



# New Phytologist

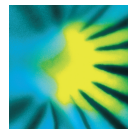
## next generation scientists

29–30 July 2014

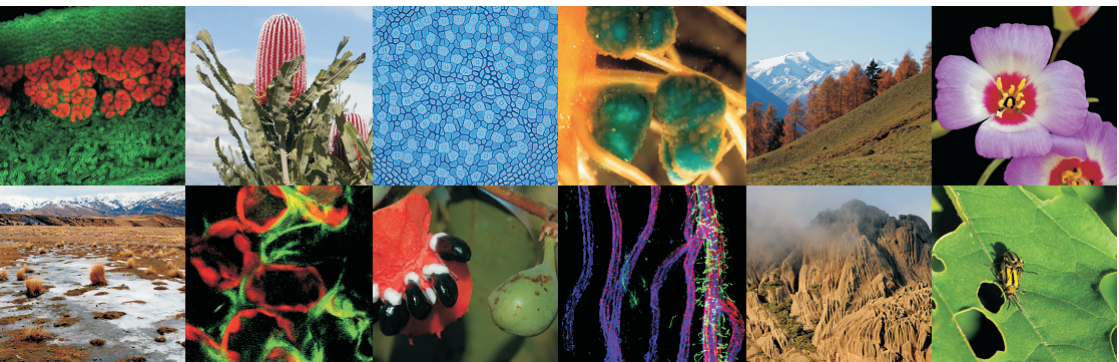
John Innes Centre, Norwich, UK

**Programme, abstracts and participants**

**WILEY**



New  
Phytologist



# **New Phytologist**

## **next generation scientists**

John Innes Centre, Norwich, UK

29–30 July 2014

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Programme, abstracts and participant list compiled by Jill Brooke  
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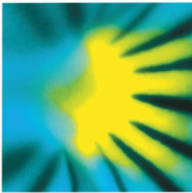
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## Acknowledgements

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### New Phytologist Trust

The New Phytologist Trust is a non-profit-making organization dedicated to the promotion of plant science. The Trust supports a number of projects, including the publication of the world-leading journal *New Phytologist*, and ensuring free access for our Tansley reviews. In addition, the Trust funds a number of other initiatives, including scientific prizes, workshops and symposia. Further information is available at <http://www.newphytologist.org/>.



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# Information for delegates

## Location

'New Phytologist next generation scientists' will take place at the John Innes Conference Centre, Norwich Research Park, Norwich, UK. It is situated opposite the main buildings of the John Innes Centre. Oral presentations will take place in the main auditorium. Posters will be displayed in the foyer on the ground floor.

## Travel directions

The John Innes Conference Centre (JIC) is a 10–15 minute walk from the University of East Anglia campus. Walk to the end of Chancellor's Drive on the UEA campus and follow the path across the field which leads to Colney Lane. Cross the road and turn right onto Colney Lane. Walk for a few minutes past the rear of JIC and take a left at the traffic lights. This road leads to the main entrance of JIC.

Full travel information can be found on the website:

<http://www.newphytologist.org/nextgensci>.

## Catering and social event

**Coffee breaks** will take place in the foyer at set times as indicated in the programme.

**Lunch** on the 29<sup>th</sup> July will be available from 12:00 to 13:25 during registration. Food is not permitted in the auditorium so please arrive early if you intend to eat before the start of the meeting at 13:25. A buffet lunch will be served at the close of the meeting from 13:30 to 14:30 on the 30<sup>th</sup> July

### **Social event:**

- A food van serving tacos will be set up outside the venue from 18:45 to 22:00. Each delegate will be provided with a coupon which can be exchanged for a meal of two tacos and a side dish. Additional food can be purchased with cash.
- An ice cream stall will be set-up outside from 20:00 to 22:00 and each delegate will receive a coupon which can be exchanged for one ice cream.
- A bar located next door to the conference centre will be open from 19:00 to 23:00. Delegates will be provided with two drink coupons which can be exchanged for (alcoholic and soft) drinks at the bar. Additional drinks can be purchased with cash.

Drink and food coupons can be found inside the delegate badges.

*Vegetarian, wheat- and gluten-free options will be available for all meals.*

## Accommodation

Accommodation for delegates will be provided at the University of East Anglia (UEA) student residences. Check-in is from 14:00 to 21:00 at the Broadview Lodge Reception or at the Security Lodge from 21:00 onwards (see UEA campus map at the end of this book). Breakfast is included and is served from 08:00–10:00. You must check-out by 10:00 on the morning of Wednesday 30<sup>th</sup> July. There will be space allocated in the conference centre where you can leave your luggage until departure. Complimentary wifi internet is available throughout UEA by connecting and registering with 'The Cloud'. There are a variety of outlets on the UEA campus that provide hot drinks and food, as well as a pub, a shop and a bank.

## Posters

Posters should be prepared so that they are no larger than A0 size, portrait orientation (118 cm high x 84 cm wide). Posters should be put up during registration (12:00–13:25 on 29<sup>th</sup> July) and will be displayed for the duration of the meeting. There will be a dedicated poster sessions at 18:40–20:10 on 29<sup>th</sup> July. We ask that poster presenters with an odd number (1, 3, 5, 7, etc.) stand by their posters from 18:40–19:25, and poster presenters with an even number (2, 4, 6, 8, etc.) should stand by their posters from 19:25–20:10. The poster hall will remain open until late on 29<sup>th</sup> July and we encourage you to return to the posters during the social event. Wine, fruit juice and snacks will be served throughout the poster session.

**Prizes:** Posters will be assessed by your peers (the other delegates) and the posters that gain the most votes will receive prizes. A scoring sheet is included in your delegate pack. Please fill out and return this sheet to one of the organisers or to the registration desk by 21:00 on Tuesday 29<sup>th</sup> July.

**Abstracts:** Poster abstracts will not be printed. Abstracts are included in the digital version of the abstract book which can be found on the USB sticks in the delegate packs or online (<http://www.newphytologist.org/nextgensci/abstracts>).

## Internet Access

Free wireless internet will be available throughout the conference centre. Login details will be provided at registration.

## Social Media

We encourage all attendees to join in discussions on social media sites. Follow @NewPhyt on Twitter and fb.com/NewPhytologist on Facebook for updates during and after the meeting. The 'New Phytologist next generation scientists' Facebook group can be found at <http://www.newphytologist.org/nextgensci/facebook>.

**Contact**

For further information, and in case of any emergencies, please contact Michael Panagopoulos. Email: [m.panagopoulos@lancaster.ac.uk](mailto:m.panagopoulos@lancaster.ac.uk), [np-symposia@lancaster.ac.uk](mailto:np-symposia@lancaster.ac.uk); tel: +44 7966 984 319.

# Meeting programme

## Tuesday 29<sup>th</sup> July

12:00–13:25	Lunch and registration	
13:25–13:40	Welcome	
13:40–14:30	Charles Godfray <i>Plenary lecture</i>	What do we mean when we talk about sustainable intensification?
14:30–14:50	Yogesh Gupta <i>Selected poster talk</i>	<b>P30:</b> The exocyst complex is required for appressorium-mediated tissue invasion by rice blast fungus, <i>Magnaporthe oryzae</i>
14:50–15:10	Aleksandr Gavrin <i>Selected poster talk</i>	<b>P24:</b> Symbiosome membrane is a crossroad of exo/endocytic pathways in infected cells of legume root nodules
15:10–15:30	<i>Break</i>	
15:30–15:50	Jing-Ke Weng <i>Tansley Medal 2013 winner</i>	The evolutionary implication of messiness in biology
15:50–16:10	Emily Breeze <i>Selected poster talk</i>	<b>P11:</b> Action of the NF-Y transcription factors in plant stress responses
16:10–16:30	Katie Becklin <i>Selected poster talk</i>	<b>P7:</b> Evolutionary history underlies plant physiological responses to global change since the Last Glacial Maximum
16:30–16:50	Kevin Dorn <i>Selected poster talk</i>	<b>P19:</b> Genomics and domestication of field pennycress ( <i>Thlaspi arvense</i> L.)
16:50–17:10	<i>Break</i>	
17:10–17:30	Marjorie Lundgren <i>Selected poster talk</i>	<b>P51:</b> An intraspecific gradient from C <sub>3</sub> to C <sub>4</sub> photosynthesis
17:30–17:50	Yoan Coudert <i>Selected poster talk</i>	<b>P16:</b> Molecular evidence for an indeterminate meristem precursor in a moss
17:50–18:40	Kirsten Bomblies <i>Plenary lecture</i>	Meiotic adaptation to whole genome duplication

18:40–20:10      *Poster session and reception*

20:10–      *Social event*

## Wednesday 30<sup>th</sup> July

08:55–09:00      Announcements

09:00–09:50	Philip Benfey <i>Plenary lecture</i>	Development rooted in regulatory networks
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09:50–10:10	Li-Qing Chen <i>Tansley Medal 2013 winner</i>	SWEET proteins are key for phloem loading and seed filling
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10:10–10:30	João Raimundo <i>Selected poster talk</i>	<b>P64:</b> A genetic triangle: how three MYB proteins determine flower asymmetry in <i>Antirrhinum</i>
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10:30–10:50	Sarah Jose <i>Selected poster talk</i>	<b>P36:</b> Waterproof plants: linking leaf wax and stomatal pore development in barley
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10:50–11:20      *Break*

11:20–11:40	Philippa Borrill <i>Selected poster talk</i>	<b>P10:</b> Identifying downstream targets of the wheat transcription factor <i>NAM-B1</i> : ChIP-seq in hexaploid wheat using custom-built reference pseudomolecules
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11:40–12:30	Workshop: How to get your work published	Co-Chairs: Professor Alistair Hetherington, Editor-in-Chief, <i>New Phytologist</i> and Dr Christopher Surridge, Chief Editor, <i>Nature Plants</i>
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12:30–13:20	Sarah O'Connor <i>Plenary lecture</i>	Understanding and engineering indole alkaloid biosynthesis
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13:20–13:30      Closing remarks

13:30–14:30      *Lunch*

14:30–15:30      *(Optional)* Tour of John Innes Centre

# Speaker abstracts

*Tuesday 29<sup>th</sup> July*

**What do we mean when we talk about sustainable intensification?**

*Plenary lecture*

**CHARLES GODFRAY**

**13:40–14:30**

*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK*

Sustainable intensification is now a ‘buzz phrase’ being used to support a wide variety of different farming practices and research priorities. But it also arouses strong opposition, especially amongst many NGOs who see it as a license to harm the environment or reduce standards of animal welfare. I will explore the arguments that led to the notion of sustainable intensification, in particular that at least part of our response to the challenge of global food security should be the capacity to increase food supply to meet increased demand, and second that this new food must be produced on our existing agricultural footprint. I will also describe what sustainable intensification might mean on the ground to farmers and in how we set research priorities. The arguments against sustainable intensification will then be examined, and I will argue that some involve framing and semantics (which is not to dismiss them – words do matter if one is seeking to change behaviour) but that others require ideas about sustainable intensification to be much better integrated with other food system priorities – landscape management, animal welfare, biodiversity, sustainable development and sustainable diets.

**P30: The exocyst complex is required for appressorium-mediated tissue invasion by rice blast fungus, *Magnaporthe oryzae***

*Selected poster talk*

**YOGESH K. GUPTA<sup>1</sup>, Y. F. DAGDAS<sup>1</sup>, G. R. LITTLEJOHN<sup>1</sup>, A. L. MARTINEZ-ROCHA<sup>1</sup>, M. C. GIRALDO<sup>2</sup>, B. VALENT<sup>2</sup>, N. J. TALBOT<sup>1</sup>**

**14:30–14:50**

<sup>1</sup>*School of Biosciences, University of Exeter, EX4 4QD, UK;* <sup>2</sup>*Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA*

*Magnaporthe oryzae* is one of the most serious fungal pathogens of cultivated rice. *M. oryzae* forms a single-celled infection structure called an appressorium which breaches the leaf cuticle and thereby invades host cells. During host colonization, the fungus secretes effector proteins via a mechanism that is not well defined in *M. oryzae*. We characterized the exocyst complex, an evolutionarily conserved octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84), which plays a crucial role in vesicle tethering to the plasma membrane. Like other fungi, *M. oryzae* exocyst components localize to the vegetative hyphal tip and during infection-related development they are found around the appressorium pore, implicating it as a site of protein secretion during infection. We generated a temperature-sensitive mutant of Sec6, which completely disrupts exocyst assembly at the appressorium pore and impairs in effector secretion. Recently, we have shown that Exo70 and Sec5 are involved in secretion of cytoplasmic (host cell-delivered) effectors, but not apoplastic effectors. Targeted gene deletion of exocyst components Exo70 and Sec5 and the temperature sensitive mutation of Sec6 also cause significant loss of virulence. Furthermore, we found that the localization of the exocyst at the appressorium pore is septin-dependent.

**P24: Symbiosome membrane is a crossroad of  
exo/endocytic pathways in infected cells of  
legume root nodules**

*Selected  
poster talk*

**ALEKSANDR GAVRIN, B. KAISER, V. CLARKE, R. OVERALL,  
P. M. C. SMITH, T. BISSELING, E. FEDOROVA**

**14:50–15:10**

*School of Biological Sciences, The University of Sydney, NSW2006, Australia;  
School of Agriculture Food and Wine, The University of Adelaide, SA5084,  
Australia; Molecular Biology Department, Wageningen University,  
Droevendaalsesteeg 1, 6708PB, The Netherlands*

In *Rhizobium*–legume symbiosis, the bacteria are enclosed in the specialized plant-derived membrane, the so called symbiosome membrane (SM). The SM is the interface between symbiotes, which facilitates the exchange of nutrients and solutes. Despite its key importance, the molecular mechanisms of SM formation are largely unknown. Cytological analysis revealed great alterations of the endomembrane system in symbiotic cells. To shed light on the mechanisms of them we focused on the key endosomal identity markers, and participators of membrane fusion machinery of the plant cell: HOPS complex, small GTPases and SNARE proteins during symbiosis development. Our results show that the Vamp72-controlled exocytosis pathway regulates the release of bacteria into the plant cell by the reorganization of the cell wall materials. Further development of symbiosis occurs by the suppression of the HOPS complex and as a consequence by the collapse and defunctionalization of vacuoles in the symbiotic cells. Simultaneous retargeting of tonoplast proteins to the SM is essential for the functional maturation of symbiotic cells. Proteomic analysis of the SM confirmed its mosaic composition. The SM combines plasma membrane and tonoplast proteins and thereby appears as a crossroad of exo/endocytic pathways in infected cells of legume root nodules.

## **The evolutionary implication of messiness in biology**

*Tansley Medal  
2013 Winner*

**JING-KE WENG**

**15:30–15:50**

*Whitehead Institute for Biomedical Research and Department of Biology,  
Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA  
02142, USA*

Metabolic pathways are often considered ‘perfected’ or at least predictable as substrates efficiently rearrange into products through the intervention of an optimized enzyme. Moreover, single catalytic steps link up, forming a myriad of metabolic circuits that are often modeled with a high degree of certainty. However, on closer examination, most enzymes are not precise with respect to their activity, using not just one substrate but often a variety and producing not just one product but a diversity. Hence, the metabolic systems assembled from enzymes possessing varying degrees of what can be termed catalytic promiscuity are not clear-cut and restrictive; rather, they may at times operate stochastically in the intracellular milieu. This ‘messiness’ complicates our understanding of normal and aberrant cellular behavior, while paradoxically sowing the seeds for future advantageous metabolic adaptations for host organisms. Orthologous enzymes refer to enzymes descended from the same ancestral enzyme separated by speciation events. Mutations, which have resulted in sequence divergence among orthologous enzymes, are often thought to be neutral, because they do not alter the presumably conserved function in orthologous enzymes. Using hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT) as a working system, we observed that although orthologous HCTs from different plant lineages contain the conserved HCT function, they vary drastically in their catalytic promiscuity landscape towards non-native substrates. We propose such difference predefines the evolvability of individual orthologous enzymes, and might have shaped the evolutionary trajectories of a number of HCT-like enzymes historically derived from HCTs through gene duplication followed by neofunctionalization.

**P11: Action of the NF-Y transcription factors in plant stress responses**

*Selected poster talk*

**EMILY BREEZE, C. HILL, J. PRUSINSKA, V. BUCHANAN-WOLLASTON, K. DENBY**

**15:50–16:10**

*Warwick Systems Biology, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK*

Environmental stresses, such as drought, high salinity and pathogen attack, cause significant crop losses worldwide. Consequently, plants have evolved complex and highly regulated stress response mechanisms. Although there are certainly stimuli-specific pathways, many genes appear to be induced by multiple stresses supporting the existence of a common ‘core stress response’ regulatory network. The NF-Y transcription factor (TF) family are likely key regulators in multiple stress responses. NF-Y TFs function as a heterotrimeric complex consisting of NF-YA, NF-YB and NF-YC subunits, which, in *Arabidopsis*, are encoded by multigene families that could theoretically combine to form 1000 unique TFs. This combinatorial diversity could enable fine-tuning of transcriptional regulation by activating specific groups of stress-responsive genes. This project aims to identify functional NF-Y complexes involved in regulating plant stress responses, and to elucidate their downstream targets and upstream regulators. Network inference, together with yeast-1 and 2-hybrid assays and microarray analysis of altered expression mutants, has enabled the generation of robust small-scale networks centred around a subset of key regulatory NF-Y subunits. Current work is focussed on the determination of a complete *Arabidopsis* NF-Y TF complex involved in the regulation of stress-induced jasmonate biosynthesis.

**P7: Evolutionary history underlies plant physiological responses to global change since the Last Glacial Maximum**

*Selected poster talk*

**KATIE M. BECKLIN, J. S. MEDEIROS, K. R. SALE, J. K. WARD**

**16:10–16:30**

*Department of Ecology and Evolutionary Biology, University of Kansas, 8028 Haworth Hall, 1200 Sunnyside Avenue, Lawrence, KS 66045, USA*

Assessing family and species-level variation in physiological responses to global change across geologic time is critical for understanding factors that underlie changes in species distributions and community composition. Ancient plant specimens preserved within packrat middens are invaluable in this context since they allow for comparisons between co-occurring plant lineages. Here we used modern and ancient plants preserved within packrat middens from the Snake Range, NV to investigate plant physiological responses to global change since the last glacial maximum (LGM). We used a conceptual model to infer relative changes in stomatal conductance ( $g_s$ ) and maximum photosynthetic capacity ( $A_{max}$ ) from measures of leaf carbon isotope, stomatal characteristics, and leaf %N. Our results indicate that most of the sampled taxa decreased  $g_s$  and/or  $A_{max}$  from glacial to modern times. However, plant families differed in the timing and magnitude of these physiological responses. Additionally, leaf-level responses were more similar within plant families than within co-occurring species assemblages. This suggests that adaptation at the level of leaf physiology may not be the main determinant of shifts in community composition, and that plant evolutionary history may drive physiological adaptation to global change over recent geologic time.

**P19: Genomics and domestication of field pennycress (*Thlaspi arvense* L.)**

*Selected poster talk*

**KEVIN M. DORN<sup>1</sup>, D. L. WYSE<sup>2</sup>, M. D. MARKS<sup>1</sup>**

**16:30–16:50**

<sup>1</sup>*Department of Plant Biology, University of Minnesota, 250 Biological Sciences Center, 1445 Gortner Avenue, St. Paul, MN 55108 USA;* <sup>2</sup>*Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA*

Large portions of the Midwestern US lack any living plant cover from the time of corn harvest in the fall until soybean and corn develop and establish a canopy cover the following June. The lack of plant cover leaves soil vulnerable to erosion and nutrient runoff, significantly impacting the health of surface waters. Planting winter cover crops has been shown to protect soil and water health, as well as limiting the growth of spring weeds. We are developing field pennycress as a fall planted, winter annual oilseed crop that can be harvested in the early spring and serve as a biodiesel feedstock.

There have been limited efforts to improve the agronomic qualities of pennycress, but we are applying modern genomic technologies to enable the rapid domestication of this species. We have configured and built a personal computer for c. \$2000 US able to perform *de novo* assembly and annotation of the pennycress transcriptome and genome from next generation sequencing data. From this work, we have identified candidate genes responsible for controlling key traits like seed dormancy and flowering time, which will guide future improvement efforts. The generation of these genomic resources will provide an unprecedented tool for the domestication of pennycress.

**P51: An intraspecific gradient from C<sub>3</sub> to C<sub>4</sub> photosynthesis**

*Selected  
poster talk*

**MARJORIE R. LUNDGREN, P. A. CHRISTIN, C. P. OSBORNE**

**17:10–17:30**

*Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK*

During the diversification of flowering plants, C<sub>4</sub> photosynthesis evolved independently from C<sub>3</sub> ancestors over 60 times in distantly related taxa, but the details of the changes responsible for C<sub>4</sub> evolution remain poorly understood. Here, we analyze the variation in photosynthetic types that exists within a single species, the grass *Alloteropsis semialata*. We characterize the distribution of <sup>13</sup>C and leaf anatomy across the species' range in a spatio-ecological context and build a phylogeographic hypothesis based on genetic markers. The <sup>13</sup>C values present a continuous distribution that suggests the presence of C<sub>3</sub>–C<sub>4</sub> intermediate physiology. Individuals at the C<sub>3</sub> and C<sub>4</sub> ends of the <sup>13</sup>C spectrum inhabit different environments, however, there is a region of overlap in eastern Africa – the region where C<sub>3</sub>–C<sub>4</sub> intermediates also occur. Some anatomical traits shift abruptly across the <sup>13</sup>C spectrum, while others form a continuum. The environmental drivers behind the variation in these traits and environmental inducibility of each C<sub>4</sub> anatomical trait are presented. Thus, anatomical and physiological continua are discussed in light of the species' phylogeography and phenotypic plasticity for C<sub>4</sub> anatomy, shedding new light on C<sub>4</sub> evolution among populations of the same species.

**P16: Molecular evidence for an indeterminate meristem precursor in a moss**

*Selected poster talk*

**YOAN COUDERT, C. J. HARRISON**

**17:30–17:50**

*Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK*

The shape of animals is determined during embryogenesis; conversely, plants develop mainly postembryonically. They have the ability to produce new organs throughout their life from meristems, populations of stem cells located at their shoot tips. As a result, the shape of most extant plants is indeterminate. Only a few extant plant groups, such as mosses, display ancestral features and have determinate shoots. The evolution of indeterminacy from a determinate ancestor was a prerequisite to the diversification of plant forms, however, the molecular mechanisms underpinning this transition are unknown. A KNOX–cytokinin regulatory loop has well characterised roles in promoting meristem indeterminacy in flowering plants. To investigate the ancestral role of this molecular module, we examined its conservation in a determinate moss shoot and show that the moss KNOX protein MKN2, acting via cytokinin biosynthesis, is necessary and sufficient to promote proliferation in a non-apical zone. We propose that this non-apical proliferative zone represents a progenitor to the evolution of an indeterminate apical meristem.

## Meiotic adaptation to whole genome duplication

Plenary lecture

KIRSTEN BOMBLIES, L. YANT, J. HOLLISTER, K. WRIGHT,  
K. XUE

17:50–18:40

*Harvard University, Cambridge, MA, USA*

Whole genome duplication (WGD) has important implications for adaptation, speciation, and crop improvement. However, doubling the number of homologs poses serious challenges to reliable chromosome segregation in meiosis. Newly formed polyploids commonly show meiotic defects such as multivalent associations among available homologs associated with reduced fertility. Nevertheless, many genome-duplicated (polyploid) species persist in nature; most have stable diploid-like chromosome segregation, indicating early problems can be overcome. We use *Arabidopsis arenosa*, an outcrossing relative of *A. thaliana* with extant diploid and autotetraploids, to understand the genic basis of adaptation to polyploidy. Autotetraploid *A. arenosa* has cytologically diploidized meiosis, while newly formed tetraploids have numerous multivalents in diakinesis and metaphase I. Reduction in multivalents in the established polyploid is associated with reduced crossover number per chromosome. We show from whole genome resequencing that there is strong ploidy-associated allelic differentiation in 39 loci with sharply elevated differentiation. Among these are eight meiosis genes whose products are known to function together to coordinate chromosome pairing, synapsis, and the number and distribution of chiasmata. We show evidence for one gene that the derived allele has a strong functional consequence in polyploid meiosis. We hypothesize that these genes together represent a co-evolved polygenic solution to WGD-associated chromosome segregation challenges, likely through increased strength of crossover interference.

**Wednesday 30<sup>th</sup> July**

**Development rooted in regulatory networks**      *Plenary lecture*

**PHILIP N. BENFEY**

**09:00–09:50**

*Biology Department and HHMI, Duke University, Durham, NC, USA*

To understand the progression from stem cells to differentiated tissues we are exploiting the simplifying aspects of root development. We have profiled mRNA, small RNAs, alternative splicing and DNA methylation at cell-type specific resolution within the Arabidopsis root. We are developing new experimental, analytical and imaging methods to identify networks functioning within different cell types and developmental stages. We are particularly interested in a subnetwork that regulates a key asymmetric cell division of a stem cell and the regulatory networks that control differentiation of the stem cell's progeny. We have uncovered a clock-like process responsible for the positioning of lateral roots along the root primary axis. Two sets of genes were identified that oscillate in opposite phases at the root tip and are involved in the production of prebranch sites, locations of future lateral roots. Finally, we are analyzing the dynamics of growth of physical root networks using novel non-invasive imaging methods with the goal of identifying the genes regulating root system architecture.

This work is supported by grants from the NIH, NSF, DARPA and the Gordon and Betty Moore Foundation.

**SWEET proteins are key for phloem loading and seed filling** *Tansley Medal  
2013 Winner*

**LI-QING CHEN, X-Q. QU, I. W. LIN, D. SOSSO, W. B. FROMMER**

**09:50–10:10**

*Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA*

Cellular sugar efflux is a critical step required for carbon allocation in multicellular organisms. Specifically, cellular sucrose efflux is required as the first step of phloem loading in photosynthetic organs, for seed filling, for nectar secretion and for pathogen nutrition access. Here we describe that SWEET sugar transporters are key player prior to phloem loading mediated by sucrose- $H^+$  symporter. SWEET11, 12 and 15 function as sucrose transporters. SWEET11 and 12 likely localize at the plasma membrane of phloem parenchyma cells and are involved in efflux of photosynthetically-derived sucrose to the apoplasm. A *sweet11;12* double mutant shows defects in phloem translocation, such as reduced rosette leaf size and elevated starch accumulation. Besides roles in phloem translocation, SWEETs also appear to be supplying the embryos with sucrose in developing seeds. We show that SWEET11, 12 and 15 are required for seed filling. *SWEET11*, 12 and 15 are differentially expressed in seed compartments. A triple *sweet11;12;15* mutant shows retarded embryo development and reduced seed weight, a wrinkled seed phenotype. These results indicate a role of SWEETs in yield potential.

**P64: A genetic triangle: how three MYB proteins determine flower asymmetry in *Antirrhinum***

*Selected poster talk*

**JOÃO. RAIMUNDO, R. SOBRAL, E. S. COEN, M. M. R. COSTA**

**10:10–10:30**

*Center for Biodiversity Functional and Integrative Genomics (BioFIG), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; Department of Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, UK*

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

Therefore, we propose that RAD antagonises DIV in a subcellular competition for a DRIF protein by inhibiting the interaction between DIV and DRIFs in the dorsal regions of the *Antirrhinum* flower in order to establish the asymmetric pattern of gene activity in the flower meristem.

This work was funded by FCT/COMPETE/FEDER with a project grant (ref. FCOMP-01-0124-FEDER-008818) and with a Royal Society International Joint Project grant (2008/R2). JR was supported by funding from FCT with a PhD grant (ref. SFRH/BD/75050/2010).

**P36: Waterproof plants: linking leaf wax and stomatal pore development in barley**

*Selected  
poster talk*

**SARAH JOSE, K. J. EDWARDS, R. WAUGH, A. M.  
HETHERINGTON**

**10:30–10:50**

*School of Biological Sciences, University of Bristol, Woodland Road, Bristol,  
BS8 1UG, UK*

The production of a waxy cuticle and the ability to close stomata in dry conditions are two important ways that plants prevent excessive water loss. Wax biosynthesis has been linked to stomatal development, however it is not currently known whether a shared genetic pathway or differences in leaf permeability are responsible. This link was first recognised in a barley flower spike wax mutant with the pleiotropic phenotype of clustered stomata, and we revisited this mutant and others with similar wax morphologies. Stomatal clusters and abnormal subsidiary cells were rare in wild type barley but comprised up to 14% of stomata in the mutant plants. GC-FID analysis of the wax composition found that beta-diketones and hydroxyl-beta-diketones were reduced in one of the mutants, suggesting a possible link between the production of these compounds with stomatal clustering in barley. We are also investigating several *Arabidopsis* mutants along the wax biosynthesis pathway for possible stomatal phenotypes, in order to determine whether the reduction of specific groups of compounds leads to abnormal stomatal development. Understanding how the production of leaf wax and stomata are connected may be crucial for food security in dry conditions and sustainable agriculture in the future.

**P10: Identifying downstream targets of the wheat transcription factor *NAM-B1*: ChIP-seq in hexaploid wheat using custom-built reference pseudomolecules**

*Selected poster talk*

**PHILIPPA BORRILL, M. TRICK, A. M. SMITH, C. UAUY**

**11:20–11:40**

*John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK*

The *NAM-B1* transcription factor is a key regulator of wheat grain nutrient content and senescence. To dissect these traits we have identified downstream targets of *NAM-B1* using ChIP-seq (Chromatin Immuno-Precipitation combined with next-generation sequencing). We created transgenic wheat lines expressing *NAM-B1* tagged with a FLAG peptide to enable specific pull-down of *NAM-B1*. DNA libraries prepared from the bound chromatin were sequenced on an Illumina Hi-seq. We aligned these reads to a custom-built pseudomolecule reference sequence, which consists of gene-rich contigs from the IWGSC, ordered with reference to a Chinese Spring x Paragon genetic map.

We have identified several hundred genes which are associated with genomic regions bound by *NAM-B1*. 50% of the regions bound by *NAM-B1* are within 1kb of the open reading frame of the nearest gene suggesting a regulatory role. A conserved 15-bp motif was observed in 86% of the binding sites. To produce a high confidence list of *NAM-B1* target genes we have compared the genes identified by ChIP-seq to genes which are differentially expressed in wheat *NAM-B1* RNAi lines (identified by RNA-seq). Our work identifies a network of genes involved in nutrient remobilisation, grain nutrient content and senescence in wheat for further study.

## Understanding and engineering indole alkaloid biosynthesis

*Plenary lecture*

**SARAH E. O'CONNOR**

**12:30–13:20**

*John Innes Centre, Department of Biological Chemistry and The University of East Anglia, School of Chemistry, Joseph Chatt Building, Colney, Norwich, Norfolk, NR4 7UH, UK*

Many enzymatic transformations are utilized in the biosynthesis of the monoterpene indole alkaloids, a group of structurally diverse natural products. Here we describe the discovery, functional characterization and mechanistic study of several enzymes involved in the biosynthesis of the monoterpene indole alkaloids in *Catharanthus roseus*. Also discussed are the implications of this work in the metabolic engineering of natural products.

# Poster abstracts

\* P=poster abstract. Bold=presenting author

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<b>Ajmera, Ishan</b>	<b>P3</b>
<b>Alagna, Fiammetta</b>	<b>P4</b>
<b>Albihlal, Waleed S.</b>	<b>P5</b>
<b>Balogianni, Vasiliki</b>	<b>P6</b>
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<b>Benbow, Harriet</b>	<b>P8</b>
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<b>van Campen, Julia</b>	<b>P12</b>
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<b>Guo, Miao</b>	<b>P29</b>
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<b>Hetherington, Flora</b>	<b>P32</b>
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<b>Lagunas, Beatriz</b>	<b>P44</b>
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*Posters P20, P55, P71, P73 and P75 have been withdrawn from the meeting.*

# Poster abstracts

*Poster abstracts are ordered alphabetically by presenting author (underlined).*

## **P1**      **Heat-wave mediated high concentration of O<sub>3</sub> only temporally affect seed germination of alpine plants**

**T. ABELI<sup>1</sup>, A. MONDONI<sup>1</sup>, S. ORSENIGO<sup>1</sup>, G. ROSSI<sup>1</sup>, D. GUASCONI<sup>1</sup>, P. CRISTOFANELLI<sup>2</sup>, P. BONASONI<sup>2</sup>**

<sup>1</sup>*Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100, Pavia, Italy;* <sup>2</sup>*ISAC-CNR, Bologna, Italy*

Heat-waves (HW) are often associated to high O<sub>3</sub> concentration. However, little is known about the effect of peaks of O<sub>3</sub> on alpine plant reproductive performance. We tested the effect of a peak in O<sub>3</sub> concentration recorded at Global GAW Meteorological Station 'ICO-OV' (Mt. Cimone, Italy), during the summer HW 2006. This peak was the highest recorded by the GAW stations across the Alps and Apennines in the decade 2002–2012. Seeds of 11 alpine plants collected at Mt. Cimone were incubated on agar at 25°C/15°C and exposed to two ozone treatments (0 and 125 ppb) for five days, according to the meteorological data referred to the HW 2006. At the end of the 125 ppb treatment, seed germination (SG) was reduced in all the species of *Festuca* (2) and *Silene* (3) and the mean germination time (MGT) was both enhanced or reduced, with respect to 0ppb treatment. After three weeks from the 125 ppb treatment, no significant differences in SG and MGT between 0 and 125 ppb treatment were detected. Results suggest that ozone may only have a temporally effect on seed germination of alpine plant even at high concentration. Thus O<sub>3</sub> seems to play a minor role in affecting alpine plant reproduction.

## P2

### **A low-cost high-resolution root phenotyping assay for phenotypic selection of phosphorus efficient *Brassica rapa* genotypes**

**M. O. ADU<sup>1,2</sup>, M. J. BENNETT<sup>2</sup>, M. R. BROADLEY<sup>2</sup>, P. J. WHITE<sup>1</sup>, L. X. DUPUY<sup>1</sup>**

<sup>1</sup>*Department of Ecological Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK;* <sup>2</sup>*Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK*

Phosphorus (P) is an essential nutrient for plant growth and is acquired as phosphate (Pi), mainly in the form of orthophosphate ( $\text{H}_2\text{PO}_4^-$ ) but the concentration of Pi in the soil solution is generally low. Insufficient phytoavailable Pi has necessitated increased application of P fertilizers, which comes mostly from finite resources of rock phosphate. To reduce P fertilizer input, the P use efficiency of crops (PUE) must be improved. PUE is the product of root P-uptake efficiency (PUpE) and physiological P-utilization efficiency (PUtE). Differences between crop genotypes in their PUE are often correlated to PUpE. Plant roots have evolved genetic modifications which correlate with PUpE. The exploration of the genetic diversity in plants and subsequent alterations in root system architecture (RSA) through breeding could improve PUpE. RSAs are however difficult to quantify, posing a major limitation to breeding by phenotypic selection. This presentation will illustrate the development of a scanner-based phenotyping assay for RSA of *Brassica* seedlings and the correlations between RSAs measured in soil and non-soil substrates. The relationships between aspects of the seedling RSA and Pi acquisition under P deficient conditions and the adoption of nonlinear mixed effects model-based statistical analyses of root growth dynamics will also be presented.

# P3

## Modelling the regulation of phosphate uptake in plants

**I. AJMERA, T. C. HODGMAN, C. LU**

*Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, LE12 5RD, UK*

The world's finite phosphate stocks have been identified as a significant concern in relation to global food security. Hence understanding plant responses to phosphate depletion is a priority for developing crop varieties that can achieve good yields despite little phosphate input. To accelerate the conventional breeding procedures, a multidisciplinary approach has been used, leading to a mechanistic mathematical model of the phosphate-uptake regulation. This ODE model accounts for the change in the expression of genes under phosphate starvation, specifically the pathway involving microRNA399-mediated degradation of the key repressor, PHO2/LTN1, of membrane phosphate-transporters. Advanced statistical tools have been employed to determine appropriate model parameters from rice whole-root transcriptomic data. The profiles of the parameterised model correspond well with their experimental counterparts observed under normal and low-phosphate conditions. However, certain model predictions point to gaps in our understanding of PHO2/LTN1 regulation, specifically an extra level of repression is needed during phosphate starvation. These observations lead to several hypotheses, PHO2 primary transcription could be repressed, the mRNA could be degraded through the binding of some other microRNA or the protein might be degraded faster. These possibilities are currently under informatics and laboratory investigation.

**F. ALAGNA<sup>1,2</sup>, F. GEU-FLORES<sup>1</sup>, S. E. O'CONNOR<sup>1</sup>, L. BALDONI<sup>2</sup>, A. OSBOURN<sup>1</sup>**

<sup>1</sup>Department of Metabolic Biology, John Innes Centre, Norwich NR4 7UH, UK;

<sup>2</sup>Institute of Biosciences and Bio-resources (IBBR), CNR, 06128 Perugia, Italy

The seco-iridoids are a class of metabolites present in olive (*Olea europaea*) that possess a wide range of properties and beneficial effects on human health. Despite their importance, the genes responsible for their biosynthesis in olive are still unknown.

Recently, the iridoid synthase (ISY), involved in the biosynthesis of the iridoid ring scaffold of monoterpene-indole alkaloids, has been identified and characterized in *Catharanthus roseus*. It belongs to a new class of cyclases which seem to couple an initial NAD(P)H-dependent 1,4-reduction step with a subsequent cyclization. Genes sharing sequence homology with this kind of reductases are common in many plant species; however, their physiological function is still unknown for most of them.

We have identified and functionally characterized the olive homolog of ISY. Our data strongly suggest that it synthesises the monoterpene scaffold of secoiridoids in olive tissues. Moreover, two other genes encoding for 1,4-reductases have been biochemically characterized; even if their physiological role still remains unknown, our results shed some light on their putative function.

## P5

### **The *Arabidopsis* transcription factor HSFA1b controls a network which undergoes dramatic change during transition from growth to stress defence**

**W. S. ALBIHLAL, I. CHERNUKHIN, S. A. YATES, U. BECHTOLD, P. M. MULLINEAUX**

*School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO2 3SQ, Essex, UK*

The *Arabidopsis* transcription factor (TF) HSFA1b influences growth and responses to abiotic and biotic stress. We set out in this study to define the HSFA1b network of regulated genes to address how it fulfils these diverse roles. Using plants over-expressing an *HSFA1b-RFP* fusion (*HSFA1bOX*), we performed chromatin immune-precipitation followed by massively parallel sequencing (ChIP-SEQ) and transcriptomics (RNA-SEQ) on heat-stressed (37°C, 30 min) and non-stressed plants. HSFA1b binds 7284 sites under normal conditions. However, only 627 differentially expressed genes (DEGs) were detected comparing wild type and *HSFA1bOX* plants. Upon heat stress, HSFA1b binding sites were reduced to 654, but there was 3394 DEGs. A large group of DEGs encoding growth and development TFs were down-regulated as a result of HSFA1b loss from their promoters under heat stress. Strikingly, most heat-responsive genes were not bound by HSFA1b under heat stress, despite being bound and differentially expressed in non-stressed plants. We hypothesise that under normal conditions the HSFA1b network regulates genes controlling growth and development and also those that initiate a rapid heat stress response. This represents an important mechanism for controlling the allocation of resources in plants between growth and stress tolerance.

**V. G. BALOGIANNI, S. D. WILSON**

*Department of Biology, University of Regina, Laboratory Building 420, 3737 Wascana Parkway, Regina, SK S4S0A2, Canada*

Root traits and their effects on ecosystem functions are only poorly understood, relative to those of shoots, even though 90% of grassland productivity occurs belowground. In the central North American grasslands, the invasive perennial grass *Agropyron cristatum* is highly productive, but does not increase soil carbon (C) sequestration. We explored how invasion-associated changes in root and shoot characteristics alter the local carbon cycle in a 7-year study. Invasion altered all belowground characteristics studied, except for soil C content. Relative to uninvaded grasslands, *Agropyron* stands had double the root length, reduced root lignin content, and increased CO<sub>2</sub> evolution from root decomposition. In contrast, shoot mass and CO<sub>2</sub> evolution did not differ significantly between uninvaded and invaded vegetation stands, even though *Agropyron* had greater leaf lignin content. Given the great biomass allocation to root in these grasslands, our results suggest that increased root length beneath *Agropyron* did not increase soil C content because its lower root lignin content led to faster rates of root decomposition. Belowground, not aboveground, traits account for the similarities in C sequestration in soils beneath uninvaded and invaded stands.

## P7

### Evolutionary history underlies plant physiological responses to global change since the Last Glacial Maximum

**K. M. BECKLIN, J. S. MEDEIROS, K. R. SALE, J. K. WARD**

*Department of Ecology and Evolutionary Biology, University of Kansas, 8028 Haworth Hall, 1200 Sunnyside Avenue, Lawrence, KS 66045, USA*

Assessing family and species-level variation in physiological responses to global change across geologic time is critical for understanding factors that underlie changes in species distributions and community composition. Ancient plant specimens preserved within packrat middens are invaluable in this context since they allow for comparisons between co-occurring plant lineages. Here we used modern and ancient plants preserved within packrat middens from the Snake Range, NV to investigate plant physiological responses to global change since the last glacial maximum (LGM). We used a conceptual model to infer relative changes in stomatal conductance ( $g_s$ ) and maximum photosynthetic capacity ( $A_{max}$ ) from measures of leaf carbon isotope, stomatal characteristics, and leaf %N. Our results indicate that most of the sampled taxa decreased  $g_s$  and/or  $A_{max}$  from glacial to modern times. However, plant families differed in the timing and magnitude of these physiological responses. Additionally, leaf-level responses were more similar within plant families than within co-occurring species assemblages. This suggests that adaptation at the level of leaf physiology may not be the main determinant of shifts in community composition, and that plant evolutionary history may drive physiological adaptation to global change over recent geologic time.

## Identifying loci association with grain weight in bread wheat, *Triticum aestivum*

**H. R. BENBOW<sup>1</sup>, A. M. ALLEN<sup>1</sup>, A. J. BURRIDGE<sup>1</sup>, R. HORSNELL<sup>2</sup>, I. MACKAY<sup>2</sup>, G. L. A. BARKER<sup>1</sup>, P. WILKINSON<sup>1</sup>, M. WINFIELD<sup>1</sup>, J. COCKRAM<sup>2</sup>, P. HOWELL<sup>2</sup>, A. BENTLEY<sup>2</sup>, A. GREENLAND<sup>2</sup>, C. BEQUAIN<sup>3</sup>, P. JACK<sup>3</sup>, K. J. EDWARDS<sup>1</sup>**

<sup>1</sup>*School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK;* <sup>2</sup>*The John Bingham Laboratory, NIAB, Huntingdon Road, Cambridge, CB3 0LE, UK;* <sup>3</sup>*RAGT Seeds LTD, Grange Road, Ickleton, Essex CB10 1TA, UK*

A large array of single-nucleotide polymorphisms (SNPs) was used to identify loci associated with thousand grain weight (TGW) in Hexaploid wheat, *Triticum aestivum*.

Two experimental populations; a doubled-haploid cross of 2 UK varieties (population 1) and the NIAB elite (8-way) winter wheat MAGIC population (population 2) were genotyped with 7721 SNPs which were used to create genetic linkage maps for quantitative trait loci (QTL) analysis and a genome-wide association study for thousand grain weight (TGW).

This study reports 7 QTLs for TGW within population 1 located on chromosomes 4B, 4D, 5A, 5B and 6A, and 124 SNPs associated with TGW within population 2. The QTLs will be investigated with regard to their proximity to known genes, such as those for reduced height and vernalisation requirement.

Further scrutiny will highlight elite alleles and their parental contributors within the MAGIC population which will be investigated, supported by sequence data from population one.

**R. E. BONE<sup>1</sup>, J. A. C. SMITH<sup>2</sup>, S. BUERKI<sup>1</sup>**

*<sup>1</sup>The Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK; <sup>2</sup>Department of Plant Science, University of Oxford, South Parks Road, Oxford OX1 3RB, UK*

Epidendroid orchids of the Eulophiinae subtribe are remarkably varied in form and occupy a variety of habitats in Madagascar, from large lush epiphytes of the humid forests of the eastern side of the island, and tall leafy terrestrials of upland grasslands, to small leathery-leaved xerophytes of dry deciduous forests. In addition to stabilizing the classification, by providing novel hypotheses for relationships among genera in this subtribe, we seek to understand adaptation of specific clades to arid environments. Carbon isotope analysis across a broad sampling of the subtribe has revealed crassulacean acid metabolism (CAM) photosynthesis in the xerophytic forms of Eulophiinae (primarily in the genus *Oeceoclades*, among species restricted to dry forests). We present a phylogeny based on plastid and nuclear regions, computed with Bayesian and Likelihood algorithms, and infer a temporal framework for shifts in photosynthetic pathway in the subtribe, providing insights into orchid evolution in relation to historic changes in climatic conditions. We suggest that evolution of CAM photosynthesis has played a role in the success of this group of terrestrial orchids in dry habitats that are not typically associated with the orchid family.

## P10

### Identifying downstream targets of the wheat transcription factor *NAM-B1*: ChIP-seq in hexaploid wheat using custom-built reference pseudomolecules

P. BORRILL, M. TRICK, A. M. SMITH, C. UAUY

*John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK*

The *NAM-B1* transcription factor is a key regulator of wheat grain nutrient content and senescence. To dissect these traits we have identified downstream targets of *NAM-B1* using ChIP-seq (Chromatin Immuno-Precipitation combined with next-generation sequencing). We created transgenic wheat lines expressing *NAM-B1* tagged with a FLAG peptide to enable specific pull-down of *NAM-B1*. DNA libraries prepared from the bound chromatin were sequenced on an Illumina Hi-seq. We aligned these reads to a custom-built pseudomolecule reference sequence, which consists of gene-rich contigs from the IWGSC, ordered with reference to a Chinese Spring x Paragon genetic map.

We have identified several hundred genes which are associated with genomic regions bound by *NAM-B1*. 50% of the regions bound by *NAM-B1* are within 1kb of the open reading frame of the nearest gene suggesting a regulatory role. A conserved 15-bp motif was observed in 86% of the binding sites. To produce a high confidence list of *NAM-B1* target genes we have compared the genes identified by ChIP-seq to genes which are differentially expressed in wheat *NAM-B1* RNAi lines (identified by RNA-seq). Our work identifies a network of genes involved in nutrient remobilisation, grain nutrient content and senescence in wheat for further study.

## P11

### Action of the NF-Y transcription factors in plant stress responses

**E. BREEZE, C. HILL, J. PRUSINSKA, V. BUCHANAN-WOLLASTON, K. DENBY**

*Warwick Systems Biology, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK*

Environmental stresses, such as drought, high salinity and pathogen attack, cause significant crop losses worldwide. Consequently, plants have evolved complex and highly regulated stress response mechanisms. Although there are certainly stimuli-specific pathways, many genes appear to be induced by multiple stresses supporting the existence of a common 'core stress response' regulatory network. The NF-Y transcription factor (TF) family are likely key regulators in multiple stress responses. NF-Y TFs function as a heterotrimeric complex consisting of NF-YA, NF-YB and NF-YC subunits, which, in *Arabidopsis*, are encoded by multigene families that could theoretically combine to form 1000 unique TFs. This combinatorial diversity could enable fine-tuning of transcriptional regulation by activating specific groups of stress-responsive genes. This project aims to identify functional NF-Y complexes involved in regulating plant stress responses, and to elucidate their downstream targets and upstream regulators. Network inference, together with yeast-1 and 2-hybrid assays and microarray analysis of altered expression mutants, has enabled the generation of robust small-scale networks centred around a subset of key regulatory NF-Y subunits. Current work is focussed on the determination of a complete *Arabidopsis* NF-Y TF complex involved in the regulation of stress-induced jasmonate biosynthesis.

## P12

### Chloroplast and vein development in leaf primordia of rice

J. C. VAN CAMPEN, X. YIN, V. THAKUR, S. WANCHANA, S. A. ROLFE, W. P. QUICK, A. J. FLEMING

*Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Sheffield, S10 2TN, UK*

In order to improve yield, water use efficiency and nitrogen use efficiency, a global consortium is seeking to develop  $C_4$  rice. However, this radical re-engineering of the rice crop requires a solid understanding of the fundamental developmental processes underlying the differences between  $C_3$  and  $C_4$  leaf structure. Thus, we aim to elucidate the timing of chloroplast and vein development in rice. Using a novel microscopic chlorophyll fluorescence imaging approach, we have identified the developmental stage at which photosynthetic electron transport is first measureable as being the P3 stage, with rapid basipetal differentiation of chloroplasts occurring at P4 stage. Vein development at these stages is being investigated by shoot apex tissue culture and treatment with inhibitors of polar auxin transport. In parallel, we are interrogating RNA-Seq data of these developmental stages to identify rice genes putatively involved in vein and chloroplast differentiation, as well as expression patterns of genes which may be co-opted for  $C_4$  metabolism. This could aid strategies to produce a  $C_4$  rice to bolster global food security.

## P13

### Natural variation for zinc deficiency tolerance in *Arabidopsis thaliana*

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Zinc is an important micronutrient for plants and humans, therefore the relevance of studying Zn homeostasis and mechanisms involved in Zn deficiency tolerance in plants aiming enhancing Zn concentration in plants edible parts and developing varieties more tolerant to Zn deficiency. In our study we used twenty diverse *A. thaliana* accessions originated from different locations around the world to characterize in detail the natural variation for Zn deficiency tolerance in shoot and root tissue. Changes at the physiological, ionomic (elements concentration) and molecular level were studied. As a follow up we selected two contrasting *A. thaliana* accessions for Zn deficiency tolerance and Col-0, which were used for a whole genome transcriptome analysis of the Zn deficiency response. Our results show that *A. thaliana* accessions show natural variation for the minimum Zn concentration required for growth. Different levels of Zn deficiency resulted in changes at the concentration of other elements in the shoot and root. Differences in Zn deficiency tolerance were also visible at the gene expression level. Based on the transcriptome analysis we identified possible new candidate genes involved in the core Zn deficiency response. Finally, we were able to identify genes clusters which are involved in the accession specific response to Zn deficiency and their roles are being further investigated at the moment.

# P14

## Convergence of stomatal CO<sub>2</sub> and ABA signalling pathways

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Exposure of plants to elevated CO<sub>2</sub> concentrations causes rapid reductions in stomatal aperture and often induces reductions in stomatal number and density in new leaves. Drought conditions promote stomatal closure and can cause lower stomatal densities, both via the hormone abscisic acid (ABA). Despite the importance of transpiration, little is known about the mechanisms responsible for the control of stomatal aperture and development by CO<sub>2</sub>. This work investigates the degree of convergence between the guard cell ABA and CO<sub>2</sub> signalling pathways and shows that CO<sub>2</sub> signalling is compromised in *Arabidopsis* mutants defective in ABA perception and reactive oxygen species (ROS) production. In addition, we show evidence to support a role for ROS in the control of stomatal development by CO<sub>2</sub>.

## **P15**      **Proteomic and transcriptomic profiling of totipotency acquisition in plant somatic embryogenesis – tamarillo a case study**

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Plant somatic embryogenesis (SE) is a well-known developmental pathway in which a somatic cell acquires totipotency and evolves into an embryo. In our work the solanaceous tree tamarillo (*Cyphomandra betacea*, syn. *Solanum betaceum*) has been used as a model to understand totipotency acquisition and characterise the early stages of SE. In this system, induced embryogenic and non-embryogenic tissues, with the same genetic background but different morphogenic abilities, can be easily separated and grown as independent cell lines. A comparative proteomic profile of tamarillo's embryogenic and non-embryogenic tissues was obtained, with the embryogenic cells showing a better ability to regulate the effects of stress conditions through an increased expression of heat-shock and energy metabolism related proteins, such as enolases or treonine synthases. Moreover, a putative inhibitor in the acquisition of embryogenic competence was isolated and characterized. No significant differences were detected at the mRNA level for most of the differentially expressed proteins, which indicates the relevance of posttranscriptional mechanisms of control. Based on these results, more detailed approaches at the transcriptomic level are being conducted. However, the amount of information so far obtained has given good indications about tamarillo's use as a model to understand the mechanisms of plant totipotency.

# P16

## Molecular evidence for an indeterminate meristem precursor in a moss

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The shape of animals is determined during embryogenesis; conversely, plants develop mainly postembryonically. They have the ability to produce new organs throughout their life from meristems, populations of stem cells located at their shoot tips. As a result, the shape of most extant plants is indeterminate. Only a few extant plant groups, such as mosses, display ancestral features and have determinate shoots. The evolution of indeterminacy from a determinate ancestor was a prerequisite to the diversification of plant forms, however, the molecular mechanisms underpinning this transition are unknown. A KNOX–cytokinin regulatory loop has well characterised roles in promoting meristem indeterminacy in flowering plants. To investigate the ancestral role of this molecular module, we examined its conservation in a determinate moss shoot and show that the moss KNOX protein MKN2, acting via cytokinin biosynthesis, is necessary and sufficient to promote proliferation in a non-apical zone. We propose that this non-apical proliferative zone represents a progenitor to the evolution of an indeterminate apical meristem.

## **P17**      **Transcriptional regulation in effector-triggered immunity *via* NB-LRR resistance genes *RPS4* and *RRS1***

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Divergently transcribed resistance (R) gene pairs, *RPS4* (*resistance to Pseudomonas syringae 4*) and *RRS1* (*resistance to Ralstonia solanacearum 1*) from *Arabidopsis thaliana*, have been reported to function together in defence against bacterial pathogens, *Pseudomonas syringae* (*AvrRps4*) and *Ralstonia solanacearum* (*PopP2*), as well as the fungus *Colletotrichum higginsianum*. Mechanism how this pair of R proteins works in effector triggered immunity has been the focus of many research groups. *slh1* (*sensitive to low humidity 1*) has been reported to encode a mutated *RRS1* protein with attenuated DNA binding activity *in vitro*. *slh1* plants display dwarf, and autoimmune phenotypes. Accordingly, an intriguing question is raised up whether *RRS1/RPS4* targets multiple defence-related genes directly, and activates the downstream events through the transcriptional regulation during effector triggered immunity. Chromatin immunoprecipitation sequencing (ChIP-seq) will be developed to unravel this question. To achieve a better dataset, *RRS1* and *RPS4* driven by enhanced promoters, fused with different epitope tags, and *AvrRps4* driven by an inducible promoter will be constructed in one binary vector through Golden Gate Modules Cloning strategy. This technique will enable us to generate various combinations of multiple-gene transgenic plants. Therefore, combined with gene expression data, we can dissect the detailed mechanism of *RPS4/RRS1* mediated immunity.

## P18

### Genomic repeat abundances contain phylogenetic signal in diverse eukaryotic groups

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A large proportion of genomic information, particularly repetitive elements, is usually ignored when researchers are using next-generation sequencing. Here we demonstrate the usefulness of this repetitive fraction in phylogenetic analyses, utilising comparative graph-based clustering of next-generation sequence reads, which results in abundance estimates of different classes of genomic repeats. Phylogenetic trees are then inferred based on the genome-wide abundance of different repeat types treated as continuously varying characters; such repeats are scattered across chromosomes, and in angiosperms can constitute a majority of nuclear genomic DNA. In six diverse examples, five angiosperms and one insect, this method provides generally well-supported relationships at interspecific and intergeneric levels. We propose that this methodology may prove especially useful in groups where there is little genetic differentiation in standard phylogenetic markers. At the same time as providing data for phylogenetic inference, this method additionally yields a wealth of data for comparative studies of genome evolution.

## P19

### Genomics and domestication of field pennycress (*Thlaspi arvense* L.)

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Large portions of the Midwestern US lack any living plant cover from the time of corn harvest in the fall until soybean and corn develop and establish a canopy cover the following June. The lack of plant cover leaves soil vulnerable to erosion and nutrient runoff, significantly impacting the health of surface waters. Planting winter cover crops has been shown to protect soil and water health, as well as limiting the growth of spring weeds. We are developing field pennycress as a fall planted, winter annual oilseed crop that can be harvested in the early spring and serve as a biodiesel feedstock.

There have been limited efforts to improve the agronomic qualities of pennycress, but we are applying modern genomic technologies to enable the rapid domestication of this species. We have configured and built a personal computer for c. \$2000 US able to perform de novo assembly and annotation of the pennycress transcriptome and genome from next generation sequencing data. From this work, we have identified candidate genes responsible for controlling key traits like seed dormancy and flowering time, which will guide future improvement efforts. The generation of these genomic resources will provide an unprecedented tool for the domestication of pennycress.

## P21

## Synthetic gene clusters for the delivery of designer traits into plants

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Gene clusters for the synthesis of specialised metabolites are a growing theme in plant biology. Our overall goal is to engineer synthetic gene clusters to answer two questions: Firstly, can functional clusters be built up from defined components? Secondly, can synthetic gene clusters be used for the introduction of multi-gene designer traits into plants?

To develop this strategy we have used a three-step pathway for the synthesis of hydroxynitrile glucosides from sorghum; also genes for the synthesis of three different coloured fluorescent proteins. Transient co-expression in *Nicotiana benthamiana* leaves (using the CaMV35S promoter) showed accumulation of hydroxynitrile glucosides and co-expression of the three fluorescent proteins as expected.

Next we will evaluate the ability of promoter sets from two different plant metabolic gene clusters (the oat avenacin cluster and the *A. thaliana* thalianol cluster, both of which are expressed in roots) to drive expression of our validated three-gene read-outs in stably transformed lines of *A. thaliana*. Having validated this approach, we will then broaden our experiments to build a library of sets of co-regulated promoters from different plant metabolic gene clusters that will enable co-expression of multi-gene ensembles on demand (e.g. at particular developmental stages or in response to biotic/abiotic stresses).

## P22

### Taking control of seed vigour in a climate change scenario

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Environmental temperature during seed set profoundly affects seed dormancy and vigour. Agricultural productivity depends on the constant supply of high vigour seeds into the market, but predictions of increased climate and weather variability threaten a continued delivery of high quality seeds by the global seed industry. Therefore, in order to secure future food supplies we need to understand the genetic and molecular mechanisms through which temperature affects seed vigour, and use this knowledge to endow the next generation of crops with seed quality that is resistant to the effects of climate change. Using *Arabidopsis thaliana*, I screened a mutagenised population of plants to discover new genes involved in temperature regulation of seed vigour. The mutants identified render the seeds insensitive to temperature variation during seed production, indicating that in the wild type plants these genes play an important role in temperature responsiveness during seed set. The genes responsible are being identified using next-generation sequencing technology which enables us to rapidly detect our mutations responsible for vigour enhancement. The first of these mutants has been identified to be deficient in a gene necessary for synthesis of the biopolymer suberin in seeds, a waxy substance responsible for sealing the space where the seed was connected to the mother plant. We can also show that suberin biosynthesis is affected by temperature during seed set and that temperature-regulation of suberin synthesis is therefore a key process through which temperature variation can reduce seed vigour. We conclude that manipulating suberin levels in crop seeds has the potential to allow seed companies to control the vigour of their seeds, and provide predictable high product performance even in the face of less predictable weather and climate variation during seed production.

**ATHB4, a transcription factor that regulates the Shade Avoidance Syndrome by an independent DNA-binding activity****M. GALLEMI<sup>1</sup>, M. J. MOLINA-CONTRERAS<sup>1</sup>, J. F. MARTINEZ-GARCIA<sup>1,2</sup>**

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Perception of vegetation proximity by the phytochrome photoreceptors activates a set of responses called the Shade Avoidance Syndrome (SAS), with the main objective to outcompete for light. Among these responses we focus in the promotion of hypocotyl elongation. Molecularly, perception of plant proximity activates a transcriptional cascade with rapid changes in gene expression. One of the genes rapidly induced by shade perception in *Arabidopsis thaliana* is *ATHB4*, which encodes for a transcription factor of the Homeo-Domain Leucine-Zipper (HD-Zip) family. Previously we have characterized *ATHB4* as a SAS regulator that, when over-expressed, represses shade-induced hypocotyl elongation and expression of shade markers. *ATHB4* over-expression also affects leaf polarity, visualized by narrow leaves that are curved upwards. To elucidate which domains are required for its biological activity, a series of truncated forms of *ATHB4* were over-expressed in *Arabidopsis* plants. In these transgenic lines, we analyzed three traits indicative of *ATHB4* biological activity: shade-induced hypocotyls elongation and changes of gene expression, both at the seedling stage, and effects on leaf polarity, in adult plants. Our results suggest that *ATHB4* can act through different mechanism depending on the process regulated: 1) as a transcription factor, 2) as a cofactor, acting in a DNA-binding independent mechanism.

## P24

### **Symbiosome membrane is a crossroad of exo/endocytic pathways in infected cells of legume root nodules**

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In *Rhizobium*–legume symbiosis, the bacteria are enclosed in the specialized plant-derived membrane, the so called symbiosome membrane (SM). The SM is the interface between symbiotes, which facilitates the exchange of nutrients and solutes. Despite its key importance, the molecular mechanisms of SM formation are largely unknown. Cytological analysis revealed great alterations of the endomembrane system in symbiotic cells. To shed light on the mechanisms of them we focused on the key endosomal identity markers, and participators of membrane fusion machinery of the plant cell: HOPS complex, small GTPases and SNARE proteins during symbiosis development. Our results show that the Vamp72-controlled exocytosis pathway regulates the release of bacteria into the plant cell by the reorganization of the cell wall materials. Further development of symbiosis occurs by the suppression of the HOPS complex and as a consequence by the collapse and defunctionalization of vacuoles in the symbiotic cells. Simultaneous retargeting of tonoplast proteins to the SM is essential for the functional maturation of symbiotic cells. Proteomic analysis of the SM confirmed its mosaic composition. The SM combines plasma membrane and tonoplast proteins and thereby appears as a crossroad of exo/endocytic pathways in infected cells of legume root nodules.

**Apoplastic pH dynamics modulate stomata aperture via abscisic acid levels in leaf apoplast and guard cells in salt-stressed *Vicia faba* L.**

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Salt stress causes the leaf apoplast to transiently alkalize, an event that is presumed to contribute to the ability of plants to survive. However, there is no evidence that apoplastic pH dynamics indeed initiate coordinated processes downstream. We hypothesize that salt stress-induced apoplastic pH dynamics modulate the concentrations of abscisic acid (ABA) in *Vicia faba* L. and, as a consequence thereof, functions on stomatal aperture during salinity. This is supposed because the pH range acts as a basic chemical requirement necessary for the compartmental distribution of ABA.

Ratio-imaging was applied for *in-planta* monitoring of pH dynamics. Using mass spectrometry techniques, ABA concentrations were determined both in the leaf apoplast and in guard cells against the formation of the apoplastic pH dynamics.

Our results demonstrate that a stress signal that initiates the pH dynamics propagates from root to leaf in a way similar to xylem-distributed water. In the leaves, it finally induces a systemic apoplastic alkalization. Subsequently, this pH alteration causes apoplastic ABA level to increase, followed by an elevation of endogenous guard cell ABA. Thus, downstream of the salt-dependent transient apoplastic alkalization, a coordinated sequence was demonstrated, that induces stomatal closure during the initial phase in salt stress.

## P26

### A novel structure in the cell wall of *Arabidopsis thaliana* trichome

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We report the discovery of a callose ring in the cell wall near the base of *Arabidopsis* trichome, which to our knowledge has not yet been described, and we propose to call this structure 'Ortmannian band' (OB). The OB forms during trichome maturation and colocalizes with a bundle of microtubules that resembles the preprophasic band; the nucleus is often found near the site of OB formation. These findings point to the possibility that OB might be a rudiment of evolutionary ancestral trichome cell division. The OB is mostly absent in the secretory pathway mutant *exo70H4*, which is defective in secondary cell wall deposition during trichome maturation. The *EXO70H4* gene expression is upregulated by UV-B and downregulated by methyl jasmonate treatment; the same is true for OB formation in wild type plants. These data together suggest that *EXO70H4* plays a key role in the formation of OB and that it might be involved in the defense against UV irradiation and/or herbivore attack.

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In the current and projected global climate, the production of crops that can withstand abiotic stresses or generate a greater yield is at the forefront of crop research. The resurrection grass *Sporobolus stapfianus* is able to withstand extreme environments by desiccating when water is scarce. A UDP-glycosyltransferase (*SDG8i*) isolated from *S. stapfianus* has been shown to glycosylate strigolactone-like compounds, this action increases plant yield and drought tolerance when *SDG8i* is expressed in *Arabidopsis thaliana*. Furthermore, expression of *SDG4i* in *A. thaliana* alters ABA-dependent senescence and ABA signalling through interaction with an ERD15-like protein in plants resulting in increased longevity, seed yield and abiotic stress tolerance. The survival of desiccation in resurrection plants in part relies on the accumulation of sugars to form intracellular glasses. While resurrection plants accumulate large levels of sucrose during desiccation, the sugar trehalose is also accumulated. Trehalose, in particular its precursor trehalose-6-phosphate (T6P), is considered an important regulator of developmental and metabolic processes in plants. Trehalose accumulation has been associated with abiotic stress tolerance in non-resurrection plants, but its precursor T6P is the main active component regulating gene expression through the protein kinase SnRK1 and priming gene expression for growth after recovery from stress-induced sink limitation. Potentially, information gained on the molecular mechanisms of hormone and sugar signalling in desiccation tolerance could be applied to agriculturally important crop species to ultimately enhance drought-tolerance and/or crop yield.

## P28

### Control of environmental stress responses by the circadian clock and abscisic acid

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Plants are exposed to a variety of abiotic stresses, including salinity and drought. These environmental stresses cause major losses in crop yield. High salinity stress alone impairs crop production on at least 20% of irrigated land worldwide. Thus, the development of stress-tolerant crops is of major importance for food security. Many physiological responses to ensure acclimation to adverse environmental conditions require the synthesis and perception of the plant hormone abscisic acid (ABA). Recent studies have uncovered an unexpectedly tight coupling between the circadian clock and ABA signalling. We found that the LHY transcription factor, which functions as a key component of the circadian clock, also binds to many of the components of the ABA signalling pathway. This is exciting because this suggests that the circadian clock is likely to be important to modulate responses to environmental stress. Elucidating this interaction between the clock and ABA signalling may therefore lead to novel strategies for improving abiotic stress tolerance in crop plants.

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Imola, an Italian poplar elite clone, grown under short/very short rotation coppice management in a plantation located at Casale Monferrato, is a potential feedstock for the production of bioethanol. This hybrid poplar clone was obtained by controlled crossing of *Populus deltoides* Bartr. with *Populus nigra* L.. To gain an in-depth understanding of the environmental profiles of Imola-derived bioethanol, an attributional LCA approach was undertaken to model potential bioethanol supply chains (combinations of various cultivation methods and processing technologies) and to compare bioethanol with gasoline. A biogeochemistry model Denitrification-Decomposition (DNDC) was modified for simulation of perennial bioenergy crops. The biomass dynamics and C pool derived from DNDC simulations were compared with the experimental observations. The DNDC-modeled soil carbon sequestration and C/N fluxes together with the processing technologies simulated using AspenPlus were incorporated into LCA model. Overall, bioethanol derived from Imola represents a promising alternative transport fuel to gasoline. These benefits increase significantly from improved poplar feedstock projected to come on-stream in the future. The overall results suggest that by introducing hybrid poplar clones with higher biomass yields, modified composition and improved cell wall accessibility, the genetic engineering and advanced breeding programme will potentially advance the environmental sustainability of lignocellulosic biorefining industry.

## P30

### The exocyst complex is required for appressorium-mediated tissue invasion by rice blast fungus, *Magnaporthe oryzae*

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*Magnaporthe oryzae*, is one of the most serious fungal pathogens of cultivated rice. *M. oryzae* forms a single-celled infection structure called an appressorium which breaches the leaf cuticle and thereby invades host cells. During host colonization, the fungus secretes effector proteins via a mechanism that is not well defined in *M. oryzae*. We characterized the exocyst complex, an evolutionarily conserved octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84), which plays a crucial role in vesicle tethering to the plasma membrane. Like other fungi, *M. oryzae*, exocyst components localize to the vegetative hyphal tip and during infection-related development they are found around the appressorium pore, implicating it as a site of protein secretion during infection. We generated a temperature-sensitive mutant of Sec6, which completely disrupts exocyst assembly at the appressorium pore and impairs in effector secretion. Recently, we have shown that Exo70 and Sec5 are involved in secretion of cytoplasmic (host cell-delivered) effectors, but not apoplastic effectors. Targeted gene deletion of exocyst components Exo70 and Sec5 and the temperature sensitive mutation of Sec6 also cause significant loss of virulence. Furthermore, we found that the localization of the exocyst at the appressorium pore is septin-dependent.

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Plant leaf epidermes are covered with pores called stomata, the apertures of which are controlled by specialised guard cells. Changes in guard cell turgor alters stomatal aperture, allowing for rapid correction for environmental changes in order to maintain an appropriate balance between carbon acquisition and water loss. The phytohormone auxin is integral to almost every aspect of plant physiology, however its mode of action in guard cells has never been fully characterised. We seek to investigate the role of auxin in the complex web of pathways that govern stomatal activity in *Arabidopsis thaliana*, paying particular attention to the involvement of the non-transcriptional auxin receptor ABP1. Hormonal bioassay of epidermal peels from mutants lacking ABP1 will elucidate this receptor's role in normal stomatal responses. Stomatal patterning is also under delicate environmental control during development, as final stomatal size and density determines the limits to the leaf's range of stomatal conductance. In order to assess the role of auxin in stomatal development we will analyse impressions of the abaxial leaf surface of auxin mutants grown under various conditions.

## P32

### Mechanisms of plant growth responses to Potassium

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Potassium deprivation leads to reduced plant growth. Soils poor in potassium result in low crop yields, and potassium fertilizers must be added to improve crop performance. Reduced potassium alters root morphology and it is hypothesized that the signalling response is mediated by hormone signalling pathways. The aim of the project is to understand better the relationship between potassium availability, hormone signalling pathways and root architecture in the model experimental organism, *Arabidopsis thaliana*.

## P33

### The synaptonemal complex protein ZYP1 is required for imposition of meiotic crossovers in Barley

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In many of our major cereal crops, meiotic crossovers predominantly occur towards the ends of chromosomes and 30-50% of genes rarely recombine. This limits the exploitation of genetic variation by plant breeding. Previous reports demonstrate that chiasma frequency can be manipulated in plants by depletion of the synaptonemal complex protein ZYP1, but conflict as to the direction of change, with fewer chiasma reported in *Arabidopsis* and more crossovers reported for rice. Here, we use of RNAi to reduce the amount of polymerised ZYP1 to only 2-17% of normal zygotene levels in transgenic barley. In the *ZYP1<sup>RNAi</sup>* lines, fewer than half of the chromosome pairs formed bivalents at metaphase and many univalents were observed, leading to chromosome non-disjunction and semi-sterility. The mean number of chiasmata per cell was reduced from 14 in control plants to 3-4 in the ZYP1-depleted lines, although the distribution of residual chiasmata was not affected. Barley ZYP1 appears to function similarly to ZIP1/ZYP1 in yeast and *Arabidopsis*, respectively, and has an opposite effect on crossover number to that reported for its orthologue, ZEP1, in rice, another member of the Poaceae.

## P34

### Hybridisation, polyploidy and allelic divergence promote the appearance of apomixis in the *Ranunculus auricomus* complex

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Hybridisation and polyploidy are major features contributing to plant evolution, often connected to shifts from sexuality to apomixis in angiosperm lineages. Gametophytic apomixis results from developmental asynchrony and altered gene expression in duplicated hybrid genomes. We analyse the consequences of interspecific hybridisation, ploidy levels and allelic divergence on embryo sac development and seed formation in the *Ranunculus auricomus* complex. RNAseq was used to sequence flower-specific transcriptomes of 5 *Ranunculus* genotypes (3 sexual and 2 apomictic biotypes). High quality single nucleotide (SNP) and insertion-deletion (indel) polymorphisms were mined from each library. Reproductive development in natural and synthetic hybrids was compared to parental taxa. Non-synonymous (dN) to synonymous (dS) substitution ratios of annotated genes between apomictic and sexual genotypes revealed outliers and diversifying selection for 324 genes, some associated with meiosis and gametogenesis. Ovule development of natural and synthetic hybrids show a delay in megaspore and embryo sac development compared to parental taxa, and high frequencies of apomixis in natural polyploids. We confirm that interspecific hybridisation triggers the initiation of apospory, but embryo sac development and functional apomixis require a rise in ploidy level. Diversifying selection on reproductive genes can drive the shift from sexuality to apomixis in natural populations.

## P35

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

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*Silene dioica* is a dioecious species where sex is determined by heteromorphic XY sex chromosomes. Dioecy has evolved independently in several different genera as an out-breeding mechanism which results in single sex flowers occurring on separate plants. Males (XY) are the heterogametic sex and females (XX) are the homogametic sex. The Y chromosome promotes stamen development and pollen development and suppresses carpel development. In the absence of a Y chromosome, female flowers develop functional carpels and stamen primordia are arrested.

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

We will present our characterisation of this locus, and progress towards identifying the transposon, as well as the identification of floral mutants from screens of plants carrying the active transposon.

## P36

### Waterproof plants: linking leaf wax and stomatal pore development in barley

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The production of a waxy cuticle and the ability to close stomata in dry conditions are two important ways that plants prevent excessive water loss. Wax biosynthesis has been linked to stomatal development, however it is not currently known whether a shared genetic pathway or differences in leaf permeability are responsible. This link was first recognised in a barley flower spike wax mutant with the pleiotropic phenotype of clustered stomata, and we revisited this mutant and others with similar wax morphologies. Stomatal clusters and abnormal subsidiary cells were rare in wild type barley but comprised up to 14% of stomata in the mutant plants. GC-FID analysis of the wax composition found that beta-diketones and hydroxyl-beta-diketones were reduced in one of the mutants, suggesting a possible link between the production of these compounds with stomatal clustering in barley. We are also investigating several *Arabidopsis* mutants along the wax biosynthesis pathway for possible stomatal phenotypes, in order to determine whether the reduction of specific groups of compounds leads to abnormal stomatal development. Understanding how the production of leaf wax and stomata are connected may be crucial for food security in dry conditions and sustainable agriculture in the future.

## P37

### A root hair assay is a useful tool for dissecting multiple signalling pathways leading to plant programmed cell death

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Programmed cell death (PCD) is activated as a result of complex signalling pathways. We have assessed the applicability of a novel technique, the root hair assay, for studying multiple signalling pathways leading to PCD activation. The root hair assay is a method for quantifying PCD rates in plants *in vivo*, based on observations of dying root hairs morphology. We used the assay to investigate the crosstalk between salicylic acid (SA), autophagy and apoptosis-like PCD (AL-PCD) in *Arabidopsis thaliana*. The root hair assay was used to determine rates of AL-PCD induced by a panel of cell death inducing treatments in wild type plants treated with chemical modulators of SA synthesis or autophagy, and in genetic lines defective in autophagy or SA signalling.

The results suggest that SA is negatively regulated by autophagy during SA and mycotoxin-induced AL-PCD. However, this crosstalk does not appear to be directly involved in PCD induced by gibberellic acid or abiotic stress. This study demonstrates that the root hair assay is an effective tool for relatively rapid investigation of complex signalling pathways leading to the activation of PCD.

## P38

### Creation of C<sub>4</sub> rice through genetic engineering

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Rice (*Oryza sativa* L.) is one of the most important crops in the world. To provide enough food to the rapidly growing population, it is imperative that the rice yields continue to increase. Climate change is happening at an unprecedented rate with more damage than anticipated posing even greater challenge to the food production. Various studies have reported that converting C<sub>3</sub> crops into C<sub>4</sub> by installing a more efficient C<sub>4</sub> photosynthetic pathway would be one of the sustainable ways of crop improvement. In order to engineer C<sub>4</sub> rice, we have transformed five core C<sub>4</sub> genes and six transporters from maize into rice. Antisense RNA and artificial microRNAs were employed to down-regulate Rubisco and Glycine decarboxylase in the rice mesophyll cells to create a favorable environment for the C<sub>4</sub> pathway. To combine all these C<sub>4</sub> genes, promising single transgenic plants were crossed. We have successfully stacked five genes into a single plant. Physiological and biochemical characterization of these transgenics is underway. The goal is to pyramid these core biochemical genes and transporters into a single plant that will form a basic C<sub>4</sub> rice prototype to validate proof of concept.

## P39

### **Analysis of photoperiod control in Bambara groundnut (*Vigna subterranea* L.), an underutilised tropical legume with potential to contribute to future food security**

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Photoperiod can regulate a diverse set of traits in crops, including flowering, pod-set, pod-filling and, hence, yield. However, research on photoperiod control of traits other than flower date is limited. In Bambara groundnut (*Vigna subterranea* L.), an African underutilised legume which thrives in drought prone and marginal soils, incorrect photoperiod can prevent pod-set and pod-filling. An understanding of photoperiod responses would facilitate the development of cultivars of this drought tolerant species with a wider geographic range. The objective of this study is to identify QTLs controlling pod production and other photoperiod regulated traits. A dense genetic map was constructed in an F<sub>2</sub> population derived from quantitative long day and qualitative short-day parents (IITA-686 and Ankpa4, respectively) based on SNP and DArT markers. The linkage map consisted of 1238 marker loci (859 SNPs and 379 DArTs), with good coverage (1185 cM spanning 11 linkage groups; one marker per 1 cM, on average). A major QTL was identified for pod number, which explained about 55% of the phenotypic variation in the cross. Furthermore, a comparative mapping approach reveals genome collinearity between *Vigna subterranea* and *Phaseolus vulgaris* in identified QTL regions. These results will be used for Marker Assisted Selection in Bambara groundnut.

## P40

### The distribution of arsenate tolerance in plant populations throughout Europe: implications for mineral nutrition

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In the grass *Holcus lanatus* L., tolerance to arsenate is thought to be controlled by a single gene polymorphism that has consequences for the ability of plants to acquire phosphate, an analogue of arsenic that is crucial for plant growth and nutrition. Here, we determined the extent of arsenate tolerance in 29 plant populations, of which 20 were monocots and 9 were dicots, to test how widespread arsenate tolerance is in other species throughout the UK and mainland Europe. The study revealed that among the monocots, 8 species were tolerant to arsenate, and this has not been recorded in 5 of the species (*Hordeum secalinum*, *Festuca filiformis*, *Phleum bertolonii*, *Koeleria macrantha* and *Agrostis vinealis*). Among the dicots, only 2 species were tolerant to arsenate, and only one of these (*Plantago media*) was a new record. Among the tolerant species, the population of *Holcus lanatus* from Aberdeen had a significantly greater proportion (73%) of individuals that were tolerant to arsenate compared to other species. Second highest proportion (72%) of tolerant individuals had in the population of *H. secalinum*. In the population of *H. lanatus* from seed-origin, 71% individuals of the population was tolerant to arsenate while it was 55% in the population of clonal plants. There was also a positive correlation ( $r = 0.6$ ,  $P < 0.01$ ) between tolerance as seedlings and tolerance as adults of *H. lanatus*. With the application of arsenate, tiller root length of non-tolerant clones of *H. lanatus* decreased significantly while the root length of tolerant clones was similar to that of control. The study might help to draw attention on new arsenate tolerant plant populations for further research to introduce them successfully in the rehabilitating and revegetating programs of the arsenate polluted sites.

## P41

### **Integrative approaches to understanding and improving drought stress tolerance in minor crop species - Bambara Groundnut**

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Bambara groundnut (*Vigna subterranea*) is an underutilised crop which has caught the attention of researchers particularly in the past twenty or so years. As a legume it can also tolerate low fertility soils, producing nitrogen through symbiosis with nitrogen fixing bacteria. Seeds also command a high market price. It is the third most important legume in Africa after groundnut and cowpea. Underutilized species could help make the world more food secure, but the lack of funding available makes it difficult to work on underutilised species. Using data from major crops to work in minor crops is one way to reuse this investment in major crop research. The main goal of the project is to identify genes of potential importance to drought tolerance. Starting off with a range of transcriptomic data from microarray generated in the new species of interest –Bambara Groundnut, and also using tools designed for major or model species to begin to form a multidimensional dataset which can facilitate working in minor crops. The aim is to profile the response of the Bambara transcriptome to drought stress using a Genomic DNA based probe selection by cross hybridising with Soybean GeneChip array. The results demonstrate that DipC Genomic DNA has identified more gene transcripts that had expression level >2 fold when hybridised to Soybean GeneChip compared with Tiga Necaru (TN) gDNA. Gene annotations based on Soybean databases identified genes that play key roles during drought stress.

## P42

### Involvement of ORA59 in *mlo*-based resistance of *Arabidopsis thaliana* against powdery mildew fungi

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The mutation of distinct members of the MLO family confers pre-penetration resistance to many plants against infection by powdery mildew fungi. Although the protective effect of *mlo* has long been known, the signaling mechanisms inducing the resistance phenotype are still not untangled. Therefore we investigated the transcriptional response of the *Arabidopsis thaliana mlo2 mlo6 mlo12* triple mutant after inoculation with *Golovinomyces orontii* in comparison to wild type plants and found enhanced and accelerated induction of genes involved in JA signaling. Responsive genes included the AP2/ERF transcription factors and integrators of Jasmonic acid and ethylene signaling *ORA59* and *ERF1*. Investigation of the T-DNA insertion mutant *ora59* resulted in a partial suppression of *mlo2*-mediated immunity and thus points to an involvement of ORA59 in mediation of *mlo*-based resistance. As a next step we investigate possible reasons for enhanced and accelerated transcription in *mlo2 mlo6 mlo12*.

# **Switchgrass root growth parameters impacted by nitrogen fertilization rate and landscape positions**

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Switchgrass (*Panicum virgatum* L.) has been extensively studied for its value as a forage, conservation, and bioenergy crop, and soil carbon sequestration. The present study was conducted near Bristol in South Dakota, USA to evaluate the nitrogen (N) fertilizer and landscape positions impacts on switchgrass root growth parameters. Switchgrass was planted in 2009 on a marginal land previously used for croplands, and root sampling was done after 4 years of planting (2013). The study site consists 2 N levels (0, and 112 kg/ha) and three landscape (shoulder, backslope and toe) positions with 4 replicates. Soil cores from 0-100 cm depth were collected from every treatment and each core was subdivided into five sections (0-15, 15-30, 30-45, 45-60, and 60-100 cm). Roots were separated from soil for these individual depth sections, and root physical properties (length, area, volume, and density) for each depth were measured using WinRHIZO software. Soil properties (such as soil organic and inorganic carbon, bulk density, and N) were related to root growth. Results from this study imply that establishment of switchgrass on marginal lands improve soil carbon accumulation and root parameters which enhance soil quality thus improving the switchgrass biomass production and environmental quality of the landscape.

## P44

### The GRAS transcription factor *NSP2* controls GA-related nitrogen responses in *Arabidopsis thaliana* roots

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Nodules and lateral roots are key organs for the uptake of nutrients by plants. During nodulation, some plants form root nodules, housing rhizobial bacteria able to fix atmospheric nitrogen, allowing the plant to use it. Lateral roots allow the root system to be extended laterally, increasing the region from which nutrients may be taken up. Formation of lateral roots and nodules share developmental features such as single cell-type origins of the primordia, and hormonal and nutrient regulatory mechanisms, so it is hypothesised that the evolution of nodulation co-opted elements of pre-existing genetics mechanisms of lateral root formation. In this work, *A.thaliana* (non-legume) homologous genes to known *M.truncatula* (legume) nodulation genes were screened for phenotypic effects on the root. A mutant of *Arabidopsis* GRAS-domain SCR-like transcription factor (*AtSCL26*, homologous to *MtNSP2*) was found to alter lateral root architecture. This mutant was examined at the cell type level using cell-type specific transcriptomics through Fluorescence-Activated Cell Sorting (FACS). *AtSCL26* was found to modulate lateral root development through pathways involving the phytohormone Gibberellic Acid (GA). Treatment with GA rescued some components of the phenotype, indicating a potential role for the gene in activating GA biosynthesis.

## P45

### *Arabidopsis* utilizes triacylglycerides as energy source for stomata movements

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Stomatal opening is an active process that requires ATP. We have recently found evidence that triacylglycerols (TAG) are broken down and metabolised to meet some of the requirement for ATP during light-induced stomatal opening. We have shown that guard cells of *Arabidopsis thaliana* contain lipid bodies and using Nile red (NR) fluorescence have observed a significant reduction in the total volume of lipid bodies during stomatal opening. This reduction is even more pronounced in the reduced starch mutant *pgm* while there is no significant reduction in NR fluorescence in the TAG lipase mutant *sdp1*. Leaf epidermal bioassay demonstrated that *sdp1* plants have delayed stomatal opening. Our data are consistent with the suggestion that guard cells utilize TAG as an energy source to help stomatal opening.

## P46

### **Dark and disturbed or just disturbed? Modelling thermal tolerance to determine habitat preferences in early angiosperms**

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Despite more than a century of research, some key aspects of habitat preference and ecology of the early angiosperms remain poorly constrained. Proposed ecology has varied widely – from opportunistic weedy species growing in full sun to slow-growing species limited to the shaded understory of gymnosperm forests. Evidence suggests that the earliest angiosperms possessed low transpiration rates – the gas exchange rates for extant basal angiosperms are low, as are the reconstructed gas exchange rates for the oldest known angiosperm leaf fossils. Leaves with low transpirational capacity are vulnerable to overheating in full sun, favouring the hypothesis that early angiosperms were limited to the shaded understory. Here, modelled leaf temperatures are used to examine the thermal tolerance of some of the earliest angiosperms. Our results indicate that small leaf size could have mitigated low transpirational cooling capacity of many early angiosperms, enabling many species to survive in full sun. We propose that during the earliest phases of angiosperm evolution, angiosperms were not limited to the understorey and that some species were able to compete with ferns and gymnosperms in both shaded and sunny habitats, especially in the absence of competition from more rapidly growing and transpiring advanced lineages of angiosperms.

## P47

### **TaR1 provides a link between chromatin remodelling and wheat defence against *Mycosphaerella graminicola***

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*Mycosphaerella graminicola* is a hemibiotrophic fungal pathogen of wheat, which causes the foliar disease Septoria Leaf Blotch (stb). Stb is one of the most economically damaging wheat diseases worldwide, and is defined by a long undetected period of growth between mesophyll cells, before the plant shows any symptoms. Despite this, very little is known about the interaction, how the fungus remains undetected or how the switch to necrotrophism and cell death is brought about. This aim of this project was to use Virus Induced Gene Silencing to knock down expression of single wheat genes and to examine their effects on the development of symptoms and spore production in this interaction. In doing so, we have found a novel PHD domain protein, which disrupts the timing of symptom development and leads to a reduction in spore production. This protein binds to specifically methylated histones, and may bring about large-scale transcriptional changes by recruiting histone modifiers to sites of active chromatin.

## P48

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

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Self-incompatibility (SI) is a key mechanism used by many flowering plants to promote outcrossing. A multi-allelic *S* locus allows discrimination between 'self' pollen from 'non-self' pollen on the stigma. Interaction of cognate pollen and pistil *S*-determinants triggers rejection of incompatible pollen. The *S*-determinants for *Papaver rhoeas* are *PrpS* and *PrsS*. *PrsS* is a secreted protein; *PrpS* is a novel transmembrane protein. Interaction between cognate *PrpS* and *PrsS* triggers programmed cell death in incompatible pollen.

We recently introduced *PrpS* into self-compatible *A. thaliana*. When transgenic pollen expressing *PrpS* was grown *in vitro* with *PrsS* protein, a remarkably similar response to that triggered in incompatible *Papaver* pollen was elicited. Whether *PrsS* can be expressed and functional in *A. thaliana* remains to be established. To establish this and ultimately to establish if the *Papaver* SI system functions *in vivo* in *A. thaliana*, we introduced *PrsS-GFP* into *A. thaliana*. Here we will provide evidence demonstrating the expression of *PrsS-GFP* in *Arabidopsis* stigmas. We will also present preliminary *in vivo* pollinations with transgenic *Arabidopsis* plants expressing *PrsS*- and *PrpS*. Our data suggest that *PrsS* is functional and can interact with cognate *PrpS* *in vivo* to inhibit 'self' pollen tubes in *A. thaliana*.

## CO<sub>2</sub> bursts from the stem and branches to the atmosphere help trees to avoid winter embolism

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Woody plants suffer from winter embolism because it decreases their water transport capacity. In winter embolism, gas bubbles are formed in xylem conduits during freezing since gases are not soluble to ice, and the bubbles are in a risk to expand and fill the conduits with air during thawing. In this study, we test if all the gas formed during freezing actually stays in the conduits or will the gas efflux from stem during freezing thus reducing the negative impacts of winter embolism? We conducted freezing experiments for three *Pinus sylvestris* and three *Picea abies* saplings in a laboratory and measured CO<sub>2</sub> efflux from the stems. We further analyzed the size of the freezing-related CO<sub>2</sub> burst relative to the modeled CO<sub>2</sub> content of the xylem. Considerable freezing-related CO<sub>2</sub> bursts were detected during freezing implying that all gases are not trapped inside the ice in the stem during freezing as previously assumed. This result adds a new dimension to the understanding of winter embolism formation. It is concluded that conduit's water volume alone does not determine the volume of bubbles formed during freezing, but also the efficiency of gas efflux out from the conduit.

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Because of resource limitations, a trade-off between growth and immunity exists in plants, suggesting that these processes are controlled by interconnected signaling pathways. This trade-off needs to be finely regulated to ensure proper allocation of resources in an efficient and timely manner. The first layer of plant immunity is based on the perception of pathogen-associated molecular patterns (PAMPs) by surface-localized pattern-recognition receptors, leading to PAMP-triggered immunity (PTI). A unidirectional antagonism between BR and PTI signaling could be recently demonstrated, but the exact underlying mechanisms remained controversial. Here, we confirm that the BR-PTI antagonism does not occur at the level of the receptor complexes. Instead, we reveal that the BR-responsive transcription factor BZR1 is required and sufficient for suppression of PTI. The BZR1-mediated suppression of PTI is particularly relevant under conditions in which fast growth is required, such as etiolation. We propose a model in which BZR1 acts as a molecular integrator of environmental cues, generating appropriate outputs to regulate the trade-off between growth and defense. Additionally, we have evidence suggesting that activation of PTI down-regulates BR biosynthesis. Together, our findings indicate that the BR and the PTI signaling pathways mutually interact to define a dynamic balance between growth and immunity.

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During the diversification of flowering plants, C<sub>4</sub> photosynthesis evolved independently from C<sub>3</sub> ancestors over 60 times in distantly related taxa, but the details of the changes responsible for C<sub>4</sub> evolution remain poorly understood. Here, we analyze the variation in photosynthetic types that exists within a single species, the grass *Alloteropsis semialata*. We characterize the distribution of <sup>13</sup>C and leaf anatomy across the species' range in a spatio-ecological context and build a phylogeographic hypothesis based on genetic markers. The <sup>13</sup>C values present a continuous distribution that suggests the presence of C<sub>3</sub>–C<sub>4</sub> intermediate physiology. Individuals at the C<sub>3</sub> and C<sub>4</sub> ends of the <sup>13</sup>C spectrum inhabit different environments, however, there is a region of overlap in eastern Africa – the region where C<sub>3</sub>–C<sub>4</sub> intermediates also occur. Some anatomical traits shift abruptly across the <sup>13</sup>C spectrum, while others form a continuum. The environmental drivers behind the variation in these traits and environmental inducibility of each C<sub>4</sub> anatomical trait are presented. Thus, anatomical and physiological continua are discussed in light of the species' phylogeography and phenotypic plasticity for C<sub>4</sub> anatomy, shedding new light on C<sub>4</sub> evolution among populations of the same species.

## P52

### High throughput phenotyping for identification of variation in drought responses of *Miscanthus* under drought stress

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*Miscanthus* is a genus of  $C_4$  perennial grasses of great interest for biorenewable energy because of high biomass potential even in temperate regions. Yield is linked to water availability and many sites across Europe where irradiation and temperature are favourable for *Miscanthus* cultivation have limited water supply. The aim of this research is to screen a range of *Miscanthus* genotypes for biomass accumulation under drought using a phenomics facility. Facility allowed the generation of high-quality time – course data for large number of plants and high throughput imaging provides a valuable tool which allows thorough analysis of plant responses to drought. 47 *Miscanthus* genotypes, identified based on collection site and genotypic data, were screened in the National Plant Phenomics Center in Aberystwyth. Plants in triplicate were subjected to three treatments (control, 20% of field capacity and withdrawn water) under controlled photoperiod and temperature. Visual spectrum images were taken on a daily basis to assess biomass accumulation. Image analysis was used to determine growth rate, plant survival and to relate drought tolerance to geographical and meteorological data. To build a model predicting actual biomass from digital biomass plants were harvested at three time points for stem and leaf wet and dry matter.

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Abscisic acid (ABA) plays a key role in coordinating plant stress responses and is well-characterized in the stomatal closure under drought stress. The plant N-end rule pathway which targets specific proteins for degradation is known to regulate ABA-induced seed dormancy in *Arabidopsis thaliana*. In this study experiments were designed to investigate whether the biochemical mechanisms associated with the N-end rule pathway of targeted proteolysis could be involved in the regulation of stomatal apertures in *Arabidopsis thaliana*. To investigate a possible role for the N-end rule pathway in regulating ABA-induced stomatal closure, mutants lacking the N-recognin PRT6 (PROTEOLYSIS6) were investigated. The results indicate that the gene encoding the plant N-recognin, *PRT6 (PROTEOLYSIS6)*, and the N-end rule pathway, are important in regulating stomatal ABA-responses in addition to their previously described roles in germination and hypoxia. Direct measurements of stomatal apertures showed that plants lacking PRT6 exhibit hypersensitive stomatal closure in response to ABA, and IR thermal imaging revealed reduced evapotranspiration under drought-stress. Together with a reduction in stomatal density, these properties result in drought tolerant plants. In conclusion, our work demonstrates the N-end rule pathway normally restricts ABA-induced stomatal closure.

## P54

### Functional analysis of the POLARIS peptide in *Arabidopsis thaliana*

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The phytohormones ethylene, auxin and cytokinin play a pivotal role in many plant processes, including differential cell elongation and division, patterning, root development and apical hook formation. The *polaris* (*pls*) mutant was created by promoter trapping in *Arabidopsis*, with homozygous seedlings showing a phenotype associated with defects in ethylene and auxin signaling: a triple response phenotype in the absence of ethylene, decreased primary root length and reduced leaf venation. Accordingly, the *POLARIS* gene is transcribed predominantly in the root and in leaf vascular tissue, and encodes an mRNA of c. 600 nucleotides, translated into a 36 amino acid peptide. In *Arabidopsis*, ethylene is detected at the endoplasmic reticulum (ER) by a family of ethylene receptors. Evidence points to the *POLARIS* peptide functioning at the level of ethylene detection, localised to the ER and physically interacting with the ETR1 receptor. PLS appears to negatively regulate ethylene downstream responses, potentially acting as a way to reset receptors after ethylene binding. *POLARIS* is a new component enmeshed in a signaling pathway network involving hormonal crosstalk; its expression regulated by, and actually regulating, ethylene, auxin and cytokinin. We discuss how, via this network, the *POLARIS* peptide is important for the control of root development.

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Perhaps one of the greatest challenges facing science and society today is to detect and predict biological responses to environmental change such as human impact and warming. Tropical and subtropical forests maintain high levels of biodiversity and are facing worldwide decline becoming one of the most threatened ecosystems. Thus, understanding the disturbances influencing their ecological dynamics is critical to prevent their decline. Much excellent work has been carried out on plant-environment interactions, providing valuable data about plant survival and adaptation (e.g. altering their metabolism and physiology) to their habitat or to a warming planet. But a palaeoecological view about the capacity to adapt in many environmental conditions is missing, although recent studies show promising results, i.e. determining rates of biodiversity responses and vegetation sensitivity to past climate change. By looking back in time through long-term datasets we highlight: (1) how dynamic (chronically hyperdynamic) are tropical forests to land-use change and which are the main drivers of change, and (2) how resilient individual plant species and their assemblages are to past and future disturbances such as drought and climate.

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When bacterial pathogens infect a plant to colonize the intercellular spaces (the apoplast), they encounter a mildly acidic environment rich in organic acids, sugars and amino acids, which induces expression of bacterial virulence genes and supports high levels of bacterial growth. Previous research demonstrated that *Pseudomonas syringae* pathovars display differing sets of metabolite transporters, metabolic enzymes and preferred substrate sources which indicate a high degree of adaptation to the specific nutritional conditions of their host plant apoplast. Plant immune responses act to strongly limit bacterial proliferation, however, little is known about how the overall composition of the apoplast changes throughout the infection process and what effect these changes have on bacterial growth and virulence. Here, apoplastic fluid was extracted from *Phaseolus vulgaris* leaves during an infection timecourse with *P. syringae* pathovar phaseolicola leading up to the hypersensitive response. Apoplastic metabolites, metal ions and proteins were quantified using several analytical techniques including atomic absorption spectroscopy, inductively couple plasma-MS, gas chromatography-MS and orbitrap-LC-MS. The results indicate that large, rapid changes in apoplast composition occur throughout early infection due contributions from both plant and bacterial activities. Specific metabolite changes, particularly iron and citrate levels, are discussed in the context of the plant-*Pseudomonas* interaction.

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Parasites induce striking changes in their hosts. How and why they do this remains to be elucidated. Insect-transmitted bacterial phytoplasmas induce developmental changes in a broad range of plant species, including the conversion of flowers into leaves and the proliferation of stems. Here we report how and why phytoplasmas cause these dramatic changes. Phytoplasma produces a small virulence protein - effector SAP54 - which triggers the formation of leaf-like flowers. We hypothesised that the SAP54-induced plant phenotype promotes insect colonisation. We performed series of dual-choice experiments with *Macrostes quadrilineatus*, the insect vector of Aster Yellows phytoplasma strain Witches' Broom (AY-WB), and quantified leafhopper progeny on the test plants to elucidate the role of SAP54 in plant-insect interactions. *M. quadrilineatus* produced significantly more nymphs on AY-WB-infected plants and plants expressing SAP54 with leaf-like flowers compared to control plants with wild-type flowers. Taken together, SAP54 alters floral development of the host plant and enhances insect vector colonization. This would improve the acquisition and transmission of the pathogen by its insect vector in nature. Future work aims to dissect the mechanisms involved in SAP54-induced enhancement of insect colonisation and thereby better understand the links between floral development and plant defences against herbivores.

## P59

### Exploiting the natural diversity of Rubisco kinetics

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Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) enables net carbon fixation during photosynthesis. Rubisco first evolved 3 billion years ago, in a CO<sub>2</sub>-rich environment with very little molecular oxygen. Some characteristics of Rubisco are still constrained by its ancient reaction mechanism, making the enzyme surprisingly inefficient and compromising photosynthetic productivity. Improving Rubisco function has the potential to deliver improved photosynthetic performance in specific crops and environments. Characterisation of only a small fraction of the diverse forms of Rubisco has provided evidence for natural variation in its catalytic properties, suggesting that further diversity in Rubisco kinetics is likely. Rubisco kinetic properties are being characterised in a diverse range of plant species to establish natural variation and identify a better enzyme for crop photosynthetic improvement. Species have been identified that - based on their native environment - are predicted to have promising Rubisco kinetic properties. The initial data set has already enabled identification of better performing Rubisco forms that might be exploitable to improve photosynthesis in crops. Key amino acid residues associated with better catalytic performance will be identified for verification studies using transgenic approaches.

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The economically important disease powdery mildew is caused by biotrophic fungal pathogens, with almost 10,000 angiosperm species being affected; for example *Blumeria graminis* infects small grain cereals such as wheat and barley. The powdery mildews compromise host immunity through ‘secretory warfare’ with fungal effectors, including *Blumeria* Effector Candidates (BEC)s, being delivered at the haustorial complex. Eight BECs have had their effector function verified, including BEC1054, which is a ribonuclease-like protein (Pliego *et al.*, 2013). Putative interactors were identified for BEC1054 through *in vitro* pulldowns from barley epidermal and whole leaf extracts. The bait was comprised of recombinant BEC1054 with an N-terminal His tag, which had been expressed in *E. coli* and purified. A total of 247 putative interactors were identified which bound uniquely to BEC1054, when compared with those that bound to the negative controls or an unrelated BEC. Within the putative interactors, elongation factor (eEF) and small-ribosomal subunit related proteins were found to be overrepresented for almost all experimental conditions used. A yeast-two-hybrid assay was used to further validate the interactors. A glutathione-S-transferase (GST) was found to interact weakly in two bait-prey orientations; a pathogenesis related protein 5 (PR5) in one bait-prey orientation, and eEF1G in one bait-prey orientation.

# P61

## A small-secreted protein of the poplar leaf rust fungus targets plant chloroplasts

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Chloroplasts are central plant organelles. Pathogens may benefit by subverting chloroplastic functions to their advantage. Indeed, some effector proteins of plant pathogenic bacteria have been reported to target chloroplasts. To date, fungal and oomycete plant pathogen effector proteins that target chloroplasts have yet to be described. We report that a 171 amino acid small-secreted protein from the poplar leaf rust fungus *Melampsora larici-populina* targets chloroplasts. This protein, termed Chloroplast-Targeted Protein 1 (CTP1), is expressed in haustoria during poplar leaf infection and carries a predicted 80 amino acid chloroplast transit peptide (cTP) downstream of its signal peptide. A CTP1 C-terminal fusion to the Green Fluorescent Protein (GFP) traffics to chloroplasts when it is transiently expressed in the cytosol in *Nicotiana benthamiana* leaf cells. The predicted cTP is cleaved *in planta*, and is sufficient to translocate GFP into chloroplasts. We conclude that CTP1 is a complex modular protein that utilises a cleavable cTP to target chloroplasts. CTP1 is part of a *Melampsora*-specific family of polymorphic small-secreted proteins. Several CTP1 homologs from *M. larici-populina* and from the flax rust fungus *M. lini* also target chloroplasts. We hypothesize that the CTP1 family has evolved recently in *Melampsora* species to manipulate host chloroplasts functions.

## P62

### Pathogen effector induced interaction of the resistance protein Cf-4-SOBIR1 complex with BAK1 initiates endocytosis-regulated plant immunity

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The plant immune system is activated by receptors that detect infectious pathogens. Cf receptor-like proteins (RLPs) induce immunity against *Cladosporium fulvum*, a fungus causing tomato leaf mold disease. Using live-cell imaging, we find that Cf-4, in complex with SUPPRESSOR OF BIR1-1 (SOBIR1), localizes at the plasma membrane. Upon elicitation with the matching *C. fulvum* effector Avr4, the receptor complex is recruited to ARA7/ARA6 late endosomes, providing a mechanism to regulate the number of active receptors at the plasma membrane. Furthermore, we discovered that the Cf-4-SOBIR1 complex behaves like a two-component RLK that interacts with the co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1) upon its activation by Avr4. Importantly, BAK1 is required both for Avr4-triggered Cf-4-SOBIR1 endocytosis and plant defense. Our observations indicate that Cf-4 immune signaling is initiated by the formation of at least a tripartite receptor complex. These findings provide a significant contribution to elucidating how RLPs, lacking a cytoplasmic signaling domain, initiate immune responses upon ligand perception. Currently, we continue investigation of the cell biology of Cf proteins, as well as the role of clathrin and kinase activity of signaling partners in RLP-mediated immunity.

## P63

### Determining Rubisco kinetic data from diverse Triticeae species

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Rubisco is the key enzyme that fixes carbon dioxide during photosynthesis. Constraints associated with its complex reaction mechanism make the enzyme surprisingly inefficient and limit photosynthetic productivity in current and projected climates. However, natural variation in Rubisco kinetic properties exists and can be exploited to improve photosynthesis in crop species. We have used biochemical activity assays to survey the kinetic properties and specificity factors of Rubisco from 25 Triticeae species, including wild relatives of wheat. Measurements were taken at two temperatures (25 and 35°C). For all genotypes, carboxylation rates were higher at 35°C (between 1.3 and 2.6 times the rate at 25°C), while specificity factor was lower at the elevated temperature. At both temperatures a positive correlation was found between carboxylation rate ( $V_c$ ) and Michaelis-Menten constant for  $\text{CO}_2$  ( $K_c$ ). Through modelling of photosynthetic rate using these newly available kinetic parameters, we have identified 4 Rubiscos from different Triticeae species that perform better than control wheat Rubisco in a model wheat leaf. These species represent useful candidates for wheat photosynthetic improvement through breeding or other techniques such as genetic engineering.

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

Therefore, we propose that RAD antagonises DIV in a subcellular competition for a DRIF protein by inhibiting the interaction between DIV and DRIFs in the dorsal regions of the *Antirrhinum* flower in order to establish the asymmetric pattern of gene activity in the flower meristem.

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## P65

### Ethylene regulation of adventitious root formation: a role in hypoxia tolerance

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Adventitious root formation is the formation of roots from non-root tissues such as stems or leaves. They are essential for clonal propagation of commercially important plants and dominate the root system of monocot crops. In addition adventitious roots form in response to flooding in an ethylene-dependent manner, improving survival and productivity. Since flooding events are set to become more frequent in some areas of food production understanding adventitious root development is essential. Building on our previous findings that strigolactone regulates adventitious roots we studied the interaction between strigolactones and ethylene. Using mutants and hormone treatments we demonstrate that strigolactones and ethylene act independently on adventitious root formation and also discovered that treatment with the ethylene precursor, ACC, changes the position of adventitious root formation in Arabidopsis. Adding GR24 inhibited the adventitious root formation in both the upper and lower region of the hypocotyl together with and without ACC treatments. To unravel the spatial and temporal nature of this regulation we used ethylene constitutive response lines in the *ebf1ebf2* double mutant and the EIN3::GFP marker line. Better understanding of the root response to hypoxia (such as adventitious root development) will allow us to engineer roots for increased food security.

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Transcriptomic research investigating plant immunity has been conducted at the organ or organism scale. Moving from this level to cell type-specific analyses revealed a much deeper insight into plant adaptation to abiotic stresses and identified regulatory principles due to a higher resolution of underlying gene networks that were not identifiable in previous analyses.

Root immunity functions in a cell type-specific manner as exemplified by the expression of immune receptors such as the *Arabidopsis thaliana* danger-associated molecular pattern (DAMP) receptor PEPR2 in the stele of roots or the broader expression pattern of its counterpart PEPR1. Our studies further revealed that mutualistic microbes such as *Piriformospora indica* selectively reconfigure root immune signalling in order to establish successful root symbioses. We will present data indicating cell-specific immune signalling patterns in roots and how the mutualistic fungus *P. indica* modulates respective signalling outcomes in different spatial contexts of the root.

## P67

### Gene identification of sorghum mutants using next generation sequencing

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Determination of factors controlling 'Kranz' anatomy has been a great challenge although these are necessary for engineering  $C_3$  crops into  $C_4$ . To identify the genetic factors regulating Kranz anatomy, we mutagenised a million seeds each using gamma rays and EMS chemical. The mutagenised seeds were grown until M2 generation for screening for target phenotypes. Candidate mutants with low vein density- like in  $C_3$  plants were back-crossed with wildtype and further grown to generate BC1F2 population. The BC1F2 progenies with and without the traits were sampled, DNA pooled and sequenced using next generation sequencing (NGS). NGS produced short reads were culled and aligned against the reference genome of *Sorghum bicolor* BTx623 and the wild type whole genome sequence. Five genes with SNP for EMS mutants and three genes with structural translocation in gamma mutants were identified as the causal factors with the highest effects. One gene was common for both mutants. The gene expected to be one of the responsible factors for Kranz anatomy is being verified through transgenic approach.

## P68

### **Liming (to recommended rates) limits legume growth and gas exchange by increasing root-to-shoot signalling of the phytohormone abscisic acid**

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Soil acidification is a natural process that can be hastened by intensive agricultural practices such as excessive use of mineral nitrogen fertilisers. If unchecked, these may negatively affect crop yield via altered nutrient availability and aluminium toxicity. Low soil pH is traditionally managed by applying lime (calcium carbonate) to target soil pH ranges (6–6.5) that optimise nutrient availability and subsequent yield. However, recommended rates of liming can decrease crop yields in susceptible soil types, possibly by reducing phosphorous availability though there is little mechanistic information on how plant physiological responses limit yield. Shoot dry weight of pot grown pea (*Pisum sativum* L. cv. Alderman) amended with lime to target pH 6.5 (recommended rate) was reduced by 38% and gas exchange (stomatal conductance and photosynthesis) was inhibited by 50% and 32% respectively when compared to un-limed control plants (pH 5.7). Xylem sap and tissue analysis suggest that reduced gas exchange is caused by an increase in the plant hormone abscisic acid (ABA) which decreases stomatal conductance. The ABA deficient mutant pea ‘*wilty*’ showed an attenuated stomatal response to liming, apparently confirming that increased ABA is mediating legume responses to low phosphorus availability under recommended rates of liming.

## P69

### Stress that cress! Osmotic stress and root development in *Arabidopsis*

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Drought is the most significant cause of crop globally and plants require a well-developed root system to cope with stress. Under osmotic stress plants use hormone-signalling systems to remodel their root system architecture.

Our group previously identified peptide called POLARIS (PLS), which is auxin responsive and inhibits of ethylene responses.

Modelling and experimental analysis of auxin, ethylene, cytokinin and PLS interactions revealed a crosstalk circuit that regulates root growth. This work uses Bayesian emulation methodology for response surfaces, to explore parameter space of the model and to help in the design of novel experiments offering new insights into root development.

Mutant analysis of *p/s* and *pro35S::PLS* reveals a root growth phenotype under osmotic stress, indicating a link to osmotic stress responses. Severe osmotic stress causes increased expression of *PLS* and reduced expression of ethylene responsive genes.

Our previous work has shown that PIN1 protein levels are greatly increased in *p/s* and reduced in *pro35S::PLS*. This work shows that osmotic stress leads to decreases in PIN1 and PIN4 levels, and a change in auxin distribution. This work indicates the critical role of an auxin, ethylene, PLS and PIN protein crosstalk circuit to integratively regulate root development under osmotic stress.

## P70

**Phosphatase and tensin homolog (PTEN) is a growth repressor of both rhizoid and gametophore development in the moss *Physcomitrella patens***

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Phosphatase and tensin homologue (PTEN) main catalytic function is to dephosphorylate PtdIns(3,4,5)P<sub>3</sub>, a potent second messenger that elicit cellular behaviours that favour oncogenesis. In animal cells, loss of PTEN leads to increased levels of PtdIns(3,4,5)P<sub>3</sub> and a potent derepression of the phosphoinositide 3-kinase (PI3K)–AKT pathway that stimulates cell growth, survival, energy metabolism and cellular architecture.

Interestingly, PtdIns(3,4,5)P<sub>3</sub> is the only known phosphoinositide so far not detected in any plant system, and the enzymes that synthesize PtdIns(3,4,5)P<sub>3</sub> in animal cells do not exist in plants. Our studies are based on the moss *Physcomitrella patens* which has four *PTEN* genes ubiquitously expressed during the whole moss life cycle. By using a knock in approach we show that these genes are expressed in actively growing tissues, specifically in caulonemal and rhizoid cells and at the subcellular level, PpPTENs are found in the cytosol and in the nucleus. Triple and quadruple *pten* knockouts are characterized by caulonemal cells with a higher growth rate than wild type, an early switch from the juvenile protonemal stage to the development of adult gametophores and enhanced rhizoid production. Our results support the role of PpPTEN as a suppressor of cell growth in plants.

## P72

### Correcting the scientific literature: A case study on the basis of the rice immune receptor XA21

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Plants possess a multi-layered innate immune system that is able to detect danger signals to prevent successful infection. At the plasma membrane receptor kinases recognize apoplastic danger signals as elicitors and initiate intracellular signaling cascades. In rice the immune receptor kinase XA21 confers broad-spectrum resistance against most isolates of the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*. In 2009 the Ronald laboratory reported the bacterial protein Ax21 as the elicitor of XA21 immune signaling. However, while attempting to build on these studies, new lab members, including myself, could not reproduce the key initial observations. We discovered that several strains were mislabeled. The correct bacterial Ax21 mutant strain does not overcome XA21-mediated immunity. In addition a key bioassay assessing the eliciting capacity of Ax21 on XA21 plants was found to be not robust. In summary we demonstrated that Ax21 is functionally unrelated to XA21 and not the elicitor. I will present how diligent teamwork and constructive efforts lead to the correction of the scientific literature. I will highlight the validity of other key bacterial genes required for activation of XA21-mediated immunity known as *Rax* genes. Lastly, I will describe new scientific approaches we have undertaken to identify the real ligand of XA21.

## Photosynthetic and antioxidant enzyme capacities as potential indicator of water stress in some tolerant and sensitive tomato genotypes

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A greenhouse experiment was conducted to assess water stress modulation in gas exchange attributes and some key enzymes of oxidative defense system in eleven local/exotic tomato genotypes at 80% of field capacity (optimum watered), 60% and 40% (water stress) of field capacity. Activities of genotypes were significantly different under these water regimes. Significant decrease was recorded for net CO<sub>2</sub> assimilation rate (*A*), transpiration rate (*E*) and stomatal conductance (*g<sub>s</sub>*) of all genotypes of tomato but this decline was less in tolerant genotypes. Concentrations of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased in water stress tolerant genotypes, whereas activities of these enzymes in water stress sensitive and moderately water stress tolerant genotypes decreased, or remained unchanged. Thus, degrees of stress tolerance in tomato genotypes cannot be related with greater activities of these antioxidant enzymes. Although wild type tomatoes were water stress tolerant, they are not preferred for market view point. Water stress tolerant genotypes Lyallpur-1 and CLN1767 were most tolerant tomato genotypes characterized with higher antioxidant and photosynthetic capacity. Overall, it was found that some tomato genotypes maintained their degree of water stress tolerance during their growth but mechanism of water stress tolerance varies in different tomato genotypes. It can be concluded that selection based on photosynthetic activity and antioxidant capacity under appropriate water stress conditions similar to target environments are critically important for improving both drought tolerance and tomato yield potential which is of great commercial importance hence these could be used as potential selection criteria for screening germplasm for drought tolerance.

## P76

### Root development and hormone interaction: a systematic view on POLARIS peptide and auxin biosynthesis

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The auxin biosynthesis pathway has been a mystery for more than a century since Charles Darwin's discovery. Even more mysterious is its interaction with other plant hormones and relevant genes to coordinate plant growth. They can work either synergistically or antagonistically to form an entangled network in which they interact with each other. We have previously shown that the POLARIS peptide has a key role in mediating the ethylene response, auxin distribution and root development in *Arabidopsis thaliana*. Recently, with a combination of molecular experimental approach and computational modeling, we have found a potential acting point of POLARIS on auxin biosynthesis pathway. Results indicate that the POLARIS peptide is a positive regulator of auxin biosynthesis, separate from a role in ethylene signalling.

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Pre-Harvest Sprouting (PHS) is the precocious germination of grains before harvest. The consequence of this on-spike germination is a reduction in grain quality resulting in significant losses to farmers (up to 25% premium loss). PHS is believed to be caused by insufficient grain dormancy depth. However, very little is known about the molecular mechanism controlling the imposition and release of dormancy in wheat grains. To understand the genetic control of PHS, we are characterising 6 QTLs for their potential to confer stable resistance to PHS. Of particular interest is the *PHS1* QTL which accounts for up to 20% of the phenotypic variation observed in the field. We show that *PHS1* confers resistance by reducing the rate of dormancy decay in grains. Thus, *PHS1*+ lines have reduced germination compared to *phs1*- lines. Importantly, by harnessing synteny, classical genetics and recent advances in wheat genomics we have fine mapped *PHS1* to a genomic region containing 3 genes in wheat. This work will shed new insight into the molecular regulation of dormancy in wheat and also demonstrates how the genetic control of traits in species with complex genomes can be unravelled.

## Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen species that limit plant cell death and promote infection

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Plants and animals produce reactive oxygen species (ROS) in response to infection. In plants, ROS not only promote cell death to limit the spread of pathogens but also restrict the amount of cell death in response to pathogen recognition. Plants also use hormones, such as salicylic acid, to mediate immune responses to infection. However, there are long-lasting biotrophic plant-pathogen interactions, such as the interaction between parasitic nematodes and plant roots during which defense responses are suppressed and root cells are reorganized to specific nurse cell systems. In plants, ROS are primarily generated by plasma membrane-localized NADPH oxidases, and loss of NADPH oxidase activity compromises immune responses and cell death. We found that infection of *Arabidopsis thaliana* by the parasitic nematode *Heterodera schachtii* activated the NADPH oxidases RbohD and RbohF to produce ROS, which was necessary to restrict infected plant cell death and promote nurse cell formation. RbohD- and RbohF-deficient plants exhibited larger regions of cell death in response to nematode infection, and nurse cell formation was greatly reduced. Thus, by stimulating NADPH oxidase-generated ROS, parasitic nematodes fine-tune the pattern of plant cell death during the destructive root invasion and may antagonize salicylic acid-induced defense responses during biotrophic life stages.

## Medicinal plants in socioeconomic context: convergence of Australian Aboriginal and Western perspectives

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Collaborative and investigative research that merges Western and traditional scientific philosophies of Australian Aboriginal medicinal plant resources may provide a mechanism for improving the socioeconomic circumstances of these peoples. Working with multiple Australian Aboriginal communities, we are exploring a number of candidate plants with therapeutic benefits in areas that include inflammation, diseases of microbial origin, cancer and immune disorders. The research is dependent on the amalgamation of multi-disciplinary fields of expertise in traditional Aboriginal knowledge, chemistry, biology, anthropology and business development. The studies have been successful in providing a Western scientific view of the medicinal properties of selected plants, complementing the underlying traditional knowledge and preserving this for future generations. The medium to long-term objective of our research partnerships is to translate basic research into opportunities for commercial development of plant-derived therapeutics. Successful execution of such endeavors will provide employment and training opportunities on traditional homelands and capacity building through the development of locally-driven and sustainable business enterprises. It is imperative that collaborative initiatives like these are implemented using culturally appropriate methods. If this holds true, the outcomes are capable of delivering benefits both to the wider community and with potential to impact on the global population alike.

## P80

### Oxidative stress induced transcriptome: alternate way to identify novel effectors from necrotrophic fungi

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One of the earliest responses towards attempted pathogen attack of plants is oxidative burst followed by localized cell death, termed as hypersensitive response (HR). This suits well for fungi deriving nutrition as true biotroph from host, but failed to check growth of those necrotrophic fungal pathogen that derive nutrition from dead plant tissue. Such fungus will need an adaptation to face early HR and may be even utilizing it for their advantage. To explore such possibility, we try to identify genes of a necrotrophic fungal pathogen *Ascochyta rabiei* through sequencing of a subtractive cDNA library generated against mild level of oxidative stress. Many stress inducible genes were found to be induced along with some novel genes of unknown identity (hypothetical protein). When one such gene was further characterized, it proved to be a major virulence factor and probably an effector for *A. rabiei*. This gene encoded a secretory protein with no characterized domain in protein sequence. Pathogenesis was drastically reduced in knockdown strains. As no confirm source of resistance available for the devastated disease caused by *A. rabiei* against chickpea, this work may help in identification of resistance source. Our work further suggests that oxidative stress inducible protein may play major role in fungal pathogenesis.

# Through the eye of a root: carbon and nitrogen dynamics across a landscape-scale grazing experiment

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Plant roots represent the largest carbon (C) input into grassland soils. Livestock grazing is seen as a potential management tool to enhance C storage within the soil, yet little is known about how grazing regulates root decomposition. To investigate the influence of livestock grazing on annual root decomposition we used a litterbag experiment (measuring loss of mass, C and nitrogen (N)) for four common upland grass species across a landscape-scale grazing experiment (including high-intensity sheep stocking (2.7 ewes ha<sup>-1</sup> yr<sup>-1</sup>), low-intensity sheep stocking, mixed cattle and sheep, and no livestock). We found that species-specific losses of C and N were similar across all grazing treatments. This suggests an overriding importance of root traits, which was further supported by a microcosm decomposition study that found that key root traits (e.g. specific root area and phosphorus content) determined rates of decomposition. Results from the decomposition experiments suggest that livestock will impact belowground C and N dynamics through their effect on plant species composition and associated root traits. Further research will focus on screening root traits across the landscape-scale grazing experiment and connecting grazing-induced changes in root characteristics with changes in soil properties, micro-organism communities, and greenhouse gas emissions.

## P82

### Three simple rules to grow a liverwort

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The earliest plants emerged on land around 470 million years ago and principally grew across a single plane. A planar growth habit is maintained in modern liverworts that have a fleshy 'thallus', which grows as a flattened forking mat to maximise surface area. The land plant radiation was underpinned by the evolution of mechanisms allowing colonisation of aerial space by shoots, and leaves subsequently evolved to maximise surface area in this context. Whilst the mechanistic basis of planar growth in leaves has been studied in several plants, it is unknown whether this is applicable to other planar structures. Based on a quantitative growth description from the liverwort *Marchantia polymorpha*, we have generated an *in silico* model of thallus morphogenesis. This suggests that three simple, biologically plausible rules can reproduce thallus growth patterns and generate a realistic overall shape. Firstly, growth decreases with distance from the apex. Secondly, the apex and base anchor a polarity field, which promotes growth parallel to apical-basal polarity. Thirdly, growth at the apex must be inhibited to give a characteristic notched shape. Our model provides a starting point for identifying molecular regulators of liverwort shape and a basis for developmental comparisons between planar growth mechanisms in plants.

## Redox mediated regulation of microRNA, hormone homeostasis and its significance for regulating salt tolerance in *Brassica juncea*

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Elucidating the transcriptional control in plants under stress conditions is of prime importance for understanding the global responses to climate change. In the proposed research, we demonstrated the use of thiourea (non physiological thiol based ROS scavenger) for understanding the role of redox mediated salt-responsive microRNA/hormones in *Brassica juncea*. Initially, post-germination phenotyping was performed under NaCl (150 mM) +/- TU (75  $\mu$ M) treatment. A genome wide scan of smallRNAs was performed on SOLID platform and then miRNAs were identified using ShortStacks and read clustering approach by mapping the smallRNAs reads to the available *Brassica rapa* genome database. In total, we identified 90 novel mature microRNAs including 40 pre-microRNAs with intact 3p' and 5p'. The bra-miR824 showed 100-fold over-expression in root as compared to shoot followed by miR156 and miR169. In contrast, miR-156 showed 10-fold overexpression as compared to roots in shoots followed by miR-167a. The functional analysis of targets using qRT-PCR revealed the involvement of different hormones such as ABA (miR393), auxin (miR394) and jasmonate (miR319). Using HPLC based techniques, the specific induction of these hormones under NaCl+TU treatment was confirmed. Thus, the study highlights the significance of redox state for regulating miRNAs/hormone mediated defense against salt stress in plants.

## P84

### Forward genetics in *Physcomitrella patens* identifies the novel ABA regulator PpANR

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Absciscic acid (ABA) is a phytohormone that regulates plant responses to water stress and was likely crucial to the successful colonisation of land by plants *ca.* 480MYA. The 'core' ABA-signalling pathway has been shown to be highly conserved between the early diverging bryophytes and angiosperms particularly through reverse genetic analysis in the model bryophyte *Physcomitrella patens*, aided by its excellent gene-targeting capabilities. Now, the recently completed chromosome-scale genome assembly and updated gene annotations allow forward genetics to be effectively implemented to identify bryophyte-specific processes. We used high-throughput genotyping to identify a novel ABA-regulator, the protein kinase 'PpANR', by map-based cloning of an UV-mutagenised **ABA Non-Responsive (***anr***)** mutant defective in ABA-regulated growth, molecular responses and drought tolerance. The role of this gene was confirmed by targeted gene knockout in wild-type *P. patens*. PpANR has similarities with the angiosperm ethylene response regulator CTR1 in possessing both an EDR and a protein kinase domain, but it also contains an additional N-terminal PAS domain which has itself been found to be involved in ABA-signalling by precision targeted point mutagenesis. This gene appears to have been lost during vascular plant evolution suggesting a more complicated evolution of the core ABA response pathway than previously thought.

## P85

### ***Brachypodium*: a new model grass species in stomatal research**

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Stomata are small pores found on leaf surfaces. Research on understanding the cellular aspects of how stomata work has concentrated in recent years on *Arabidopsis thaliana*. However stomatal function and development of grasses have not been fully studied in the past. Fortunately, a recently sequenced model grass, *Brachypodium distachyon*, with its short life cycle is suitable for carrying out such work. We evaluated the suitability of this species for use in stomatal research. Since *Brachypodium* and *Hordeum vulgare* (barley) are both from the same subfamily (Pooideae) and barley is also another model species, these two candidates were compared and tested using bioassay of epidermal peels to study stomatal function. In the stomatal function part of this poster, the results of stomatal response to abscisic acid high concentration of CO<sub>2</sub> and darkness will be presented. In the stomatal development the results of increased photon irradiance is shown to increase stomatal density and stomatal index but higher CO<sub>2</sub> concentration (1000ppm) failed to change the stomata number of *Brachypodium*.

## P86

### Elucidating the nutrient resorption in senescing plant tissues using metabolomics approach

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Nutrient resorption is a fundamental ecosystem process that not only affects the fitness of perennial species but also influences ecosystem nutrient cycling. Currently nutrient resorption is evaluated using elemental ratios. However, the identity of compounds within these broader ratios is fundamental in deciding the fitness of plants. Climatic and edaphic conditions can largely affect resorption with climatic stress predisposing plants to produce and resorb compounds that favor the fitness of the species. We evaluated the nutrient resorption at a metabolic level in *Quercus rubra* subjected to a factorial combination of warming and altered precipitation at the Boston-Area Climate Experiment, Waltham, MA. The green and senesced leaf tissues were analyzed for both polar and non-polar metabolites using gas chromatography mass spectrometry platforms. Plants exposed to drought with high warming treatments induced the production of nitrogen based metabolites while those exposed to wet precipitation treatment under ambient temperature invested more in carbon based metabolites. Drought increased the resorption efficiency (RE) of metabolites while warming decreased RE of metabolites indicating that temperature stress might be detrimental to plant fitness. Our results suggest that warming and altered precipitation would differentially affect both production and resorption of metabolites affecting plant fitness and terrestrial nutrient cycling.

## P87

### **Plant surface texture: a comparative analysis of R2R3 MYB subgroup 9 gene function in *Marchantia* and *Nicotiana***

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The subgroup 9 gene family of R2R3 MYB transcription factors is an ancient gene lineage that arose prior to the origin of the land plants. However, they have been functionally characterised only within the flowering plants, where they regulate diverse epidermal processes including petal epidermal cell outgrowths, trichome development, and the initiation and elongation of cotton fibres. Broadly, my PhD seeks to understand the ancestral function of these genes prior to their role in the mediation of epidermal processes in flowering plants. I will present data suggesting that the role of subgroup 9 homologs in affecting epidermal cell shape arose early on in land plant evolution. Using semi-quantitative PCR, overexpression and amiRNA knock-down in the liverwort model *Marchantia polymorpha*, I will show that the single *Marchantia* subgroup 9 homolog (*Mpsg9*) is likely to be mediating the development of thallus epidermal structures.

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Due to the appearance of a multicarpellary-ovary, *Thermopsis turcica* possesses a valuable character in the breeding of fruit crops among the plant species of the family *Fabaceae*. Although this endemic endangered plant species is very important for crop improvement, based upon our review of the literature, no hybridization technique has to date been performed on this species. To understand whether gene transfer is possible between *T. turcica* and another species (*Vicia faba*), crosses in this study were first accomplished by a classic technique. Cross- and self-pollination were performed at Nezahat Gokyigit Botanical Garden of Istanbul, during the pollination period of May and June 2012. Pistil samples were collected from the first to tenth day after pollination to perform pollination possibilities. Following staining and squashing, the samples were then observed under fluorescent microscope. As a result, in all samples of one day after pollination, pollen tubes reached the ovary. Ovule development tended to increase from 1 to 5 days after pollination. To determine embryo formation, paraffin block analysis was implemented and globular hybrid embryos were observed. The findings presented have implications for crop improvement and use of this endangered rare species in further plant breeding research.

**From the <sub>low</sub> past to the <sup>high</sup> future: plant growth across CO<sub>2</sub> levels****A. A. TEMME, W. K. CORNWELL, J. H. C. CORNELISSEN, R. AERTS***Department of Ecological Science, VU University Amsterdam, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands*

Since the industrial revolution atmospheric CO<sub>2</sub> concentration has increased from 280 ppm to nearly 400 ppm, a value not experienced by plants for over 10 million years. Over much of that period CO<sub>2</sub> levels have been even lower than preindustrial levels, down to 180 ppm. Plants' recent history has thus been under carbon starvation while over the next 90 years atmospheric CO<sub>2</sub> is expected to rise to a bountiful ~800 ppm. Very little is known on how plant morphology and physiology drive growth at low CO<sub>2</sub> and how these relationships may shift with increasing CO<sub>2</sub>. In a climate chamber experiment we germinated and grew seedlings of 30 species (C<sub>3</sub>, C<sub>4</sub>, woody, herbaceous) at past low (150ppm), ambient, and future high CO<sub>2</sub> (750ppm). Our aim was to understand how plant traits are affected by CO<sub>2</sub> and if and why winners and losers in terms of growth performance shift going from past to future CO<sub>2</sub> concentrations. Results show the effect of morphological traits common to fast growth to be diminished at low CO<sub>2</sub> suggesting physiology to be of greater importance at poor CO<sub>2</sub> conditions. Ongoing work focuses on chemical composition and photosynthesis and the interaction between CO<sub>2</sub> and drought with promising results.

## P90

### **Growing urban agriculture: elucidating the mechanisms of tip burn in Vertical Farming Systems**

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Vertical Farming Systems (VFS) are capable of increasing yield per unit area. We have recently identified tipburn of lettuce as key limitation to crop quality in a VFS designed by Saturn Bioponics Ltd. Tipburn symptoms were observed in the top layers of the VFS, which were exposed to supra-optimal environmental conditions. Although these conditions have been reported to induce tipburn, the underlying mechanisms are not fully understood. This work aims to elucidate the mechanistic basis of tipburn by investigating the roles of ROS and localised calcium deficiency in inducing the disorder under supra-optimal environmental conditions. VFS are prone to marked gradients in the growing environment; a deep flow hydroponic system was used to eliminate these gradients. Two light intensities ( $450 \mu\text{mol m}^{-2}\text{s}^{-1}$  and  $170 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) were applied, simulating the conditions within the top and bottom layers of the VFS. Biochemical assays were used to quantify MDA content and antioxidant enzyme activity. Preliminary data showed that plants grown under high-light intensity exhibited oxidative stress and upregulation of antioxidant machinery. This study has established a deep flow hydroponic system as a model for studying tipburn. This will enable the underlying mechanisms behind the tipburn observed in the VFS to be investigated.

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Climate change effects on many ecosystem processes depend on how atmospheric warming alters near surface and soil microclimate. This is complicated by the plant community, which also regulates microclimate. Indeed, the extent to which the plant community modifies atmospheric warming effects on microclimate is currently unknown. Here, we used a five-year dataset from a unique peatland atmospheric warming and plant functional type (PFT) removal experiment to determine whether distinct PFTs differentially modify warming effects on canopy temperature, soil temperature, and water level. We found that dwarf-shrubs lowered canopy and soil temperature by up to 0.65 °C, but also dampened warming effects on both air and soil temperature. This was particularly striking in the soil, where dwarf-shrub presence entirely reversed the effects of warming on soil temperature. Graminoids and bryophytes also reduced soil temperature (0.13–0.30 °C), and additionally lowered water level (2.05–3.27 cm). Furthermore, warming effects on canopy and soil temperature were exacerbated by graminoid presence. Our findings show that distinct PFTs strongly modify atmospheric warming effects on microclimate. Consequently, we reveal that vegetation composition plays a key role in regulating the full impact of climate change at a scale relevant to the functioning of terrestrial ecosystems.

## P92

### Cross-talk between formation of lignin and suberin through the ABA signal during the cell wall modification

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Water and solutes at the root surface are transported to the central xylem vessels by extracellular space (apoplastic) or via cell-to-cell transport (symplastic). In the early developmental stages of *Arabidopsis*, epidermal cell walls don't represent a significant barrier to the diffusion of solutes, but endodermal layer cell wall modifications (Suberin and Casparian strip) play a more important role in realizing the specificity of both water and solute transport without completely blocking their diffusion. Recently, a dirigent-domain containing protein, ESB1 was identified. ESB1 plays an essential role in the correct formation of Casparian strips. In the absence of ESB1 disordered and defective Casparian strips are formed, and ectopic deposition of suberin can also be found. Through the observation of lateral root development of *esb1-1* under the NaCl stress and the suberin deposition response to the exogenous hormone, we propose that the ectopic deposition of suberin is a consequence of the defect in Casparian strip formation, and induction of ABA signal through the kinase is the bridge of these two developmental process. The ionomic analysis in *esb1-1* also shows that the formation of Casparian strip determine the concentrations of some solutes in the shoot, which may link the ABA signal pathway to regulate the deposition of suberin in the stage II.

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Mutualistic ectomycorrhizal interactions (ECM) that exist between soil fungi and tree fine roots are essential to forest sustainability. However, very little is known on how the ECM symbiosis is initiated in both partners. The colonisation of the root rhizodermis and cortex by the fungal hyphae is precisely tuned and tightly controlled to allow proliferation of hyphae in the host roots and avoid plant defence reactions. The ECM fungus *Laccaria bicolor* secretes the effector protein MiSSP7 which is required for symbiosis development<sup>1</sup>. In the present study, we identified two poplar Jasmonate-ZIM-domain proteins (JAZ) as direct interactors of MiSSP7. JAZ proteins are involved in hormonal homeostasis and act as jasmonic acid co-receptors. JAZ proteins mediate signalling pathways in response to biotic and abiotic stresses and act to modulate the development of plant organs, such as roots. Thus, these JAZ proteins are likely targets of *Laccaria* MiSSP7 to allow root colonization. Here, we present the results of protein-protein interaction studies to decipher the roles of both MiSSP7-targeted JAZ proteins in root development, i.e. the identification of their interaction partners within the JA-signaling cascade. Further, we will discuss the effects of RNAi knock-down and overexpression of those proteins in poplar.

<sup>1</sup>Plett JM, Kemppainen M, Kale SD, Kohler A, Legué V, Brun A, Tyler BM, Pardo AG, Martin F. 2011. A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology* 21: 1197-1203

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Most plant resistance genes encode nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors that recognize effectors secreted by pathogens. However, little information is known about NB-LRRs involved in important crop pathosystems. *Phytophthora infestans* is a notorious oomycete plant pathogen that causes late blight on potato and tomato. Rpi-blb2, an NB-LRR protein from wild potato *Solanum bulbocastanum*, recognizes effector AVRblb2 from *P. infestans*, and confers late blight resistance in potato and the model solanaceous plant *Nicotiana benthamiana*. In this study, we found that an additional NB-LRR protein, we termed NRB1, is required for the function of Rpi-blb2 as well as some other immune receptors. Using immunoprecipitation and mass spectrometry, we identified that Rpi-blb2 associates with NRB1 in *N. benthamiana* leaf lysates. The association between Rpi-blb2 and NRB1 was validated by *in planta* co-immunoprecipitation. By using gene silencing, we showed that NRB1 is genetically required for Rpi-blb2-mediated resistance and cell death. Remarkably, NRB1 is also required for the activities of two additional NB-LRR proteins, potato blight resistance protein R1 and nematode resistance protein Mi. Furthermore, tomato and potato NRB1 homologs complemented NRB1 function in *N. benthamiana*. We conclude that NRB1 is a signaling hub for a subset of NB-LRR immune receptors in solanaceous plants.

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Individual grain weight is an important component of yield in wheat; larger grains have been a key breeding target to boost yield. In this study, we identified the potential physiological traits associated with grain weight, and subsequently the quantitative trait loci (QTL) for these traits in a recombinant inbred line population derived from a cross between bread wheat and spelt. The stem water soluble carbohydrate (WSC) content and carpel size at anthesis, grain filling rate, maximum grain water mass, grain length, width and height, and grain volume were positively correlated with grain weight. Nine QTL were detected for grain weight, individually explaining 6.3–20.9% of the phenotypic variation. Three QTL for carpel dry weight, three for WSC content per shoot, four for grain filling rate, five for maximum grain water mass, four for both grain length and height, and six for grain volume were detected. QTL coincidence was found on chromosomes 2A, 3B, 4A, 5B and 7B, indicating pleiotropy or tight gene linkage. Therefore, these traits are the physiological determinants of grain weight and the potential targets to enlarge sink size. The QTL associated with these traits will enable marker-assisted selection in breeding for grain weight improvement.

## P96

### **MTR1, a rice fasciclin glycoprotein, controls the development of reproductive and somatic cells in anther**

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In the angiosperm anther, the reproductive cells and tapetum have direct contact and show postmeiotic synchronous development, which is crucial to the formation of fertile pollen. However the underlying mechanism for this communication remains poorly understood. In rice (*Oryza sativa*), MICROSPORE AND TAPETUM REGULATOR1 (MTR1), a secretory, plasma membrane localized, fasciclin glycoprotein, is specifically expressed in the male reproductive cells, but its mutant exhibits defects in the tapetum causing complete male sterility. Identification and characterization of MTR1 interacting factors is the key to reveal how MTR1 controls both development of the sporophytic and reproductive cells. A series of putative MTR1 interacting proteins were identified by a previous yeast-two-hybrid screen of a rice anther cDNA library. *N. benthamiana* leaf transient fluorescence resonance energy transfer (FRET) assay was carried out to confirm these interactions. Additionally, phylogenetic analysis was conducted on the Fasciclin-Like Arabinogalactan-Protein (FLA) family both in rice and Arabidopsis, putative Rice paralogs and Arabidopsis orthologs of MTR1 were identified. Among the putative interacting targets, Anther 7 and 17 have the highest possibility to genuinely interact with MTR1. The phylogenetic analysis indicates that MTR1 is not the only functioning FLA protein in tapetum or microspore development. Orthologs of MTR1 in Arabidopsis and paralogs of that in rice may play a conserved role in anther development.

**‘Safe harbors’ for foreign gene targeted insertion in rice were identified from transgenic rice plants expressing maize C<sub>4</sub> photosynthetic genes.**

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Agriculture always seeks enhanced crop performance over history. C<sub>4</sub> photosynthesis has higher efficiency in using water and nitrogen and higher average yield under tropical/subtropical climate conditions. Scientists have been aiming to import C<sub>4</sub> traits into rice (C<sub>3</sub> plant) to confer higher rice yield potentials. To establish C<sub>4</sub> photosynthesis in rice involves engineering large number of genes to modify the metabolic pathway and leaf anatomy. Conventional *Agrobacterium*-mediated plant transformation delivers genetic materials with T-DNA randomly into plant genome. Recent advances in genome editing using TALEN, ZFN and CRISPR/Cas allowed researchers to manipulate genome in a precise manner and to avoid unwanted effects in gene expression. Several genetic loci in rice IR64 genome were studied here to provide researchers candidate ‘safe harbours’ for safer and more efficient foreign gene expressions. The selected events were single copy events with high protein expression over generations. A series of analysis identified 2 loci at non-coding regions, and are capable of harbouring foreign DNA size of up to 9 kb. Plants showed no unexpected phenotypes over generations. Possible TALEN pairs showed no off-target. The discovery and validation of ‘safe harbours’ provide researchers available gene target loci, and will benefit plant cell engineering to improve crop performance.

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Rice yields are strongly influenced by changing biotic (i.e. presence of arbuscular mycorrhizal fungi (AMF)) and abiotic factors (i.e. nutrient availability) due to anthropic activities, including AMF inoculation and nutrient fertilization. The direction and degree of phenotypic plasticity to such changing factors is of interest, particularly in biomass allocation and traits influencing seed number (e.g. panicle number per hill, spikelet number per panicle and percentage of filled spikelets). Using a field experiment in NE China, we explored the direction and degree of the plasticity in traits indicating biomass allocation and seed number, using rice exposed to six fertilizer levels, with or without inoculation with AMF. At maturity, we quantified shoot: root ratios, panicle:shoot ratios, seed weights and traits influencing seed number. In response to variation in nutrient supply or AMF inoculation, changes in rice yield occurred via the plasticity at whole-plant, shoot, and reproductive organ levels. Our results also demonstrate that rice yield can be regulated largely by changing panicle number per hill, while little improvement in rice yields can be achieved by plasticity in seed weights. Conclusively, understanding the regulation of rice yields would benefit from a dual focus on traits *per se* and the plasticity in these traits.

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Plant secondary metabolites are derived from primary metabolism and have been targets for plant metabolic engineering for a long time. Using transcription factors to activate/suppress secondary metabolism has been used a major strategy to engineer plants with desired secondary metabolites. However, the importance of primary metabolism pathway in secondary metabolites engineering only began to emerge very recently. Needs of engineering phenylpropanoid compounds in plants are increasing, but the yielding of desired compounds are often very low. We found when overexpressed in tomato, in addition to up-regulate secondary metabolism genes, *AtMYB12* also activates genes in primary metabolism to drive the carbon flux towards phenylpropanoid pathway. Co-expression of *AtMYB12* with other transcription factors or structural genes in tomato fruit can significantly enhance the production of target compounds. *AtMYB12* can regulate both primary and secondary metabolism in tomato and can be used as a general tool for phenylpropanoid compounds engineering.

## **P100**      **Isolation and characteration of two *FNS II* genes reveals a novel pathway for flavone biosynthesis in roots of the medical plant, *Scutellaria baicalensis***

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*Scutellaria baicalensis* root specific flavones (RFSs) were reported to have a variety of specific health benefits such as anti-fibrotic to liver, anti-virus and anti-cancer properties. We report here the isolation and characteration of two *FNS II* genes. FNSII-1 has broad specificity for flavanones with or without 4'-OH. However this isoform had a higher expression in aerial tissues of *Scutellaria* and is, most likely, involved in the synthesis of scutellarin. FNSII-2 is most highly expressed in roots, is specific for pinocembrin (flavanone without a 4'-OH) and is induced in its expression by methyl jasmonate. RNAi experiments showed that FNSII-2 is responsible for the synthesis of RSFs like wogonin, wogonoside, baicalein and baicalin in roots. The results also indicate that there is a novel flavones synthesis pathway in the root of *Scutellaria baicalensis*, in which pinocembrin was used as the key intermediate rather than naringenin, and also suggest that a specific isoforms of 4CL could convert cinnamic acid and maybe isoforms of CHS and CHI are required for the formation of pinocembrin.