMANCY REGULATION IN THE REAL WORLD: HOW DO MECHANISMS IDENTIFIED IN THE LABORATORY OPERATE IN VARIABLE FIELD ENVIRONMENTS?

THURSDAY 7 JULY, 2016 13:50

WILLIAM E FINCH-SAVAGE (UNIVERSITY OF WARWICK, UNITED KINGDOM), STEVEN FOOTITT (UNIVERSITY OF WARWICK, UNITED KINGDOM)

Many molecular mechanisms that regulate dormancy have been identified individually in controlled laboratory studies. However, little is known about how the seed employs this complex suite of mechanisms during dormancy cycling in the variable environment of the soil seed bank. Nevertheless, this behaviour is essential to ensure germination takes place in a favourable habitat and climate space, and in the correct season for the resulting plant to complete its life cycle. Dormancy cycling is therefore also central to the competitiveness of weeds in crop production practice and the development of more environmentally-benign weed management. During their time in the soil seed-bank seeds continually adjust their dormancy status by sensing and integrating a range of environmental signals. The signals related to slow seasonal change are used for temporal sensing to determine the time of year and depth of dormancy. This alters their sensitivity to other spatial environmental signals that indicate in a more immediate way that conditions are suitable for germination, and so trigger the termination of dormancy. We have carried out molecular ecophysiological studies of dormancy cycling in field soils and in the laboratory with *Arabidopsis* ecotypes that behave as winter (Cvi) and summer annuals. This work has provided new insight into the role of temperature as a temporal signal in the coordination of mechanisms and signaling networks that control dormancy cycling in an ecological context.

### P3.28 NOVEL INSIGHTS INTO THE CELLULAR BASIS OF APOMIXIS IN *HIERACIUM* THROUGH CELL WALL FEATURES

THURSDAY 7 JULY, 2016 14:30

MARTINA JURANIC (CSIRO, AUSTRALIA), MATTHEW TUCKER (UNIVERSITY OF ADELAIDE, AUSTRALIA), CAROLYN SCHULTZ (UNIVERSITY OF ADELAIDE, AUSTRALIA), VINCENT BULONE (UNIVERSITY OF ADELAIDE, AUSTRALIA), ANDREW SPRIGGS (CSIRO, AUSTRALIA), JEN TAYLOR (CSIRO, AUSTRALIA), ANNA KOLTUNOW (CSIRO, AUSTRALIA)

MARTINA.JURANIC@CSIRO.AU

Apomixis is an attractive trait for the enhancement of crop species as it mediates the formation of maternal clones and has the potential to preserve hybrid vigour through successive seed generations. Apomictic *Hieracium* plants form their female gametes asexually in the ovules of the flower. This asexual process begins with the formation of aposporous initial (AI) cells in the vicinity of sexual cells undergoing meiosis. The AI cell displaces sexual cells causing termination of the sexual pathway, and directly initiates embryo

sac formation without undergoing meiosis. The resultant egg in the asexual embryo sac is diploid and maternal in genotype. We are interested in understanding cell fate switches that occur during the specification of a somatic cell into an AI cell and the subsequent growth dynamics of the AI cell governed by changes in molecular architecture of the primary cell wall. We have explored the transcriptomes of ovules and laser captured enlarging AI cells and found enrichment in transcripts associated with cell wall ontology. Furthermore, antibodies that recognize different cell wall components were used to compare cell wall composition in apomictic and sexual *Hieracium* ovules during female gamete development. This has determined that both the sexual and asexual gamete lineages are marked by specific cell wall polysaccharides. These tools have enabled us to identify AI cells prior to their enlargement, identify a zone within the ovule where AI cells originate and led us to propose a model of how the AI cell displaces sexual cells.

### P3.29 SEED LONGEVITY AND BIOTIC DEFENSE: TWO SIDES OF THE SAME COIN

THURSDAY 7 JULY, 2016 14:45

JULIA BUITINK (IRHS-INRA ANGERS, FRANCE), JOSEPH LY VU (IRHS-INRA ANGERS, FRANCE), KARIMA RIGHETTI (IRHS-INRA ANGERS, FRANCE), OLIVIER LEPRINCE (IRHS-ACO ANGERS, FRANCE)

JULIA.BUITINK@ANGERS.INRA.FR

The maturation phase is described as a developmental program encompassing the accumulation of storage reserves, drying and abscission from the mother plant. Besides regulatory pathways involved in seed filling, additional pathways must be activated to confer to orthodox seeds its dispersal characteristics, namely germination capacity, dormancy and the ability to survive in the dry state. The molecular pathways underlying the acquisition of longevity during late seed maturation and its interaction with the environment have received little attention. Yet, this trait is an important factor in the preservation of seed viability and quality during dry storage and an essential trait to ensure fast and homogenous seedling establishment. In developing seeds of *Medicago truncatula*, the long maturation period makes it feasible to distinguish seed filling from the acquisition of longevity, making it a powerful model to unravel the pathways regulating the acquisition of longevity. Using a systems biology approach, we will show how integration of phenotypic data on longevity in a conditional-dependent network of global gene expression obtained during seed maturation led to the isolation of a gene module linked to longevity. Genes in this module show also high connectivity in Arabidopsis. This module was enriched with genes playing a crucial role in defense against biotic stress. We will present evidence supporting a link between mechanisms implicated in defense against pathogens and survival in the dry state and discuss how seeds activate a developmentally regulated defense response during maturation that is also beneficial to long-term survival in the dry state.

### P3.30 FROM WEED SEED HETEROMORPHISM TO CROP SEED UNIFORMITY: PIONEER WORK INTO THE FULL SPREAD OF ADAPTATION MECHANISMS TO UNPREDICTABLE ENVIRONMENTS

THURSDAY 7 JULY, 2016 15:45

GERHARD LEUBNER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), KAI GRAEBER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), TINA STEINBRECHER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), SAFINA KHAN (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), WAHEED ARSHAD (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), GILES GRAINGE (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), THOMAS HOLLOWAY (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), JAMES HOURSTON (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MICHAEL IGNATZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), KAZUMI NAKABAYASHI (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MARTA PEREZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), CHRISTINA SCHULZE (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), LORNA RAVENHILL (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), TERESA LENSER (FRIEDRICH SCHILLER UNIVERSITY JENA, GERMANY), GÜNTHER THEISSEN (FRIEDRICH SCHILLER UNIVERSITY JENA, GERMANY), KATJA SPERBER (UNIVERSITY OF OSNABRÜCK, GERMANY), SARA MAYLAND-QUELLHORST (UNIVERSITY OF OSNABRÜCK, GERMANY), KLAUS MUMMENHOFF (UNIVERSITY OF OSNABRÜCK, GERMANY), SETAREH MOHAMMADIN (WAGENINGEN UNIVERSITY, NETHERLANDS), THU-PHUONG NGUYEN (WAGENINGEN UNIVERSITY, NETHERLANDS), ERIC SCHRANZ (WAGENINGEN UNIVERSITY, NETHERLANDS), ZSUZANNA MERAI (GREGOR MENDEL INSTITUTE VIENNA, AUSTRIA), ORTRUN MITTELSTEN SCHEID (GREGOR MENDEL INSTITUTE VIENNA, AUSTRIA), CHRISTOPHER GROSCHE (UNIVERSITY OF MARBURG, GERMANY), PER WILHELMSSON (UNIVERSITY OF MARBURG, GERMANY), CHRISTIAN ULLRICH (UNIVERSITY OF MARBURG, GERMANY), STEFAN RENSING (UNIVERSITY OF MARBURG, GERMANY), DANUSE TARKOWSKA (PALACKY UNIVERSITY OLOMOUC, CZECH REPUBLIC), MIROSLAV STRNAD (PALACKY UNIVERSITY OLOMOUC, CZECH REPUBLIC)

@ GERHARD.LEUBNER@RHUL.AC.UK

A requirement for maximum crop yield potential is successful germination and seedling establishment and this is under threat due to the increased incidence of erratic weather (climate change). Adaptation of weed germination phenology could evolve quickly to keep pace with climate change. Diverse mechanisms to time germination evolved to enable plant survival in unpredictable environments. Among them diasporic bet-hedging and phenotypic plasticity are thought to represent important adaptation strategies. However, little is known about the underpinning mechanisms of these phenomena, partly due to the lack of suitable model systems. The Seed Adapt Consortium ([www.seedapt.eu](http://www.seedapt.eu)) investigates diasporic heteromorphism, a typical bet-hedging strategy, in the annual Brassicaceae *Aethionema arabicum*. We found that *Ae. arabicum* is dimorphic and that the proportions of the two distinct fruit and seed morphs change in response to environmental conditions indicating phenotypic plasticity of this bet-hedging strategy. This dimorphism is associated with anatomic, biomechanical,

physiological, hormone and transcriptome differences between the morphs and the dimorphic dispersal units differ considerably in their germination timing. Together with the available genomic resources this will establish *Ae. arabicum* as a model system to unravel the molecular mechanisms underpinning the seed/fruit dimorphism. This diasporic heteromorphism represents a monophyletic trait within the genus *Aethionema* associated with its annual species. Seed/fruit heteromorphism evolved independently in other Brassicaceae and other plant families where it exhibits distinct degrees of phenotypic plasticity and developmental constraint. An interplay between conserved and specific mechanisms may mediate this diversity of diasporic bet-hedging adaptation strategies to cope with unpredictable environments.

### P3.31 AUTOMATED HIGH THROUGHPUT PHENOTYPING OF CROP SEED VIGOUR: IDENTIFICATION OF GENES ASSOCIATED WITH EARLY VIGOUR IN OILSEED RAPE

THURSDAY 7 JULY, 2016 16:15

STEVEN PENFIELD (JOHN INNES CENTRE, UNITED KINGDOM), JI ZHOU (TGAC, UNITED KINGDOM), CARMEL O'NEIL (JOHN INNES CENTRE, UNITED KINGDOM), RACHEL WELLS (JOHN INNES CENTRE, UNITED KINGDOM), RENE BENJAMINS (SYNGENTA SEEDS, NETHERLANDS)

@ STEVEN.PENFIELD@JIC.AC.UK

Seed germination and seedling growth phenotyping is critical in both research and commercial seed production. Traditionally this is achieved using tets carried out by eye with technicians counting seed and seedling attributes at fixed time intervals. This is low throughput and labour intensive, leads to problems with capturing events outside of normal working hours, and can contain elements of bias and subjectivity particular to individual observers. Here we describe a new simple low cost automated phenotyping platform technology developed at the John Innes Centre and show how we have used this system to phenotype 100 varieties in the Brassica napus Diversity Fixed Foundation Set. We show that we can capture germination and establishment parameters without manual inputs and use these to identify gene expression changes in Brassica napus seedlings associated with vigour differences among varieties using Associative Transcriptomics, as well as candidate polymorphisms underlying these differences.



### P3.3 USING NATURAL VARIATION TO UNCOVER ENVIRONMENTAL REGULATORS OF SEED DORMANCY

📅 WEDNESDAY 6 JULY, 2016 🕒 POSTER SESSION

👤 DANA R MACGREGOR (JOHN INNES CENTRE, UNITED KINGDOM), NAICHAO ZHANG (JOHN INNES CENTRE, UNITED KINGDOM), STEVEN D PENFIELD (JOHN INNES CENTRE, UNITED KINGDOM)

@ DANA.MACGREGOR@JIC.AC.UK

When a seed germinates determines the environment it will face during its own life, and importantly the environment in which it sets its seeds. Seed dormancy allows plants to appropriately control the timing of seed germination and is under tight genetic and environmental control. As such natural populations show a range of dormancy phenotypes. To uncover allelic variants controlling environmental regulation of seed dormancy we used a panel of 270 natural accessions of *Arabidopsis* and tested their primary seed dormancy under two contrasting temperature regimes during seed production. These data allowed us to categorise the accessions into 1) accessions that are responsive to lowered temperature during maturation, and 2) a small number where dormancy was similar regardless of the seed maturation environment. Based on our previous observations that seed coat permeability correlates with seed dormancy, we tested this for all the accessions set at both environmental conditions. Around half of the tested accessions showed no change in seed coat permeability in response to temperature indicating the activity of maternal pathway is attenuated in these lines. The other half of the accessions showed reductions in permeability under lower temperatures. The dormancy and permeability data were analysed with Genome Wide Association Mapping programs allowing us to identify putative regulators of seed dormancy and/or seed coat permeability, which are being tested using reverse genetic approaches. Further analyses of representatives of the dormancy and permeability categories will allow us to identify how temperature information is interpreted resulting in the appropriate level of dormancy for local adaptation.

### P3.4 CAN NON-THERMAL ATMOSPHERIC GAS PLASMA INFLUENCE THE BALANCE OF FORCES THAT GOVERN SEED GERMINATION?

📅 WEDNESDAY 6 JULY, 2016 🕒 POSTER SESSION

👤 GILES GRAINGE (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), ADRIAN DUNFORD (ELSOMS SEEDS LTD, UNITED KINGDOM), SUE KENNEDY (ELSOMS SEEDS LTD, UNITED KINGDOM), GILBERT SHARMA (LOUGHBOROUGH UNIVERSITY, UNITED KINGDOM), FELIPE IZA (LOUGHBOROUGH UNIVERSITY, UNITED KINGDOM), TINA STEINBRECHER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), GERHARD LEUBNER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

@ GILES.GRAINGE.2010@LIVE.RHUL.AC.UK

The development of novel methods to improve global crop yields is a requirement to achieve sustainable intensification. Seeds, as one of the major cornerstones in agriculture, are a prime target for research aiming to devise such novel methods. In particular, developments in "agri"-technology targeting successful germination and seedling

establishment over a broad range of abiotic conditions, a trait defined as seed vigour, is seen as a priority for the seed industry. The visible events during the germination of many crop seeds involves a two-step process of successive testa and endosperm rupture. This is regulated by the balance of two opposing forces; the growth potential of the radicle (embryonic root) and the mechanical restraint of the seeds covering layers (testa, endosperm, pericarp). The accumulation of reactive oxygen species (ROS) induces germination by contributing to the cell-wall loosening in the cell growth zone of the radicle and the weakening of the seed's covering layers by direct scission of site specific cell-wall polysaccharides. This results in the protrusion of the radicle which concludes the germination process. Our working hypothesis is that treatment of seeds with non-thermal atmospheric gas plasma (NTAGP) under conducive ROS forming conditions can enhance the natural ROS mediated embryo growth and weaken the seed covering layer, improving germination speed and uniformity.

### P3.5 THE EFFECT OF SMOKEWATER ON GERMINATION AND DORMANCY OF BLACKGRASS SEEDS

📅 WEDNESDAY 6 JULY, 2016 🕒 POSTER SESSION

👤 THOMAS HOLLOWAY (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), KAZUMI NAKABAYASHI (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), GERHARD LEUBNER-METZGER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

@ THOMAS.HOLLOWAY.2012@LIVE.RHUL.AC.UK

Wild fires have been known to play a role in the dynamics of plant communities by enhancing the regeneration of vegetation. More recently, isolated compounds from smoke have been shown to stimulate the germination of model and crop species. In an agricultural context, there is potential to use smoke-derived compounds for the depletion of soil seed banks to prevent weed infestation. *Alopecurus myosuroides* Huds. (blackgrass) is a serious herbicide resistant weed of high-input cereal fields. Its success is due in part to its dormancy profile, which allows it to synchronise its emergence with the cropping cycle, reducing the effectiveness of traditional chemical control methods. Seeds are shed in early autumn at the in time with the cereal harvest, and persist in the soil with a two to three week period of primary dormancy. In this poster we show that smokewater is able to break this primary dormancy and stimulate the germination of non-dormant blackgrass seeds. The cardinal temperatures for the germination of blackgrass seeds are described in a quantitative physiological manner and defined assays are used to determine the dose-response relationship of smokewater on germination. The work demonstrates the potential for smoke-derived compounds to be used as part of integrated weed control strategies.

### P3.6 WATER STRESS-INDUCED PATTERNS OF GENE EXPRESSION ASSOCIATED WITH SEED PRIMING IN ARABIDOPSIS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

JACK MITCHELL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), GEORGE W BASSEL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

@ JXM316@STUDENT.BHAM.AC.UK

Within seed populations, extensive variation in the timing to germination is observed. This represents an obstacle to agriculture and optimizing yields as it results in non-uniform crop establishment. As this problem persists, a key objective of the seed industry is to enhance the synchronization of germination within seed lots. One method used to achieve this is hydropriming, which involves placing seeds under water-limiting conditions for extended periods. Following this treatment there is a dramatic and significant enhancement in the synchronization of germination of seeds. Despite the common use of hydropriming, the mechanisms that underlie this process remain largely unknown. We examined the spatiotemporal patterns of gene expression associated with the seed to seedling transition over a time course of hydroprimed Arabidopsis seeds to examine what impact this treatment had at a molecular level within seeds. Using this approach, we have identified the stage in the progression of the seed to seedling transition at which hydropriming arrests seed germination. This has also revealed the cellular sites within the embryo where hydropriming is acting, and provides targets for the further manipulation of this system to enhance seed performance in agriculture.

### P3.7 NOVEL BIOMATERIAL ENGINEERING, MOLECULAR AND HORMONAL ANALYSES TO IMPROVE VEGETABLE SEED QUALITIES, PRIMING AND PRODUCTION IN STRESSFUL ENVIRONMENTS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

KAZUMI NAKABAYASHI (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MARTA PÉREZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), TINA STEINBRECHER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), GERHARD LEUBNER (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM)

@ KAZUMI.NAKABAYASHI@RHUL.AC.UK

Plant seeds, fruits and tubers are at the beginning and the end of all important supply chains for various kinds of horticultural products including food, feed and garden flowers. Seed and bud dormancy, germination and sprouting properties are key quality traits for secure, sustained and resilient horticulture. The long term goal of our research group is to fundamentally understand the molecular mechanisms underlying these quality traits and to apply that knowledge in agriculture and horticulture. Our current major focus is to control dormancy and ageing of seeds, to improve seed quality and seedling performance of horticultural and agricultural

crops, and also further to develop improved tools and strategies for weed management for efficient crop production. This knowledge overall is the key for high quality and yield of crops even in stressful environments for production, and consequently adaptation to a changing climate. Aiming at a solid characterisation of the biological basis for the traits of interest, we interdisciplinary combine different levels of approaches: Physiological observation, germination monitoring and modelling, endogenous hormone measurements, biomechanical measurements and molecular analysis from targeted gene expression to whole transcriptome analysis. Based on the integrated data, potential targets are exploited further for breeding and development of technologies. A few examples of our approaches and outcome will be introduced. ([www.seedbiology.eu](http://www.seedbiology.eu))

### P3.8 EPIGENETIC AND PHYSIOLOGICAL CHANGES DURING THE MATURATION OF QUERCUS SUBER L. SOMATIC EMBRYOS

WEDNESDAY 6 JULY, 2016 POSTER SESSION

MARTA PEREZ (ROYAL HOLLOWAY UNIVERSITY OF LONDON, UNITED KINGDOM), MARCOS VIEJO (UNIVERSITY OF OVIEDO, SPAIN), MARIA JESUS CAÑAL (UNIVERSITY OF OVIEDO, SPAIN)

@ MARTA.PEREZ@RHUL.AC.UK

*Quercus suber* L. (cork oak) is considered one of the most important multipurpose species in the Mediterranean ecosystem. In spite of its importance, conventional tree breeding methods as well as successful vegetative propagation are limited. Additionally, seeds are considered recalcitrant and cannot be stored. Somatic embryogenesis has been developed in cork oak as an alternative to conventional propagation techniques and is considered the most effective *in vitro* regeneration method for oak species. Nevertheless, the poor quality and incomplete maturation of somatic embryos seems to be the most important factors that limit the production that this technique offers. Embryo development, maturation and further germination require the coordinate action of hormonal and metabolic signals as well as epigenetic mechanisms that regulate the activation and repression of different gene expression programs. Therefore, the objective of the present study was to monitor epigenetic and hormonal changes during cork oak somatic embryo maturation by quantifying global DNA methylation and endogenous abscisic acid (ABA) levels. Moreover, immunodetection of 5-methyldeoxycytidine (5-mdC) and ABA signals during the process were also recorded. The results indicate that, in addition to ABA, epigenetic control appears to play an important role for the correct maturation and subsequent germination of cork oak somatic embryos with a decrease in 5-mdC levels during development while endogenous ABA content showed a transient increase with a peak in immature E2 embryos denoting the onset of the maturation phase. Moreover, the results showed that there is a specific spatial-temporal regulation during embryogenesis, particularly after cold treatment.

### P3.9 INFLUENCE OF THE GERMINATION ENVIRONMENT ON SEED SALT TOLERANCE: TEMPERATURE MODULATION OF CHLORIDE TOXICITY IN THE HALOPHYTE *SUAEDA MARITIMA*

WEDNESDAY 6 JULY, 2016 POSTER SESSION

CHARLOTTE E SEAL (ROYAL BOTANIC GARDENS KEW, UNITED KINGDOM), TIMOTHY J FLOWERS (UNIVERSITY OF SUSSEX, UNITED KINGDOM), ADELE MUSCOLO (MEDITERRANEA UNIVERSITY, ITALY)

@ C.SEAL@KEW.ORG

The economic cost of salinity to agriculture is high as the majority of common agricultural crops are sensitive to salt. Salt tolerant plants, halophytes, are increasingly considered as non-conventional cash crops due to their ability to overcome the osmotic and toxic challenges of salinity. The germination physiology of halophytes is complex, with some species reported to avoid (e.g. dormancy) and/or tolerate (e.g. ion compartmentalisation) salt to maximise the success of germination and early seedling establishment. In this study, we investigated whether the chloride concentration of different salts and the growth temperature were associated with the germination and early seedling performance of the halophyte *Suaeda maritima* (Amaranthaceae). The salts we considered were artificial sea water and equivalent concentrations of its major salt components (NaCl, MgCl<sub>2</sub> and CaCl<sub>2</sub>). At 5°C, germination was highest in distilled water and any ungerminated seeds recovered fully when transferred to fresh water, indicating an osmotic limitation. In contrast, at 25°C, germination rapidly declined with chloride concentration and the recovery of ungerminated seeds was poor, indicative of ion toxicity. Seedling dry mass was also negatively associated with chloride concentration. Through observing such a significant temperature-salinity interaction, we recommend that laboratory studies on the salt tolerance of germination consider both the chloride concentration of the germination medium and temperature, which will have downstream effects on the underlying mechanisms of seeds salt tolerance.

### P3.10 GERMINATION RESPONSES OF PINUS DENSIFLORA SEEDS TO TEMPERATURE AND LIGHT

WEDNESDAY 6 JULY, 2016 POSTER SESSION

CHAE SUN NA (INSTITUTE OF BOTANY UNIVERSITY OF INNSBRUCK, AUSTRIA), THOMAS ROACH (INSTITUTE OF BOTANY UNIVERSITY OF INNSBRUCK, AUSTRIA), CHARLOTTE SEAL (ROYAL BOTANIC GARDEN KEW, UNITED KINGDOM), ILSE KRANNER (INSTITUTE OF BOTANY UNIVERSITY OF INNSBRUCK, AUSTRIA)

@ CHAESUN.NA@UIBK.AC.AT

The germination responses of *Pinus densiflora*, a species occurred over East Asia including Japan and Korea, were investigated with respect to temperature and light. Seeds were collected from South Korea in October 2015 and dried to 5.0% moisture content. A thermal gradient plate was used to evaluate the germination response to constant and fluctuating temperatures in range of 5 to 35°C, and

the effect of light on germination was tested in comparison to darkness under four temperature conditions: 20°C, 25°C, 15/25°C and 20/30°C (day/night; 12h/12h). Results from the thermal gradient plate showed that *P. densiflora* had the highest germination success when daily temperature averaged between 18°C and 24°C, with a maximum total germination of 92% (at 23.2°C). Below an average of 15°C and above 32°C germination was less than 10%. However, pre-treated seeds at 45°C and 100% RH for up to 5 d improved germination speed and total germination. Total germination of *P. densiflora* was much greater under 12h light compared to darkness at all temperature regimes tested. It was insignificant whether a seed experienced a higher temperature during the day or night showing a separation of the responses to light and temperature. In conclusion, this species is not sensitive to temperature fluctuations, but light is necessary for germination. Seeds may possess shallow dormancy as vigour could be improved via a heat shock. The results provide baseline knowledge for future research on *P. densiflora* seeds.

### P3.11 IDENTIFICATION OF GENE REGULATORY NETWORKS DRIVING CHANGES IN THE BIOMECHANICAL PROPERTIES OF EMBRYO CELLS AND THE SEED TO SEEDLING TRANSITION

WEDNESDAY 6 JULY, 2016 POSTER SESSION

HAO XU (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), GEORGE W BASSEL (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM)

@ HAOXUPLANT@GMAIL.COM

The seed to seedling transition in *Arabidopsis* is driven exclusively through changes in cell shape. Changes in the mechanical properties of the embryo cell wall therefore underlie this transition, and cell wall modifying gene expression represents the downstream targets of the germination process. Using targeted yeast interaction screening, we have identified transcription factors which directly bind the upstream sequences of the genes which promote cell expansion during *Arabidopsis* seed germination. These transcription factors mediate the penultimate step of the germination process through their regulation of gene expression which directly alters the physical properties of the cell wall. The functional validation of these interactions is ongoing, and will ultimately provide genetic targets for the enhancement of seed vigour and seedling establishment.



# P4 THE PLANT ENDOPLASMIC RETICULUM: A DYNAMIC MULTITASKING ORGANELLE

**ORGANISED BY:** PROF CHRIS HAWES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), DR LORENZO FRIGERIO (UNIVERSITY OF WARWICK, UNITED KINGDOM), DR PATRICK SCHÄFER (UNIVERSITY OF WARWICK, UNITED KINGDOM), DR VERENA KRIECHBAUMER (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM) AND DR EMILY BREEZE (UNIVERSITY OF WARWICK, UNITED KINGDOM)

## P4.1 STRUCTURE AND DYNAMICS OF THE ENDOPLASMIC RETICULUM IN CULTURED MAMMALIAN CELLS

📅 TUESDAY 5 JULY, 2016 ⌚ 10:30

👤 EIJA JOKITALO (UNIVERSITY OF HELSINKI, FINLAND)

@ EIJA.JOKITALO@HELSINKI.FI

We are studying molecular determinants involved in organelle maintenance and dynamic interplay between organelles. Endoplasmic reticulum (ER) is the most extensive cellular membrane organelle and constitutes the biosynthetic compartment for lipids and secreted and integral membrane proteins. It also plays a key role as a reservoir of Ca<sup>2+</sup> ions and a hub controlling calcium signaling and homeostasis. It is thus not surprising that the ER directly participates in most membrane contact sites of the cell. To accommodate the vast range of functions, the ER network spreads throughout the cell, and its functions are distributed into structural subdomains according to their specific requirements. The main aim of my research team is to understand:

- how different functions are distributed to the various subdomains,
- how the dynamics of the ER is orchestrated, and
- how the ER sheet/tubule-balance is maintained.

We are utilizing several advanced imaging techniques including two 3D-EM techniques and correlative light electron microscopy to appreciate the dynamic nature of the ER and membrane contact sites and to visualize the membranes and all organelles within cellular context at once. Knowledge of the mechanisms behind the structural maintenance and dynamics will be the key towards deeper understanding of ER functions and their regulation.

## P4.2 QUANTIFYING THE PLANT ENDOPLASMIC RETICULUM, ARE WE NEARLY THERE?

📅 TUESDAY 5 JULY, 2016 ⌚ 11:10

👤 IMOGEN SPARKES (UNIVERSITY OF EXETER, UNITED KINGDOM), CONGPING LIN (UNIVERSITY OF EXETER, UNITED KINGDOM), PETER ASHWIN (UNIVERSITY OF EXETER, UNITED KINGDOM), RHIANNON WHITE (UNIVERSITY OF EXETER, UNITED KINGDOM), LAWRENCE GRIFFING (TEXAS A AND M UNIVERSITY, UNITED STATES)

@ I.SPARKES@EXETER.AC.UK

The ER is a highly dynamic network comprised of tubules and cisternal regions that readily grow, shrink and interconvert over short timescales. The ER plays essential roles in the cell that are linked, to a certain extent, to the morphology and dynamics of the organelle itself. To be able to better understand the functional role, we are trying to unpick the molecular components that drive ER dynamics. One of the main obstacles in tackling this complex problem is being able to quantify ER morphology and dynamics. Here, I will discuss some of the current techniques and advances that we have made in this area.

### P4.3 A C-TERMINAL AMPHIPATHIC HELIX IS NECESSARY FOR THE IN VIVO TUBULE-SHAPING FUNCTION OF A PLANT RETICULON

TUESDAY 5 JULY, 2016 11:25

EMILY BREEZE (UNIVERSITY OF WARWICK, UNITED KINGDOM), NATASHA DZIMITROWICZ (UNIVERSITY OF WARWICK, UNITED KINGDOM), RHIANNON BROOKS (UNIVERSITY OF WARWICK, UNITED KINGDOM), STANLEY W BOTCHWAY (RUTHERFORD APPLETON LABORATORY, UNITED KINGDOM), JACOB P BRADY (UNIVERSITY OF OXFORD, UNITED KINGDOM), CHRIS HAWES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), ANN DIXON (UNIVERSITY OF WARWICK, UNITED KINGDOM), JASON R SCHNELL (UNIVERSITY OF OXFORD, UNITED KINGDOM), LORENZO FRIGERIO (UNIVERSITY OF WARWICK, UNITED KINGDOM)

@ EMILY.BREEZE@WARWICK.AC.UK

Reticulons (RTNs) are a class of endoplasmic reticulum (ER) membrane proteins that are capable of maintaining high membrane curvature, thus helping shape the ER membrane into tubules. The mechanism of action of RTNs is hypothesised to be a combination of wedging, resulting from the transmembrane topology of their conserved reticulon homology domain, and scaffolding, arising from the ability of RTNs to form low-mobility homo-oligomers within the membrane. We have studied the plant RTN isoform RTN13, which has previously been shown to locate to ER tubules and the edges of ER cisternae and to induce constrictions in ER tubules when overexpressed, and have identified a region in the C-terminus containing a putative amphipathic helix (APH). Here we show that deletion of this region or disruption of the hydrophobic face of the predicted helix abolishes the ability of RTN13 to induce constrictions of ER tubules *in vivo*. These mutants, however, still retain their ability to interact and form low-mobility oligomers in the ER membrane. Hence, our evidence indicates that the conserved APH is a key structural feature for RTN13 function *in vivo* and we propose that RTN, like other membrane morphogens, rely on APHs for their function.

### P4.4 THE DYNAMIC BEHAVIOR OF STORAGE ORGANELLES IN DEVELOPING CEREAL SEEDS

TUESDAY 5 JULY, 2016 11:40

EVA STÖGER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), ELSA ARCALIS (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), VERENA IBL (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), STANISLAV MELNIK (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA), JENNY PETERS (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES (BOKU), AUSTRIA)

@ EVA.STOEGER@BOKU.AC.AT

Cereal endosperm is a short-lived tissue adapted for nutrient storage, and its endomembrane system is clearly influenced by this functional specialization. During endosperm development specialized organelles are formed for the accumulation of storage proteins, which are ultimately deposited either within protein bodies derived from the endoplasmic reticulum, or in protein storage vacuoles (PSVs). During seed maturation the endosperm tissue undergoes extensive endomembrane system reorganization and rearrangement including interactions between organelles, while protein transport occurs via several developmentally regulated trafficking routes. *In vivo* imaging and the use of tonoplast markers and fluorescent organelle tracers provide further insight into these dynamic membrane reorganization events in the developing endosperm of different cereal species.

The analysis of recombinant protein transport, deposition and glycosylation provides additional information about cargo trafficking to storage organelles since the specialized architecture and dynamic behavior of the endomembrane system in storage cells may influence the subcellular fate and post-translational modification of recombinant glycoproteins. On the other hand, a better understanding of the peculiar rules of protein trafficking to endogenous and ectopically induced storage organelles is desirable to design optimized production strategies for molecular farming applications that exploit the unique bioencapsulation properties of storage organelles for the delivery of recombinant vaccines and other pharmaceuticals.

### P4.5 THE NATURE OF THE ASSOCIATION OF THE ENDOPLASMIC RETICULUM WITH THE CYTOSKELETON DURING PLASMOLYSIS

TUESDAY 5 JULY, 2016 12:10

LAWRENCE GRIFFING (TEXAS A&M UNIVERSITY, UNITED STATES), XIAOHANG CHENG (TEXAS A&M UNIVERSITY, UNITED STATES), INGEBORG LANG (UNIVERSITY OF VIENNA, AUSTRIA)

@ GRIFFING@TAMU.EDU

Plasmolysis, whereby the protoplast withdraws from the cell wall, provides an experimental approach to determine the nature of the linkage between the endoplasmic reticulum (ER) at the plasma membrane/cell wall interface and the accompanying cortical cytoskeletal elements, filamentous actin and microtubules. During treatment with high osmolarity solute (0.7 M mannitol),

the protoplast of hypocotyl cells in *Arabidopsis* can undergo concave plasmolysis, whereby large concave invaginations occur along the longitudinal sides of the cell; this can develop into convex plasmolysis, whereby the round protoplast withdraws from the apical/basal ends of the cells. During both concave and convex plasmolysis, ER-containing strands continue to reside adjacent to the cell wall, and are a large part of the network called the Hechtian reticulum. The ER in the Hechtian reticulum becomes exclusively associated with cortical microtubular structures, which are dramatically altered from the cortical microtubular array in non-plasmolysed cells, and the ER no longer associates with actin filament bundles. On the other hand, during convex plasmolysis, a cap of actin filament bundles forms in the withdrawing protoplast which contains the bulk of the ER network. Large strands which contain ER and link the shrinking protoplast with the cell wall, the Hechtian strands, sometimes also contain actin filaments. Accompanying these changes in ER-cytoskeleton association are changes in the both the luminal flow of ER protein and changes in the persistent cisternalization of the ER network. Protoplast ER becomes more cisternal and more persistent during plasmolysis.

## P4.6 METABOLIC PLASTICITY AS MEDIATED BY THE DHURRIN METABOLON

TUESDAY 5 JULY, 2016 13:40

BIRGER L. MØLLER (UNIVERSITY OF COPENHAGEN, DENMARK), TOMAS LAURSEN (UNIVERSITY OF COPENHAGEN, DENMARK), KRUTIKA BAVISHI (UNIVERSITY OF COPENHAGEN, DENMARK), CAMILLA KNUDSEN (UNIVERSITY OF COPENHAGEN, DENMARK), TIMOTHY DAFFORN (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), JONAS BORCH (NOVO NORDISK, DENMARK), JEAN-ETIENNE BASSARD (UNIVERSITY OF COPENHAGEN, DENMARK)

@ BLM@PLEN.KU.DK

Cyanogenic glucosides are a class of specialized metabolites found in numerous crop plants with dhurrin being present in *Sorghum bicolor*. Dhurrin is formed from tyrosine in a pathway catalyzed by CYP79A1, CYP71E1, UGT85B1 and P450 oxidoreductase (POR). The three membrane bound proteins are situated in the ER membrane and the biosynthetic enzymes are thought to organize in enzyme complexes facilitating channeling of toxic and labile intermediates. In vivo studies based on transient expression in *Nicotiana benthamiana* demonstrated that all enzymes were active when expressed as eGFP fusion proteins and catalyzed efficient channeling of intermediates. Protein-protein interactions and channeling was studied in planta using fluorescence lifetime imaging microscopy, fluorescence correlation spectroscopy and metabolite analysis. We demonstrate that the enzymes catalyzing dhurrin biosynthesis are organized within dynamic metabolons enabling plants to adapt to environmental challenges. Using the styrene maleic acid (SMA) copolymer, discrete lipid particles (SMALPs) were excised from the ER membrane enabling purification of the dhurrin metabolon by affinity chromatography and characterization by mass spectrometry based proteomics. Functional importance of identified protein-protein interactions and lipid environment was studied by reconstitution of the dhurrin pathway in proteoliposomes. UGT85B1 binding to liposomes was dependent of the presence of CYP79A1 and CYP71E1. A model for the organization of the dhurrin metabolon in multi-enzyme clusters is presented. This SMALP nanodisc approach may be generally employed for detergent free isolation of entire biosynthetic pathways organized within metabolons to identify enzymes catalyzing missing steps and to identify the presence of lipids stabilizing protein entanglement.

## P4.7 POLYPRENOL REDUCTASE2 DEFICIENCY IS LETHAL IN ARABIDOPSIS DUE TO MALE STERILITY

TUESDAY 5 JULY, 2016 14:20

MALGORZATA GUTKOWSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), ADAM JOZWIAK (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), KATARZYNA GAWARECKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), LILIANA SURMACZ (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), ANNA BUCZKOWSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), MALGORZATA LICHOCKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), JULITA NOWAKOWSKA (WARSAW UNIVERSITY, POLAND), EWA SWIEZEWSKA (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND)

@ GOSIAG@IBB.WAW.PL

Dolichol is a required cofactor for protein glycosylation, the most common posttranslational modification modulating the stability and biological activity of proteins in all eukaryotic cells. We have identified and characterized two genes, PPRD1 and -2, which are orthologous to human SRD5A3 (steroid 5 $\alpha$  reductase type 3) and encode polyprenol reductases responsible for conversion of polyprenol to dolichol in *Arabidopsis thaliana*. PPRD1 and -2 play dedicated roles in plant metabolism. PPRD2 is essential for plant viability; its deficiency results in aberrant development of the male gametophyte and sporophyte. Impaired protein glycosylation seems to be the major factor underlying these defects although disturbances in other cellular dolichol-dependent processes could also contribute. Shortage of dolichol in PPRD2-deficient cells is partially rescued by PPRD1 overexpression or by supplementation with dolichol.

## P4.8 DISTINCT ER-GOLGI RECYCLING PATHWAYS AND ER SUB-COMPARTMENTALIZATION ARE REVEALED BY HIGH-RESOLUTION SEPARATION OF THE SECRETORY PATHWAY AND PROTEOMIC ANALYSIS

TUESDAY 5 JULY, 2016 14:35

HARRIET T PARSONS (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), TIM J STEVENS (MRC LABORATORY OF MOLECULAR BIOLOGY, UNITED KINGDOM), HEATHER E MCFARLANE (MELBOURNE UNIVERSITY, AUSTRALIA), LAURENT GATTO (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), WILLIAM G. T. WILLATS (COPENHAGEN UNIVERSITY, DENMARK), CHRISTOPHER J. PETZOLD (LAWRENCE BERKELEY NATIONAL LABORATORY, UNITED STATES), KATHRYN S. LILLEY (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), JOSHUA L. HEAZLEWOOD (MELBOURNE UNIVERSITY, AUSTRALIA)

@ TEMPEPARSONS@GMAIL.COM

A membrane-enriched fraction from *Arabidopsis thaliana* cell-suspension culture has been separated along an electrophoretic gradient, at sufficiently high resolution to resolve protein components of the ER, different Golgi cisternae and trafficking



vesicles. A gradient of surface charge that exists across the secretory pathway enabled compartments to be separated from early (ER &ndash; compartment 1) to late (trans-Golgi network [TGN]). This allowed the fate of differentially resolving groups of ER proteins to be tracked across the whole secretory pathway. Results clearly showed proteins from ER – group 1 cycling between the ER and early-medial Golgi, whereas ER-group 2 proteins extended to the trans-Golgi, and occasionally the TGN. Evidence for direct ER to PM trafficking pathways was also detected. Consistent identifications across multiple biological replicates allowed compilation of ER and Golgi sub-compartment proteomes. Comparisons of protein sequence features between proteomes revealed the distribution of retention motifs across ER compartments, as well as differences in transmembrane span properties, cytosolic and luminal charge, and amino acid enrichment. ER proteins were less positively charged, with shorter trans-membrane spans, and more aromatic side chains than medial or trans-Golgi proteins but differences also existed between ER groups. This study has delivered an unprecedented amount of new, high-resolution secretory system data, setting the ER in context and allowing the most detailed characterization to date of a eukaryotic ER proteome.

### P4.9 LIPID PHYSICAL PROPERTIES, MEMBRANE DYNAMICS AND TRANSPORT, AND LYSOPHOSPHOLIPID ACYLTRANSFERASES AT THE ER-GOLGI INTERFACE IN PLANT CELLS

**TUESDAY 5 JULY, 2016** **14:50**

**PATRICK MOREAU** (CNRS-UNIVERSITY OF BORDEAUX, FRANCE), **VALÉRIE WATTELET-BOYER** (CNRS-UNIVERSITY OF BORDEAUX, FRANCE), **MARINA LE GUÉDARD** (CNRS-UNIVERSITY OF BORDEAUX, FRANCE), **JEAN-JACQUES BESSOULE** (CNRS-UNIVERSITY OF BORDEAUX, FRANCE)

**@ PATRICK.MOREAU@U-BORDEAUX.FR**

Membrane lipids are very critical for cell and organelle compartmentalization in all eukaryotic cells. In plant cells we, and other teams, have evidenced the involvement of lipid metabolism in the regulation of the plant secretory/retrograde pathways. Physical properties of lipids and their interactions with other partners (lipids or proteins) reveal these molecules as key actors of membrane curvature regulation in many cellular processes and especially in secretory/retrograde pathways. Lipids could play critical roles in endomembrane morphodynamics regulation, organelle morphology and transport vesicle formation/fusion like it has been indicated within *in vivo*/in vitro studies in animal and yeast models.

For example, a role for several enzymes of lipid metabolism such as phospholipases and acyltransferases in the function of the secretory/retrograde pathways has been largely highlighted in animal cells. Lysophospholipid acyltransferases could be critical for the plant secretory pathway as well and especially at the ER/Golgi interface. Several lysophosphatidic acyltransferases have been identified in *A. thaliana*. These enzymes have an acyltransferase activity and subcellularly localize to the ER for 3 of them and to the Golgi for one of them. The full characterization of the acyltransferase activities using recombinant proteins is on progress, mutagenesis, reverse genetics and imaging approaches will also be developed to establish their potential roles at the ER-Golgi interface in plant cells.

### P4.10 ORCHESTRATION OF THE GLUCOSINOLATE BIOSYNTHETIC PATHWAY

**TUESDAY 5 JULY, 2016** **16:00**

**BARBARA HALKIER** (UNIVERSITY OF COPENHAGEN, DENMARK)

In our research we aimed at providing evidence for whether the glucosinolate biosynthetic pathway organized in a metabolon. In a targeted Y2H approach where biosynthetic enzymes were used as bait and prey, respectively, only weak interactions were seen between membrane-bound P450s and a soluble GGP1 enzyme, which also bound the soluble UGTs and SOTs. Untargeted yeast-2-hybrid identified 54 interacting proteins, including a small family of interactors potentially providing a direct link to defense signaling. Noticeable, none of the proteins identified in the yeast-2-hybrid screens was the enzymes in the biosynthetic pathway. Neither were there apparent scaffolding proteins and assembly chaperones amongst our candidate genes. The findings indicated that most of the enzymes are only transiently interacting. Our current thinking is that rather than viewing the individual steps as part of a metabolon with tight physical interactions, we now view the orchestration of the pathway as a dynamic cluster of enzymes that may self-assemble stochastically through transient interactions in highly organized cytoplasmic microenvironments. As weak or transient interactions between two proteins in &lsquo;split&rsquo; reporter systems may give false negative results, we have implemented a tool for plant research where reporters can be chemically induced to dimerize the FKBP12 and the FRB domains independently of the investigated interactions. The chemically-induced dimerization serves as a built-in positive internal control, thereby overcoming many of the drawbacks associated with the evaluation of protein-protein interactions between two proteins of interest.

### P4.11 AUXIN BIOSYNTHESIS AND THE ENDOPLASMIC RETICULUM

**TUESDAY 5 JULY, 2016** **16:30**

**VERENA KRIECHBAUMER** (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM)

**@ VKRIECHBAUMER@BROOKES.AC.UK**

The growth regulator auxin is involved in all key developmental processes in plants. A complex network of a multiplicity of potential auxin biosynthetic pathways as well as transport, signalling plus conjugation and deconjugation lead to a complicated system of auxin function. This raises the question how such a complex and multifaceted system producing such a powerful and important molecule as auxin can be effectively organised and controlled. We investigated the subcellular localisation of auxin biosynthetic enzymes in the TAA/YUC route and found that a subset of these enzymes is localised to the endoplasmic reticulum (ER). ER microsomal fractions also contain a significant percentage of auxin biosynthetic activity. We show specific protein-protein interactions between some of the enzymes in the TAA/YUC route of auxin biosynthesis. Taken together this could point toward a model of auxin function using ER membrane location and subcellular compartmentation for supplementary layers of regulation.

## P4.12 TWO SIGNALING PATHWAYS OF THE ER STRESS RESPONSE IN ARABIDOPSIS

WEDNESDAY 6 JULY, 2016 09:00

NOZOMU KOIZUMI (OSAKA PREFECTURE UNIVERSITY, JAPAN), YUJI IWATA (OSAKA PREFECTURE UNIVERSITY, JAPAN)

NKOIZUMI@PLANT.OSAKAFU-U.AC.JP

The ER stress response or the unfolded protein response (UPR) is a cellular response to cope with accumulation of abnormally folded proteins in the ER. In the ER stress response, genes coding for ER chaperones and foldases are coordinately induced. IRE1, an ER stress sensor well conserved among eukaryotic cells, catalyses cytoplasmic splicing of mRNA encoding bZIP transcription factors. Arabidopsis bZIP60 is translated as an ER-membrane-bound protein that is inactive under unstressed condition. When cells are exposed to stresses, the RNase domain of IRE1 cleaves the bZIP60 mRNA at two positions and an intron of 23 nucleotides in length is spliced out. This splicing causes frameshift, resulting in the translation of bZIP60 protein without a transmembrane domain, which locates to the nucleus. It has also been found that a tRNA ligase is necessary to complete the splicing. In addition to bZIP60, bZIP28, another membrane-bound transcription factor, is involved in the UPR. Different from the bZIP60, bZIP28 is regulated by proteolysis. Recent progress of the molecular mechanism of these pathways will be discussed.

## P4.13 STUDYING THE ER IN 3-D

WEDNESDAY 6 JULY, 2016 09:40

CHRIS HAWES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), LOUISE HUGHES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), MAIKE KITTELMANN (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM)

CHAWES@BROOKES.AC.UK

Recent advances in high resolution field emission scanning electron microscopy combined with the ability to section resin blocks within the microscope specimen chamber have revolutionised our ability to study cell structure in three dimensions. Serial Block Face Scanning Electron Microscopy (SBFSEM) permits the collection of large three dimensional data sets from resin embedded material. One of the major limitations of the technique is generating sufficient contrast in the specimen prior to resin embedding to permit sequential collection of relatively high magnification images. We use selective staining of membranes using the zinc iodide/osmium tetroxide impregnation technique to selectively highlight the endoplasmic reticulum. In this way the 3-D organisation of the endoplasmic reticulum can be studied through reconstructions of data sets obtained from the Gatan 3-View system and reconstructed using suitable software. Here we will describe the use of this technique to investigate the organisation of the endoplasmic reticulum in dividing root meristematic cells and leaves.

## P4.14 UNDERSTANDING SNARE INSERTION IN PLANTS: GENETIC ANALYSIS OF GET PATHWAY COMPONENTS IN ARABIDOPSIS THALIANA

WEDNESDAY 6 JULY, 2016 09:55

CHRISTOPHER GREFEN (UNIVERSITY OF TUEBINGEN, GERMANY), SHUPING XING (UNIVERSITY OF TUEBINGEN, GERMANY), DIETMAR MEHLHORN (UNIVERSITY OF TUEBINGEN, GERMANY), NIKLAS WALLMEROH (UNIVERSITY OF TUEBINGEN, GERMANY), LISA ASSECK (UNIVERSITY OF TUEBINGEN, GERMANY), RITWIKI KAR (UNIVERSITY OF TUEBINGEN, GERMANY)

CHRISTOPHER.GREFEN@UNI-TUEBINGEN.DE

SNARE proteins catalyse the final step in membrane fusion with their cognate SNARE partners through tight interaction via their cytosolic N-terminal domains. Their C-terminal membrane anchor pulls the opposite membranes together, overcoming the strong dehydration forces associated with the lipid bilayer and ultimately leading to fusion of the two membranes. This important function is prerequisite to a multitude of vital cellular functions such as trafficking of cargo to the outside of the cell or adding additional membrane material to the plasma membrane for expansion. In yeast and mammals integration of tail-anchored (TA) membrane proteins seems to be facilitated via cytosolic components in an ATP-dependent fashion. This 'Guided-Entry of TA proteins' (GET) pathway has not been described in plants where research focusses on import pathways into chloroplasts and mitochondria. How the abundance of SNARE and other TA proteins are integrated into the ER membrane in plants is currently unknown. We have identified the candidates involved in a putative GET pathway of Arabidopsis. Our data show that plants have evolved multiple orthologues of specific GET pathway components, albeit in a compartment-specific manner. In contrast, others seem to be absent in plants suggesting differences in the protein insertion mechanism or the development of alternative pathways. The latter hypothesis is supported by highly specific rather than general phenotypes associated with loss-of-function lines highlighting the plant's need for backup insertion mechanisms.

## P4.15 ARABIDOPSIS NAP1 REGULATES THE FORMATION OF AUTOPHAGOSOMES

WEDNESDAY 6 JULY, 2016 10:10

PENGWEI WANG (DURHAM UNIVERSITY, UNITED KINGDOM), CHRISTINE RICHARDSON (DURHAM UNIVERSITY, UNITED KINGDOM), CHRIS HAWES (OXFORD BROOKES UNIVERSITY, UNITED KINGDOM), PATRICK J. HUSSEY (DURHAM UNIVERSITY, UNITED KINGDOM)

PENGWEI.WANG@DURHAM.AC.UK

The SCAR/WAVE complex is required for ARP2/3 mediated actin nucleation, and this mechanism is highly conserved in plants and animals. Proteins from the SCAR/WAVE complex have been found to be membrane associated in plants. Using fluorescent protein fusions we have found that NAP1, a component of the SCAR/WAVE complex, locates to vesicles/puncta that appear upon applied pressure. These NAP1 vesicles can be ER associated, can co-align with the cytoskeleton and fuse to each other homotypically. More

interestingly, the majority co-localize with the autophagosome marker, ATG8. Anti-NAP1 identifies autophagosomes in immune-TEM. Autophagy is enhanced upon Nitrogen starvation and upon salt stress. However, less autophagosomes are generated in nap1 mutants during starvation stress, indicating that the formation of autophagosomes is dependent on the presence of a functioning SCAR/WAVE complex. NAP1 knockout mutants (and KO mutants of other components of the SCAR/WAVE and ARP2/3 complexes) are more susceptible to Nitrogen starvation and are less salt tolerant. This indicates that the NAP1 mutant is defective in autophagy. Taken together our data show that NAP1 has another function in plant cells, other than in cell expansion, and that is as a regulator of autophagy.

## P4.16 THE ENDOPLASMIC RETICULUM AS MEDIATOR OF STRESS ADAPTATION AND CELL DEATH IN PLANTS

WEDNESDAY 6 JULY, 2016 11:00

PATRICK SCHÄFER (UNIVERSITY OF WARWICK, UNITED KINGDOM), CHARLOTTE RICH (UNIVERSITY OF WARWICK, UNITED KINGDOM), RUTH EICHMANN (UNIVERSITY OF WARWICK, UNITED KINGDOM)

P.SCHAFFER@WARWICK.AC.UK

The endoplasmic reticulum (ER) takes a central role in the integration of processes regulating plant growth and stress adaptation. Stress and certain growth stages induce ER stress in plants since the ER is transiently unable to process a suddenly increased demand for secretory proteins. Adapting ER function therefore bears a high potential to improve plant performance under diverse conditions. Cells take the accumulation of unfolded proteins in the ER as an indicator for ER stress. To reinstall proper ER function, ER stress sensors are translocated to the nucleus to induce the unfolded protein response (UPR) upon mild ER stress. However, plants and animals apparently use some of these sensors to activate programmed cell death (PCD) upon failed UPR activation or severe ER stress. In plants, the mutualistic fungus *Piriformospora indica* (Pi) triggers ER stress but simultaneously suppresses the adaptive UPR to initiate, after a lag phase, ER-PCD. We present data how Pi can serve us as a tool to dissect ER stress signalling and to identify key regulators of the UPR and ER stress induced PCD.

## P4.17 ESCRTING DURING BARLEY ENDOSPERM DEVELOPMENT

WEDNESDAY 6 JULY, 2016 11:30

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

VERENA.IBL@BOKU.AC.AT

The significance of the Endosomal sorting complex required for transport (ESCRT)-III in cereal endosperm, comprising transfer cells, embryo surrounding tissue cells, aleurone and starchy endosperm, has been shown by the identification of the recessive mutant supernumerary aleurone layer1 (SAL1) in maize.

ESCRT-III is a huge subcomplex within the ESCRT machinery and is indispensable in the final membrane fission step during biogenesis of multivesicular bodies (MVBs), responsible for protein sorting to vacuoles and to the cell surface.

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) derived from the endoplasmic reticulum (ER). In addition, trafficking may follow a number of different routes, depending on cell type, developmental stage and environment, showing that the endomembrane system is capable of massive reorganization.

Recently, we have annotated barley ESCRT-III members in the (model) crop *Hordeum vulgare* (Hv) and show that all identified members are expressed in developing barley endosperm. Here, we will present first results concerning the functional organization of ESCRT-III in barley endosperm tissue and its putative protein sorting role in seed storage protein trafficking in barley endosperm by cell biological, molecular biological, bioinformatic and biochemical studies.

## P4.18 WORKSHOP: PLANT ER MEMBRANE LIPIDS: ANALYSIS, COMPOSITION AND FUNCTIONAL ASPECTS

WEDNESDAY 6 JULY, 2016 14:30

PATRICK MOREAU (CNRS, FRANCE), LILLY MANETA-PEYRET (UNIVERSITY OF BORDEAUX, FRANCE), LAETITIA FOUILLEN (CNRS, FRANCE)

PATRICK.MOREAU@U-BORDEAUX.FR

Plant ER membranes are the major site of biosynthesis of several lipid families (phospholipids, sphingolipids, neutral lipids such as sterols and triacylglycerols...) and contributes to the overall biosynthesis of lipids of many other organelles in plant cells. The structural diversity of lipids presents considerable challenges to comprehensive lipid analysis. The talk will present rapidly the various biosynthetic pathways and several aspects of lipid analyses: lipids extraction, handling, separation, detection,



identification and data presentation. Different examples will be given on different tools/approaches of lipid analyses in relation to the studies to be carried out (lipid metabolism, trafficking, domains...), and relationships between lipid properties and some functional aspects will also be discussed.

## P4.19 WORKSHOP: QUANTITATION OF THE ER NETWORK

📅 WEDNESDAY 6 JULY, 2016 ⌚ 15:45

👤 MARK FRICKER (UNIVERSITY OF OXFORD, UNITED KINGDOM),  
BOGUSLAW OBARA (DURHAM UNIVERSITY, UNITED KINGDOM),  
LUKE HEATON (UNIVERSITY OF OXFORD, UNITED KINGDOM),  
NICK JONES (IMPERIAL COLLEGE, UNITED KINGDOM)

@ MARK.FRICKER@PLANTS.OX.AC.UK

The complex interconnected tubular-cisternal organisation of the endoplasmic reticulum poses particular challenges for quantitative image analysis. Here we apply a network analysis pipeline using a combination of phase-congruency tensors, as an intensity-independent image enhancement for the tubular elements, and active-contour delineation of the cisternal compartments. The segmented structure is converted to a weighted graph representation with nodes at junctions and within the cisternae, linked by edges representing the length and diameter of the tubular components. This approach provides additional metrics describing the ER structure that are more robust to segmentation errors than conventional measures of surface area and volume and capture the macroscopic organisation better. Full parametric description of the network also allows mathematical modelling of internal transport processes. Nevertheless, the computational demands of network segmentation and analysis are considerably higher, and it is non-trivial to fully describe network dynamics from a succession of graph-representations.

# P5 MAKING CONNECTIONS – PLANT VASCULAR TISSUE DEVELOPMENT

**ORGANISED BY:** DR PETER ETHELLES (DURHAM UNIVERSITY, UNITED KINGDOM) & PROF SIMON TURNER (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

**SESSION SPONSORED BY:** JOURNAL OF EXPERIMENTAL BOTANY

## P5.1 SIGNALING THAT REGULATES VASCULAR CELL FATES IN PLANTS

📅 MONDAY 4 JULY, 2016 ⌚ 11:00

👤 HIROO FUKUDA (THE UNIVERSITY OF TOKYO, JAPAN)

@ FUKUDA@BS.S.U-TOKYO.AC.JP

In vascular meristem, procambial and cambial cells act as vascular stem cells to give rise to phloem cells and xylem cells. A CLE peptide, tracheary element differentiation inhibitory factor (TDIF) is a crucial factor to regulate vascular stem cell fate. TDIF suppresses the differentiation of procambial cells into xylem cells and promotes their proliferation in non-cell-autonomous fashion through a specific membrane-associated receptor for TDIF (TDR/PXY). Glycogen synthase kinase 3 proteins (GSK3s) and BES1 act at suppressing xylem differentiation downstream of TDIF-TDR signaling. Recently, we found that these factors also regulate phloem differentiation. Using an inhibitor of plant GSK3s, we established the VISUAL system, in which *Arabidopsis* mesophyll cells differentiate into tracheary elements and sieve elements via procambial cells. In this report, I will show novel results about differentiation into procambial and phloem cells and discuss about regulation of vascular cell fates.

## P5.2 PEPTIDES, REACTIVE OXYGEN SPECIES AND PINS SHOULD CROSSTALK TO BE INVOLVED IN INITIATION OF THE PLANT VASCULAR TISSUE DEVELOPMENT

📅 MONDAY 4 JULY, 2016 ⌚ 11:40

👤 IRINA G STRIZH (M.V. LOMONOSOV MOSCOW STATE UNIVERSITY, RUSSIA), SERGEY KOVALCHUK (SHEMYAKIN-OVCHINNIKOV INSTITUTE OF BIOORGANIC CHEMISTRY, RUSSIA), ALEXANDER SKRIPNIKOV (M.V. LOMONOSOV MOSCOW STATE UNIVERSITY, RUSSIA)

@ IRINA.STRIZH@MAIL.RU

Several CLE peptides are known to be involved in vascular tissue development. It is also well known that auxin distribution causes initiation of the plant vascular tissue development. Though reactive oxygen species (ROS) are known to be involved in root growth, there is no clear role for them in vascular tissue development. We have observed that typical nitro tetrazolium blue (NBT) staining occurs not only in the root apical meristem as previously identified

but in vascular tissues as well. Secondly, we have found that CLE-like chemically synthesized dodecapeptide CLV3p affect ROS distribution and that these peptides affect the distribution of PINs. We suggest that CLE-like dodecapeptides can be the trigger and signal molecular that affect auxin transporters redistribution, auxin flow and ROS generation that is involved in plant vascular development. There is also the question of whether CLE peptides directly affect transporters, NADPH-oxidases or peroxidases. We are working on the molecular connections between peptides, ROSes and auxin redistribution.

## P5.3 SPATIOTEMPORAL ANALYSIS OF BRASSINOSTEROID RECEPTOR BRL3 WITHIN THE PROVASCULAR TISSUES OF THE ARABIDOPSIS PRIMARY ROOT

📅 MONDAY 4 JULY, 2016 ⌚ 11:55

👤 ANA CANO-DELGADO (CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), JORGE E SALAZAR-HENAO (CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), REINHARD LEHNER (CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), ISABEL BETEGÓN-PUTZÉ (CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), JOSEP VILARRASA-BLASI (CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN)

@ ANA.CANO@CRAGENOMICA.ES

Brassinosteroid (BR) hormones are important regulators of plant growth and development. Recent studies revealed the cell-specific role of Brassinosteroids in vascular and stem cell development by the action of cell-specific of BR receptor complexes and downstream signaling components in *Arabidopsis thaliana* (*Arabidopsis*). Despite the importance of spatiotemporal regulation of hormone signaling in the control of plant vascular development, the mechanisms that confine cellular specificity to BR receptors within the vascular cells are not yet understood. Our recent data shows that BRI1-like receptors 1 and 3 (*BRL1* and *BRL3*) are differentially regulated by BRs. By using promoter deletions constructs of *BRL1* and *BRL3* receptors fused to GFP/GUS reporters in *Arabidopsis*, analysis of their cell-specific expression and regulation by BRs in the root apex has been carried out. We found that *BRL3* expression is finely modulated by BRs in different root cell types, whereas the location of *BRL1* appears to be independent of this hormone. Physiological and genetic analysis show a BR-dependent expression of *BRL3* in the root meristem. In particular, *BRL3* expression requires active BES1, a central transcriptional effector within the BRI1 pathway. Overall our study unveils the existence of a cell-specific negative

feedback loop from BRI1-mediated BES1 transcription factor of *BRL3* in phloem cells, while contributing to a general understanding of the spatial control of brassinosteroid signaling in plant development.

## P5.4 THE ROLE OF S-ACYLATION IN CELLULOSE SYNTHESIS IN DEVELOPING XYLEM

📅 MONDAY 4 JULY, 2016 ⌚ 12:25

👤 SIMON TURNER (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), MANOJ KUMAR (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), IVAN ATANASSOV (AGROBIOINSTITUTE UNIVERSITY OF MANCHESTER, BULGARIA), PIERS HEMSLEY (SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM), ANJALI GUPTA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), RAYMOND WIGHTMAN (SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE UNIVERSITY OF MANCHESTER, UNITED KINGDOM), PAUL CARR (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), LIAM BLACKLOCK (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ SIMON.TURNER@MANCHESTER.AC.UK

CESA proteins are the catalytic subunits of the cellulose synthase complex (CSC), with each CSC likely to contain at least 18-24 CESAs. We present our recent results about the importance of cysteine modifications of CESA proteins in the functioning of CSCs. S-acylation is the addition of a fatty acid, usually stearate or palmitate, to a cysteine residue. We are able to demonstrate that CESA proteins are extensively modified by the addition of acyl groups to cysteines. We demonstrate that all 4 cysteines in the VR2 plus the 2 at the carboxyl terminus are acylated. If the CSC is composed of 18 CESA proteins and they are acylated at least 6 times on average, a single complex would contain up to 108 acyl groups and may also explain why the CSC aggregates and has proved so hard to purify. CESA7 mutants where acylation is completely abolished are still able to assemble with other CESA proteins and are trafficked around the cell, but they appear unable to integrate into the plasma membrane. We believe acylation has an essential role in locking the CSC into the plasma membrane and preventing it 'popping out' as the complex moves through the membrane. Plant cells are also faced with another logistical problem, as the plasma membrane is crowded with many other components. We propose a model whereby the properties conferred by the acylation of CESAs might contribute to partition CESA proteins within membrane domains and contribute to the co-alignment of CESAs and microtubules.

## P5.5 INTEGRATION OF HORMONAL AND TRANSCRIPTIONAL CONTROL DURING VASCULAR DEVELOPMENT

📅 MONDAY 4 JULY, 2016 ⌚ 13:55

👤 YRJÖ HELARIUTTA (UNIVERSITY OF CAMBRIDGE, UNITED KINGDOM)

@ YRJO.HELARIUTTA@SLCU.CAM.AC.UK

Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. During root primary development, xylem is specified early as an axis of vessel element cell files, whereas phloem is established through a set of asymmetric

cell divisions also contributing to the intervening procambial tissue (Mähönen et al. 2000 Genes Dev). Auxin and cytokinins interact to specify the xylem and phloem/procambial domains, respectively (Mähönen et al. 2006 Science; Bishopp et al. 2011 Curr Biol). Furthermore, auxin promotes expression of the class III HD-ZIP genes (whose expression domain is specified by the ground-tissue originating miR165/6) to promote xylem identity (Carlsbecker et al. 2010 Nature, Ursache et al. 2012 Development). By taking advantage of a tool with which we can control plasmodesmatal trafficking (Vaten et al. 2011 Developmental Cell), we identified a group of mobile transcription factors that mediate the specification of phloem/procambial domain downstream of cytokinins. Their interaction with the class III-ZIP genes to integrate the hormonal signaling is discussed.

## P5.6 KEY PLAYERS OF A SECONDARY MERISTEM IN *ARABIDOPSIS* - THE PLETHORA TRANSCRIPTION FACTORS

📅 MONDAY 4 JULY, 2016 ⌚ 14:35

👤 GUGAN ESWARAN (UNIVERSITY OF HELSINKI, FINLAND), ONDREJ SMETANA (UNIVERSITY OF HELSINKI, FINLAND), ARI PEKKA MÄHÖNEN (UNIVERSITY OF HELSINKI, FINLAND)

@ GUGAN.ESWARAN@HELSINKI.FI

The vascular cambium - a secondary meristem in plants produces secondary xylem (wood) and secondary phloem. Meristematic activity in vascular cambium ensures the production of phloem and xylem, which are essential for transportation of nutrients and water. Thus understanding the molecular mechanism behind the maintenance of meristematic state of vascular cambium and its development becomes essential. The PLETHORA (PLT) transcription factors are the central regulators of the primary meristems. Recent research works on PLT/AINTEGUMENTA (AIL) and/or AINTEGUMENTA (ANT) genes provide the insight of stem cell maintenance in plant primary meristems and their role in phyllotaxis and rhizotaxis. However, their functional role in a secondary meristem is largely unknown and needs to be elucidated. Therefore, we studied whether the PLT/AIL factors have a function also in the vascular cambium. In our work, we observed that several PLT/AIL family members are expressed in cambium, and when we generated mutant combinations from the cambium-expressed PLT/AILs, we found defects in vascular patterning and cambial cell maintenance in a few double and triple mutant combinations. In addition, overexpression of PLT/AILs inhibit the differentiation of the cambial cells. We aim to further specify the role of PLT/AIL in cambial maintenance, as well as how it is interacting with other known cambial regulators. Such knowledge will significantly contribute to the improvement of the quality, biomass of the forest industry and bioenergy.



## P5.7 THE CAMBIUM: HOW TO ORGANIZE A BIDIRECTIONAL PRODUCTION OF TISSUES?

📅 MONDAY 4 JULY, 2016 ⌚ 14:50

👤 THOMAS GREB (UNIVERSITY OF HEIDELBERG, GERMANY), IVAN LBOVKA (UNIVERSITY OF HEIDELBERG, GERMANY), NIAL GURSANSKY (GREGOR MENDEL INSTITUTE, AUSTRIA), VIRGINIE JOUANNET (UNIVERSITY OF HEIDELBERG, GERMANY)

@ THOMAS.GREB@COS.UNI-HEIDELBERG.DE

Lateral plant growth is mediated by the cambium, a stem cell niche continuously producing wood (xylem) and bast (phloem) in a strictly bifacial manner at the periphery of plant growth axes. Due to obstacles for live cell imaging, knowledge of cambium organization and dynamics is scarce. This is in spite of the fact that the cambium contributes a large part to land biomass and that it can serve as a paradigm for bifacial niche organization in multicellular organisms. Here, we present the identification of essential cambium subdomains with the help of a computational model describing cambium dynamics. The model is able to reproduce cambium anatomy in wild type and previously described mutants and highlights the importance of intercellular communication along the radial sequence of cambium domains. Our model predicts that only a limited number of factors are sufficient to create a stable system from which not all have been identified experimentally. In particular, we postulate a factor counteracting cell division and expansion. By investigating transcriptome remodeling in individual cambium domains upon manipulation of intra- and intercellular signaling we are in the process of characterizing the molecular basis of the spatio-temporal organization of the cambium.

## P5.8 MOLECULAR MECHANISMS OF PERIDERM DEVELOPMENT

📅 MONDAY 4 JULY, 2016 ⌚ 16:10

👤 LAURA RAGNI (ZMBP UNIVERSITY OF TÜBINGEN, GERMANY), ANNA WUNDERLING (ZMBP UNIVERISTY OF TÜBINGEN, GERMANY), DAGMAR RIPPER (ZMBP UNIVERISTY OF TÜBINGEN, GERMANY)

@ LAURA.RAGNI@ZMBP.UNI-TUEBINGEN.DE

During secondary growth, the periderm replaces the epidermis as a protective layer once the latter cannot longer accompany radial growth, acting as a physical barrier to protect the plant from the loss of water and difficult environmental conditions. These barrier properties are mainly conferred by suberin. A periderm is formed in stems, branches and roots of most dicotyledons and gymnosperms. The periderm is a three-tissue system, comprising the cork cambium/phellogen that produces outwards the cork/phellem and inwards the phellogen. The molecular mechanisms underlying periderm establishment and maintenance are largely unknown even in model organisms.

In *Arabidopsis*, the periderm occurs in the root and hypocotyl but is absent in the stem. We characterised periderm establishment at cellular levels using the 'quantitative histology approach'. Our analyses pointed out that periderm growth is tightly connected with the loosening of the 'outside tissues' (endodermis, cortex and epidermis), which follows a specific developmental pattern. As the

periderm arises from the pericycle, the same layer that originates lateral roots, we investigated whether lateral roots and periderm formation share a common regulatory network. However, the analyses of mutants lacking lateral roots and plants with impaired periderms suggested two independent mechanisms. In order to follow the dynamics of cork formation and phellogen activity live and to ultimately obtain the periderm transcriptome, we established fluorescence marker lines for those tissues. We are currently investigating the impact of an impaired periderm on plant growth and in response to abiotic stress.

## P5.9 ABSCISIC ACID STIMULATES XYLEM FIBRE PRODUCTION IN THE ARABIDOPSIS HYPOCOTYL

📅 MONDAY 4 JULY, 2016 ⌚ 16:40

👤 LIAM CAMPBELL (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), SIMON TURNER (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

@ LIAM.CAMPBELL@POSTGRAD.MANCHESTER.AC.UK

The *Arabidopsis* hypocotyl has two phases of secondary growth, separated at the onset of flowering. The second phase is known as 'xylem expansion' due to the increased rate of xylem production relative to phloem. It is during this phase that xylary fibres begin to differentiate as providers of structural support, producing vascular tissue remarkably similar to the wood of trees. Abscisic acid (ABA) is a phytohormone known to have a major role in various plant developmental processes, particularly in the response to abiotic stresses and in the promotion of seed dormancy. Through reverse genetic screens based upon microarray data, we have identified that ABA promotes the onset of fibre differentiation in the hypocotyl. We found that T-DNA insertional mutagenesis of ABA-biosynthesis enzymes inhibits fibre production without affecting the xylem:phloem ratio. This is reflected at the transcript level, with a reduced expression of fibre formation marker genes in the *aba1* mutant. Furthermore, the application of exogenous ABA to the mutant rescues the phenotype, restoring fibre differentiation to wild-type levels. We are currently attempting to identify some downstream components of this pathway.

## P5.10 MOLECULAR CONTROL OF WOOD FORMATION IN HYBRID ASPEN

📅 MONDAY 4 JULY, 2016 ⌚ 16:55

👤 RISHIKESH BHALERAO (UMEÅ PLANT SCIENCE CENTRE, SWEDEN)

@ RISHI.BHALERAO@SLU.SE

The cambial meristem gives rise to secondary xylem and phloem. The secondary xylem undergoes a sequential developmental program involving cell division, cell expansion, secondary cell wall formation and cell death. The plant hormone indole-acetic acid (auxin) plays a key role in controlling diverse aspects of secondary xylem development. For example, auxin distribution influences the secondary xylem development. Moreover, the seasonal control of cambial activity is mediated by auxin in response to change in day length. I will discuss our results which provide insight into the auxin mediated control of cambial activity and secondary xylem

development. I will also discuss transcriptional network analysis of gene expression in wood forming tissues in hybrid aspen and its use for identifying genes controlling various aspects of wood formation.

## P5.11 TRANSCRIPTIONAL REGULATION OF XYLEM DEVELOPMENT

TUESDAY 5 JULY, 2016 10:30

SIOBHAN BRADY (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), KAISA KAJALA (UC DAVIS, UNITED STATES), GINA TURCO (UC DAVIS, UNITED STATES), PETER ETHELLS (DURHAM UNIVERSITY, UNITED KINGDOM)

SBRADY@UCDAVIS.EDU

Roots are the 'hidden half' of a plant, yet, without them, plants would not grow. In particular, xylem cells in the root are the starting point for providing water, mineral nutrients and mechanical support to the above ground part of the plant, and are also necessary for human health through the supply of vitamins and fiber. My research group determines the underlying molecular mechanisms that provide the instructions and signals to organize root xylem cell development using genomic and systems biology approaches. We have mapped a root xylem cell transcriptional regulatory network that contains an over-representation of feed forward loops. In order to determine the dynamic behavior of these feed forward loops we have generated tools to perturb transcription factors in six feed forward loops critical for xylem cell development. These perturbation experiments have demonstrated complex, non-linear regulatory behavior. In addition, we will describe efforts to map differences and similarities in regulation of xylem cell development in Sorghum relative to Arabidopsis and maize and the potential importance of DNA methylation in this process.

## P5.12 THE ROLE OF EPIGENETIC REGULATION IN XYLEM VESSEL DIFFERENTIATION

TUESDAY 5 JULY, 2016 11:10

HITOSHI ENDO (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), MISATO OHTANI (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), SIOBHAN M. BRADY (UNIVERSITY OF CALIFORNIA, UNITED STATES), TAKU DEMURA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN)

H-ENDO@BS.NAIST.JP

Xylem vessels are critical conductive tissues that transport water and minerals throughout the body in vascular plants. We identified the master transcriptional switch for the formation of xylem vessels, VASCULAR-RELATED NAC-DOMAIN7 (VND7), which is a member of plant-specific NAC-domain transcription factors. Extensive works on VND7-based transcriptional networks and the downstream genes of VND7 have revealed the details of molecular system of xylem vessel cell differentiation. However, it has yet to be shown how the expression of VND7 is spatio-temporally regulated during plant development. Therefore, we aimed to address the question of how the expression of VND7, a master transcriptional switch for the formation of xylem vessels, is spatio-temporally regulated during plant development. Firstly, to decipher the regulatory mechanism

underlying VND7 expression, we revealed the transcription factors that act upstream of VND7 and succeeded in identifying several potential transcription factors that positively regulate the expression of VND7. However, this study also revealed another unknown potential mechanism that contributes to the tight control of VND7 expression in planta. To explore this possible mechanism, we secondly focused on the epigenetic control of the expression of VND genes as epigenetic control is a well-studied mechanism for the tight regulation of certain genes in plants and animals. We found that the VND7 locus is marked by at least two epigenetic marks, histone H3 lysine 27 try-methylation (H3K27me3) and DNA methylation. In particular, both epigenetic marks seem to repress VND7 expression.

## P5.13 PHYTOZOME-WIDE SCREENING OF CLAVATA3/ESR-RELATED (CLE) PEPTIDES

TUESDAY 5 JULY, 2016 11:25

SOPHIE MOGG (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), SIMON TURNER (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

SOPHIE.MOGG@POSTGRAD.MANCHESTER.AC.UK

CLAVATA3/ESR-related (CLE) peptides are known to be involved with numerous developmental processes including xylem differentiation. While many CLEs act on the shoot and root apical meristems, a subset of CLE peptides have been shown to act within the cambium, negatively regulating the development of xylem tissue and promoting cambial cell divisions. While it has been shown that the ectopic over-expression of CLE41 results in the loss of cell division orientation causing the intercalation of the xylem and phloem tissues, loss of function mutant yield no observable phenotype probably as a result of redundancy within the CLE peptide family. Recent studies in pines and legume species have revealed greater CLE gene diversity than previously thought. Therefore, with the use of bioinformatics tools such as BLAST and MEME-FIMO a search for putative peptides has been conducted using all plant species available in phytozome. MEME has also been used to identify motifs within the full length proteins. This approach has yielded a comprehensive classification of CLE genes and revealed large variation on the organisation on CLE peptides within a single gene. This approach has also identified novel CLE genes even in many species including Arabidopsis and includes potential candidate for further investigation in the regulation of xylem development.

## P5.14 FROM MOLECULES TO TISSUE: ORGANISATION OF ROOT VASCULAR PATTERN

📅 TUESDAY 5 JULY, 2016 ⌚ 11:40

👤 ANTHONY BISHOPP (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), NATHAN MELLOR (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), BRITTA KÜMPERS (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), BEN GOODALL (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM)

@ ANTHONY.BISHOPP@NOTTINGHAM.AC.UK

A central question in developmental biology is how multicellular organisms are patterned, so that a seemingly homogenous initial population of cells can give rise to the complex patterns of differentiated cells observed. New organs can be patterned either de-novo through symmetry breaking or by propagation of existing patterns in other tissues.

In my group we use the patterning of root vascular tissues (xylem and phloem) as a model for pattern formation in plants. The xylem and phloem form a vital transport network and it is essential that these cells differentiate in the correct position to form a single continuous network connecting organs. In this talk I will discuss our experimental research and modeling approaches to investigate the genetic network controlling vascular patterning. I will also discuss whether this network is sufficient to generate pattern de-novo or whether other inputs are required.

## P5.15 PREDICTIVE MODELLING OF HORMONE SIGNALLING SYSTEMS IN THE ARABIDOPSIS ROOT

📅 TUESDAY 5 JULY, 2016 ⌚ 12:10

👤 KEITH LINDSEY (DURHAM UNIVERSITY, UNITED KINGDOM), SIMON MOORE (DURHAM UNIVERSITY, UNITED KINGDOM), JUNLI LIU (DURHAM UNIVERSITY, UNITED KINGDOM)

@ KEITH.LINDSEY@DURHAM.AC.UK

The Arabidopsis root represents an excellent model to study the relationship between meristem identity, meristem function and the transition to differentiation, because there is a clear spatial pattern that represents the events in a temporal pattern. The identity of stem cells within the meristem is regulated by signalling from the quiescent centre (QC), a group of cells originally identified by their low rates of division. The stem cells (initials) that surround the QC in turn divide to give rise to the files of cells, both proximal and distal to the meristem, that differentiate into all cells of the root. The control of identity and activity of the meristem, and the transition to differentiation, is regulated by an interacting network of genes and hormonal signalling systems. Key players include the hormones auxin, cytokinin and ethylene, the relative distribution and activities of which pattern the expression of regulatory genes. To understand better the nature and behaviour of this complex network, we have developed computational models that simulate the relationships between these hormones and predicts the level and patterning of hormones and gene expression, both in a virtual single cell and, more recently, in a more realistic 2-dimensional root model. We will discuss recent results that inform our understanding of the interactions between auxin transporter function (influx and efflux), and the link with other hormonal and gene components, to regulate patterning in the Arabidopsis root.



## P5.16 A NETWORK ANALYSIS OF THE ROLE OF PXY SIGNALLING IN VASCULAR DEVELOPMENT

📅 TUESDAY 5 JULY, 2016 ⌚ 12:25

👤 PETER J ETHELLES (DURHAM UNIVERSITY, UNITED KINGDOM), MARGOT E SMIT (WAGENINGEN UNIVERSITY, NETHERLANDS), SIOBHAN M BRADY (UC DAVIS, UNITED STATES)

@ PETER.ETHELLES@DURHAM.AC.UK

In plant vasculature tissue, xylem and phloem, specialist tissues for transport of water and nutrients, differentiate from initials derived from divisions in the vascular meristem known as the cambium. encodes a phloem-expressed peptide ligand that signals to a cambium-expressed receptor, PXY. This signalling pathway has complex outputs and is thought to influence vascular organisation, the rate of cell division, and repression of xylem differentiation.

In order to understand how PXY signalling is integrated with other factors controlling vascular development, we combined a transcriptomic approach to identify novel genes that act downstream of PXY signalling with Yeast One Hybrid, to identify both putative direct regulatory connections, and upstream regulators of PXY signalling components. The complete network contains a total of 312 genes present in 690 binding interactions. Hypotheses arising from this analysis suggest that PXY may coordinate vascular growth and development by influencing expression of transcription factors that also targets of the ARF transcription factor MONOPTEROS.

## P5.17 DISTURBANCE OF CORTICAL MICROTUBULE ORIENTATION BY SULFAMETHIZOLE LEADS TO ABNORMAL SECONDARY CELL WALL PATTERNING DURING XYLEM VESSEL CELL DIFFERENTIATION

📅 TUESDAY 5 JULY, 2016 POSTER SESSION

👤 ERI KAMON (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), ARATA YONEDA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), MISATO OHTANI (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN), TAKU DEMURA (NARA INSTITUTE OF SCIENCE AND TECHNOLOGY, JAPAN)

@ E-KAMON@BS.NAIST.JP

Xylem vessel cells have secondary cell walls (SCWs) with specific ordered patterns, such as the spiral pattern found in protoxylem vessel cells, and reticulate and pitted patterns in metaxylem vessel cells. It has been shown that cortical microtubule (CMT) arrays principally determine the SCW patterns, through controlling the trajectory of cellulose synthase complexes (CSCs). Recently several key linker proteins between CMT and CSCs were reported, however, the molecular mechanism of SCW patterning by CMT is not fully understood, especially for protoxylem vessel cells. VASCULER-RELATED NAC-DOMAIN 7 (VND7) acts as a master regulator of xylem vessel cell differentiation in *Arabidopsis thaliana*. In this study, we utilized VND7 inducible tobacco BY-2 cells, in which most cells become transdifferentiated into protoxylem-like cells with the spiral SCWs. By screening a chemical library, we have isolated the unique compound, sulfamethizole (SMZ), as the chemical that disturbs SCW patterns. SMZ caused the prominent branching and curling of CMT patterns, and the SCW deposition was followed these CMT alignments, indicating that SMZ didn't affect the interaction between CMT and SCW deposition. The analysis on CMT revealed that SMZ altered microtubule dynamics, but not the depolymerization of CMT. The *Arabidopsis* mutant with decreased microtubule dynamics showed similar abnormal patterns of SCW to the SMZ-treated VND7 inducible BY-2 cells, thus, the microtubule dynamics is one of important factors for the determination of SCW patterns during xylem vessel cell differentiation. Based on these results, we will discuss the relationship between SCW patterning and microtubule dynamics.