

# New Phytologist next generation scientists

24–26 July 2017 John Innes Centre, Norwich, UK

Programme, abstracts and participants

WILEY





# **New Phytologist**

### next generation scientists

John Innes Centre, Norwich, UK

24-26 July 2017

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Programme, abstracts and participant list compiled by Jill Brooke and Sarah Jose 'New Phytologist next generation scientists' logo by A.P.P.S., Lancaster, UK

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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 <a href="http://www.newphytologist.org/">http://www.newphytologist.org/</a>.



### Wiley

Wiley is the world's leading society publisher and a market leader in publishing plant science research.

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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:

https://www.newphytologist.org/nextgeneventpages/index/9

### Catering and social event

**Coffee breaks** will take place in the foyer at set times as indicated in the programme. **Lunch** on the 24<sup>th</sup> July will be available from 12:00 to 13:30 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 13:30. A buffet lunch will be served 12:30–13:30 on the 25<sup>th</sup> July and lunch will be available at the close of the meeting from 12:20 to 13:20 on the 26<sup>th</sup> July.

### Social event Monday evening:

- A food gazebo serving pizza will be set up outside the venue from 19:00 to 22:00. Each delegate will be provided with a coupon which can be exchanged for a meal (2 slices of pizza and one side). Additional food can be purchased with cash.
- An ice cream stall will be set-up outside from 20:00 to 21:30. Each delegate will receive a coupon which can be exchanged for one ice cream/sorbet.

### Social event Tuesday evening:

A BBQ will be set up outside the venue from 19:00 to 22:00.

On both evenings a bar located next door to the conference centre will be open from 19:00 to 22:00. Delegates will be provided with two drink coupons for each evening 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, vegan, 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 at the Lodge Reception (see UEA campus map <a href="https://www.uea.ac.uk/documents/3154295/3352666/UEA+Campus+Map">https://www.uea.ac.uk/documents/3154295/3352666/UEA+Campus+Map</a>). Breakfast is included and is served in Zest. You must check-out by 10:00 on the morning of Wednesday 26<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 (12:00–13:30 on 24<sup>th</sup> July) and will be displayed for the duration of the meeting. There will be a dedicated poster sessions at 18:00–19:30 on Monday 24<sup>th</sup> and Tuesday 25<sup>th</sup> July. We ask that poster presenters with an odd number (1, 3, 5, 7, etc.) stand by their posters on Monday and poster presenters with an even number (2, 4, 6, 8, etc.) should stand by their posters on Tuesday. The poster hall will remain open until late on the 24<sup>th</sup> and 25<sup>th</sup> July and we encourage you to return to the posters during the social event. Drinks 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 the registration desk by 21:00 on Tuesday 25<sup>th</sup> July.

**Abstracts:** Abstracts are included in the digital/print 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 <a href="http://www.newphytologist.org/nextgensci/facebook">http://www.newphytologist.org/nextgensci/facebook</a>. Please include #NPNextGen in your tweets.

### Filming and photography

Photography and / or filming will take place at New Phytologist next generation scientists 2017.

The resulting photographs and video footage will be used by the New Phytologist Trust for the purpose of promoting its activities, and may be published on the New Phytologist Trust's website and social media channels.

If you do not wish to appear in the photographs or video footage, please speak to one of the organisers.

### Code of conduct

The New Phytologist Trust celebrates diversity and we expect participants in our meetings to be respectful, considerate and supportive of each other, to offer constructive critiques and embrace the variety of opinions on offer. New Phytologist next generation scientists 2017 is an opportunity to share, develop and broaden our viewpoints within a safe and inclusive setting, and we hope that you will enjoy the meeting. If you have any concerns or suggestions, please speak to one of the organisers.

#### Contact

For further information, and in case of any emergencies, please contact Helen Pinfield-Wells. Email: h.pinfield-wells@lancaster.ac.uk, np-symposia@lancaster.ac.uk; tel: +44 7966 450389.

# **Meeting programme**

### Monday 24th July

12:00-13:30	Lunch and registration	
13:30–13:40	Dale Sanders and Silke Robatzek	Welcome
13:40–14:30	Caroline Dean Plenary lecture	Sensing and remembering winter
14:30–14:50	Jonathan Cocker Selected poster talk	<b>P16:</b> Floral heteromorphy in <i>Primula</i> : new insights for an old model
14:50–15:10	Lucas Frungillo Selected poster talk	<b>P32:</b> S-nitrosothiol impact on hormonal trade-offs in plant immunity
15:10–15:40	Tea/Coffee Break	
15:40–16:10	William Anderegg Tansley Medal 2014 winner	Linking stomata and plant hydraulics to understand plant responses to drought
16:10–16:30	Hanna Hõrak Selected poster talk	<b>P47:</b> MPK12 and MPK4 regulate HT1 kinase in stomatal CO <sub>2</sub> -signalling
16:30–16:40	Break	
16:40–17:30	David Beerling Plenary lecture	We need to talk about climate change. How can we harness global croplands for climate change and food security?
17:30–18:00	Flash talks	
18:00–19:30	Poster session	
	Social event	
19:30		

### Tuesday 25<sup>th</sup> July

08:55-09:00	Announcements	
09:00–09:50	Liam Dolan Plenary lecture	Evolution and development of the plant soil interface
9.50–10:10	Scott Hayes Selected poster talk	<b>P41:</b> Soil salinity limits plant shade-avoidance
10:10-10:45	Tea/Coffee Break	
10:45-12:30	<b>Workshop:</b> How to get published	John Christie ( <i>New Phytologist</i> ), Chris Surridge ( <i>Nature Plants</i> ), Anne Knowlton ( <i>Current Biology</i> ), Adam Wheeler ( <i>Wiley</i> ) and Ashlynne Merrifield ( <i>Taylor &amp; Francis</i> ).
12:30-13:30	Lunch	
13:30–14:20	Dominique Bergmann Plenary lecture	Modulation of asymmetric division diversity through cytokinin and SPEECHLESS regulatory interactions, or, how to make different differences
14:20–14:50	Alexander Jones Tansley Medal 2015 winner	Dynamic regulation of gibberellin gradients influencing plant growth patterning
14:50–15:20	Tea/Coffee Break	
15:20-17:00	Workshop: Publishing ethics	Chris Graf (COPE)
17:00-17:10	Break	
17:10–18:00	Beverley Glover Plenary lecture	How do petals influence pollinator behaviour?
18:00–19:30	Poster session	
19:30	BBQ and band	

### Wednesday 26th July

08:55–09:00	Announcements	
09:00–09:50	June Medford Plenary lecture	Synthetic biology: using 'phytodetectors'
09:50–10:10	Qin He Selected poster talk	<b>P42</b> : <i>Phytophthora infestans</i> RXLR effector interacts with S factor NRL1 to promote turnover of SWAP70
10:10–10:30	Peter Marhavý Selected poster talk	<b>P63:</b> Short distance cell-to-cell communication in response to wound stress in Arabidopsis root
10:30-11:00	Break	
11:00–11:20	Ann Carla Staver Selected poster talk	<b>P94:</b> The evolution of flammability among grasses with spatially explicit fire spread
11:20–12:10	Ian Baldwin Plenary lecture	On becoming (and remaining) a plant scientist in the genomics era
12:10–12:20	Final comments	
12:20–13:20	Lunch	

### **Speaker abstracts**

### Monday 24th July

Sensing and remembering winter

Plenary lecture

**CAROLINE DEAN** 

13:40-14:30

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

Plants use seasonal cues to judge when winter is over and the time is right to flower. These cues are chiefly photoperiod and exposure to prolonged cold; plants need to integrate fluctuating temperature signals over many months and not get confused by a short period of cold in autumn. The sensing and remembering of long-term temperature exposure is a process known as vernalization.

Molecular genetic approaches in Arabidopsis have been instrumental in dissecting vernalization. In Arabidopsis, the vernalization pathways exert their effects chiefly through regulation of one gene. This encodes FLC, a transcriptional regulator that represses genes required to switch the meristem to a floral fate. Prolonged cold epigenetically silences FLC. Molecular analysis of the vernalization pathways has led us into the dissection of conserved non-coding RNA and chromatin mechanisms underlying epigenetic switches. The talk will describe our latest understanding of these conserved mechanisms, how they intersect to give robust and quantitative regulation of this developmental regulator and how these mechanisms have been modulated during adaptation.

# P16: Floral heteromorphy in *Primula*: new insights for an old model

Selected poster talk

JONATHAN M. COCKER<sup>1,2</sup>, J. LI<sup>1,2</sup>, J. WRIGHT<sup>3</sup>, M.A. WEBSTER<sup>1,2</sup>, M. MCMULLAN<sup>3</sup>, S. DYER<sup>4</sup>, D. SWARBRECK<sup>3</sup>, M. CACCAMO<sup>4</sup>, C.V. OOSTERHOUT<sup>1</sup>, P.M. GILMARTIN<sup>1,2</sup>

14:30-14:50

<sup>1</sup>School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK; <sup>2</sup>John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK; <sup>3</sup>Earlham Institute, Norwich Research Park, Norwich, NR4 7UH, UK; <sup>4</sup>National Institute for Agricultural Botany, Huntingdon Road, Cambridge, CB3 OLE, UK

The genetic and evolutionary basis of floral heteromorphy in *Primula* has been debated for 150 years. Darwin demonstrated how reciprocal anther and stigma positions in the two floral morphs, pin and thrum, serve to physically promote insect-mediated outcrossing. This phenomenon evolved independently in over 28 angiosperm families. The *Primula S* locus, which regulates heterostyly and self-incompatibility, is recognised as a "supergene", a cluster of tightly-linked genes inherited as one unit; self-fertile homostyle primroses, with anthers and stigma at the same height, were predicted to arise through rare recombination events in heterozygous thrums. This model underpins 60 years of research into heterostyly. To characterise the S locus we undertook assembly and annotation of the Primula vulgaris genome, alongside RNA-Seg and cross-species comparisons. We show the S locus is hemizygous in thrums, not heterozygous, comprising five thrum-specific genes absent from pin; homostyles result from mutation, not recombination. We identify the S locus genes, estimate assembly of the supergene at 51.7 MYA, and reveal conserved genetic architecture across the Primulaceae. These findings represent insight into the structure and origin of the *Primula S* locus, providing a platform for identification and evolutionary analysis of the genes regulating outcrossing in *Primula* and other heterostylous genera.

# P32: S-nitrosothiol impact on hormonal trade-offs in plant immunity

**LUCAS FRUNGILLO**, S.H. SPOEL

Selected poster talk

14:50-15:10

Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, EH9 3BF, UK

The bacterial pathogen *Pseudomonas syringae* (Psm) hijacks the plant immune system by secreting the phytotoxin coronatine, a highly active jasmonate (JA) analogue that promotes virulence by counteracting salicylate (SA) signalling. Conspicuous to immune responses, plants trigger marked cellular redox fluctuations that coordinate defence. Particularly, the redox active molecule, nitric oxide (NO), is extensively involved in shaping hormonal signalling during immunity. NO bioactivity is mediated through protein S-nitrosylation, i.e. the covalent attachment of a NO moiety to reactive thiol groups of proteins, forming a protein-SNO. Recently, Thioredoxin-h5 (TRX-h5) was reported to determine fate and amplitude of SA-mediated immune responses by manipulating specific branches of protein-SNO. However, it remains largely unknown if TRX-h5 controls other aspects of plant immune signalling. Here we investigated the role of TRX-h5 in providing specificity to protein-SNO signalling in SA/JA trade-offs during plant immunity. Infiltration of (S)NO mutants with Psm with or without COR revealed an unexpected interplay between protein-SNO and resistance. Additionally, TRX-h5 overexpressing lines in different backgrounds were tested for resistance against Psm +/-COR and transcription of SA- and JAmarker genes analysed. Overall, our data suggests that specific protein-SNO are manipulated by virulent pathogens to assist in the development of disease.

# Linking stomata and plant hydraulics to understand plant responses to drought

Tansley Medal 2014 winner

**WILLIAM ANDEREGG** 

15:40-16:10

Department of Biology, University of Utah, Salt Lake City, UT, USA

Forests absorb a quarter of human carbon dioxide emissions, greatly slowing down climate change, but the future of Earth's forests in a rapidly changing climate is highly uncertain. Forests' future will be greatly influenced by the race between increasing carbon dioxide concentrations and increasing climate stress, including drought, temperature, and fire. Scientific understanding and predictive ability of many of these processes, particularly drought stress on plants, is limited, which hinders our ability to forecast the future of forests. I will highlight several broad challenges in the field of predictive understanding of plant responses to drought stress. I will further show recent and ongoing research from our group on 1) the traits that influence plant mortality during drought, 2) spatial patterns and processes that underlie plant recovery after drought across the world, and 3) a new evolutionary approach that links plant carbon uptake and plant hydraulics in a predictive framework that has strong potential for improving ecosystem and vegetation models to shed light on the future of Earth's forests this century.

# P47: MPK12 and MPK4 regulate HT1 kinase in stomatal CO<sub>2</sub>-signalling

Selected poster talk

HANNA HÕRAK<sup>1</sup>, L. JAKOBSON<sup>1</sup>, M. SIERLA<sup>2</sup>, K. TÕLDSEPP<sup>1</sup>, L. VAAHTERA<sup>2</sup>, C. WANG<sup>3</sup>, Y.-S. WANG<sup>1</sup>, Y. SINDAROVSKA<sup>1</sup>, M. NUHKAT<sup>1</sup>, E. VALK<sup>1</sup>, P. PECHTER<sup>1</sup>, E. MERILO<sup>1</sup>, J. SALOJÄRVI<sup>2</sup>, K. OVERMYER<sup>2</sup>, M. LOOG<sup>1</sup>, J. KANGASJÄRVI<sup>2</sup>, J.I. SCHROEDER<sup>3</sup>, M. BROSCHÉ<sup>1,2</sup>, H. KOLLIST<sup>1</sup>

16:10-16:30

<sup>1</sup>Institute of Technology, University of Tartu, Nooruse 1, 50411, Tartu, Estonia; <sup>2</sup>Division of Plant Biology, Department of Biosciences, University of Helsinki, Viikinkaari 1, 00014, Helsinki, Finland; <sup>3</sup>Division of Biological Sciences, Cell and Developmental Biology Section and Center for Food and Fuel for the 21<sup>st</sup> Century, University of California San Diego, 9500 Gilman Drive #0116, La Jolla CA 92093-0116, San Diego, CA, USA

Plant gas-exchange with the environment occurs via stomata, small pores on the leaves and stems. Adequate stomatal responses to changes in environmental factors ensure efficient uptake of CO<sub>2</sub> for photosynthesis with minimal water loss. Increase and decrease in CO2 concentration cause stomatal closure and opening, respectively. The signal transduction events underlying these responses have largely remained elusive. We identified key regulators CO<sub>2</sub>-induced stomatal closure via analysis of ozone-sensitive mutants of Arabidopsis thaliana. A dominant mutation in HIGH LEAF TEMPERATURE1 (HT1) kinase, a regulator of stomatal CO<sub>2</sub> responses, caused high stomatal conductance and complete loss of stomatal CO<sub>2</sub>responsiveness. We also showed that MITOGEN- ACTIVATED PROTEIN KINASE12 (MPK12) is a regulator of stomatal CO<sub>2</sub> signalling as plants deficient in MPK12 had impaired stomatal responses to CO<sub>2</sub>. MPK12 and MPK4 interacted with HT1 and inhibited its activity in vitro. Lack of both MPK12 and MPK4 in guard cells caused complete CO<sub>2</sub>-insensitivity of stomata. These data indicate that MPK12 and MPK4 are negative regulators of HT1 kinase in stomatal CO<sub>2</sub>-signalling. Characterization of HT1-MPK interaction in crop plants will help to further understand how plants respond to the changing atmospheric CO<sub>2</sub> concentration and contributes to breeding of crop plants with higher water use efficiency.

We need to talk about climate change. How can we harness global croplands for climate change and food security? Plenary lecture

**DAVID BEERLING** 

16:40-17:30

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

The United Nations 21<sup>st</sup> Conference of the Parties in Paris marked a turning point in the climate change debate in which the focus shifted from describing climate change to a commitment to seek innovative, sustainable solutions. Achieved by amending soils of intensively managed croplands with crushed calcium and magnesium-bearing silicate and carbonate rocks, enhanced weathering is a natural mechanism for carbon sequestration and soluble alkalinity generation that ultimately reduces ocean acidification. With nearly 11% of the terrestrial surface annually managed for crop production, this could offer an opportunity to deploy a means of carbon sequestration at scale within a decade or two. Undertaken carefully, could a well-designed program capture carbon whilst simultaneously helping to restore soils, improve crop yields, and conserve geologic fertilizer resources? Donald J. Trump may have given up on climate change, David J. Beerling hasn't, as this presentation will explain.

### Tuesday 25th July

## Evolution and development of the plant soil interface

Plenary lecture

LIAM DOLAN 09:00-09:50

Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK

The evolution of the first rooting systems some time before 400 million years was a key innovation that occurred when the first complex multicellular eukaryotic photosynthetic organisms – plants – colonized the land. Rooting systems are important because they facilitate the uptake of most chemical elements that are required for plant growth. The rooting systems of the earliest diverging group of extant land plants comprised unicellular tipgrowing filaments called rhizoids and are morphologically similar to cells that develop at the interface between the plant and the soil in vascular plants – root hairs.

An aim of our research is to use genetics to define the regulatory mechanisms that controlled the development of the first land plant root system and determine how these mechanisms changed during the course of evolution. We identified regulatory components of the ancient control mechanism that positively regulated the formation of rhizoids in the extinct common ancestor of the land plants. This mechanism is preserved in most land plant lineages. By contrast, negative regulatory components evolved independently in different lineages and some are more than 370 million years old.

By combining evidence from paleontology, genetics and development we can construct a picture for the evolution of rooting systems in the 100 million years after plants colonized the land and radiated across the continental surfaces.

### P41: Soil salinity limits plant shadeavoidance

Selected poster talk

SCOTT HAYES<sup>1</sup>, A. TWEEN<sup>1</sup>, C. TESTERINK<sup>2</sup>, S. PRAT<sup>3</sup>, R. PIERIK<sup>1</sup>

9.50-10:10

<sup>1</sup>Plant Ecophysiology, Utrecht University, Padualaan 8, Utrecht, The Netherlands; <sup>2</sup>SILS, University of Amsterdam, Science Park 904, Amsterdam, The Netherlands; <sup>3</sup>Centro Nacional de Biotecnología, CSIC, Calle Darwin 3, Madrid, Spain

Global food production is set to keep increasing despite a predicted decrease in total arable land. To achieve this, denser planting will be required on increasingly degraded soils. In dense canopies, plants perceive shade through alterations in light quality, which then causes them to elongate until sunlight is reached. Shade-induced elongation is mediated by the bHLH transcription factors, PHYTOCHROME INTERACTING FACTOR4 (PIF4), PIF5 and PIF7. Here we demonstrate that very low levels of NaCl in soil strongly impair the ability of plants to respond to the threat of shade.

Using a combination of phenotypic, genetic and biochemical approaches, we show interaction between salt, abscisic acid (ABA), brassinosteroid (BR) and light signal cascades. The inhibition of shade-avoidance by salt is dependent upon ABA perception and signalling and requires the BR signalling kinase BRASSIONSTEROID INSENSITIVE 2 (BIN2). BIN2 is known to suppress PIF4 function. It is proposed that salt-mediated increases in ABA signalling enhances BIN2 action against the PIFs, thereby limiting shade-avoidance. The results represent a substantial step forward in our understanding of how multiple environmental factors are integrated towards optimal growth in variable environments.

Modulation of asymmetric division diversity through cytokinin and SPEECHLESS regulatory interactions, or, how to make different differences

Plenary lecture

### **DOMINIQUE BERGMANN**

13:30-14:20

Department of Biology, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA

The Arabidopsis stomatal lineage is a useful model to dissect cell fate decisions, cell polarity and cell communication in plants. This lineage not only produces essential cell types, but its stem cell-like precursor stage has the potential to modulate the ratios and overall numbers of these cell types, enabling the developmental flexibility plants exhibit in response to environment change (e.g., bigger or smaller leaves, more or fewer stomata). We have uncovered a regulatory circuit involving the hormone cytokinin (CK) and the key stomatal transcription factor, SPEECHLESS (SPCH) that modulates stomatal stem cell proliferation. This regulatory circuit dictates how often cells divide and, moreover, influences the type of asymmetric divisions made in the stomatal lineage. Expression analysis of TCSn, a reporter of CK response, revealed a spatially and temporally dynamic landscape of CK response during stomatal lineage progression, and we show that this landscape is sculpted by SPCH-dependent expression of CK-related signaling peptides and negative-acting regulatory elements. This system works orthogonally to the peptide-receptor-MAPK signaling previously described to regulate cell fate, cell polarity and cell patterning, thereby allowing plants to conditionally modulate leaf anatomy, yet maintain appropriate tissue pattern.

# Dynamic regulation of gibberellin gradients influencing plant growth patterning

Tansley Medal 2015 winner

### **ALEXANDER JONES**

14:20-14:50

The Sainsbury Laboratory, Cambridge University, Bateman St., Cambridge, CB2 1LR, UK

The phytohormone gibberellin (GA) is a key regulator of plant growth and development. An ensemble of enzymatic and transport steps control the cellular distribution of GA and these steps are themselves influenced by myriad endogenous and exogenous signals. Although the upstream regulation and downstream responses to GA vary across cells and tissues, developmental stages, and environmental conditions, the spatiotemporal distribution of gibberellin in vivo remains unclear. We engineered a highaffinity optogenetic biosensor, Gibberellin Perception Sensor 1 (GPS1), that senses nanomolar levels of bioactive gibberellins (K<sub>d</sub> = 24 nM for GA<sub>4</sub>). Arabidopsis thaliana plants expressing a nuclear localised GPS1 report on gibberellins at the cellular level. In rapidly elongating tissues in which GA promotes rapid cell elongation, i.e. root tips and dark-grown hypocotyls, GA levels correlated with cell length resulting in longitudinal gradients of GA. In roots, exogenous GA accumulation was also correlated with cell length, suggesting that a root GA distribution gradient can be generated independent of GA biosynthesis. In hypocotyls, GA levels were reduced in a phytochrome interacting factor (PIF) quadruple mutant in the dark and increased in a phytochrome double mutant in the light, suggesting that PIFs elevate GA in the dark and that phytochrome inhibition of PIFs lowers GA levels in the light. This result is in marked contrast to regulation of GA in seeds, where PIFs lower GA levels in the dark and phytochrome inhibition of PIFs elevates GA in the light. Better knowledge of cellular GA distributions and how they are determined will enable a deeper understanding of the signal integration upstream and growth programming downstream of GA, a small, mobile signalling molecule with substantial influence over plant growth and development.

# How do petals influence pollinator behaviour?

Plenary lecture

### **BEVERLEY GLOVER**

17:10-18:00

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

Flowers and the animals that pollinate them interact at a single key point — the petal epidermis. It is this single layer of tissue that provides the visual surface that advertises nectar and pollen rewards. It is on this layer of tissue that pollinators land, finding grip or slipping, and using tactile cues to locate rewards. And it is often from this layer of tissue that the scents that attract pollinators over longer ranges are released. We take an integrated evo-devo approach to understanding the petal epidermis, and our recent research has focused on its optical and tactile properties. I will present recent work on the nanoscale and microscale properties of the petal surface, describing a combination of developmental genetic, evolutionary and pollinator behavioural perspectives.

### Wednesday 26th July

Synthetic biology: using 'phytodetectors'

Plenary lecture

<u>JUNE MEDFORD</u> 09:00–09:50

Department of Biology, Colorado State University, Fort Collins, CO, 80523-1878, USA

Plants make exceptional chassis for synthetic biology in that they offer a platform that provides pathway to design and build sustainable systems for life on earth. For example, plants have their own onboard energy generating system (photosynthesis) and systems that enable them to extract other resources they need (such as water and nutrients) from their environment. The fact that plant platforms are also able to fully self-assemble and selfrepair makes them one of the most unique synthetic biology platforms. We have developed synthetic biology tools that enable amplification and memory of signals as well as "reset". When these signals are combined with computationally designed sensor proteins, a new detection technology, "phytodetectors". We have expanded our synthetic biology work to technologies that can provide humans and the environment with essential needs. Critical to development of new plant technologies is a fundamental understanding of plant anatomy. We will elaborate on the details of this work, with both near term applications to crops such as rice and longer-term applications to sustainable systems.

# P42: Phytophthora infestans RXLR effector interacts with S factor NRL1 to promote turnover of SWAP70

Selected poster talk

QIN HE<sup>1\*</sup>, SHAISTA NAQVI<sup>1\*</sup>, HAZEL McLELLAN<sup>1</sup>, PETRA BOEVINK<sup>2</sup>, ELEANOR GILROY<sup>2</sup>, INGO HEIN<sup>1, 2</sup>, PAUL R.J. BIRCH<sup>1, 2</sup>

09:50-10:10

<sup>1</sup>Division of Plant Science, University of Dundee; and <sup>2</sup>Cell and Molecular Sciences; both located at James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

Plant pathogens deliver effectors into plant cells to manipulate host processes. Much attention has been focused on identifying their host targets. Recently, we reported that *Phytophthora infestans* RXLR effector Pi02860 targets NRL1, an NPH3/RPT2-like protein, in the host cytoplasm and at the cell plasma membrane (Yang et al 2016 Plant Physiol). NRL1 is a susceptibility (S) factor that suppresses INF1-triggered cell death. A dimerization-deficient NRL1 mutant loses its ability to suppress INF1-triggered cell death. NRL1-mut reduces the ability of Pi02860 to attenuate INF1-mediated HR, demonstrating that host NRL1 activity is required for Pi02860 to promote disease. NRL1 interacts with a guanine nucleotide exchange factor (GEF), SWAP70, which localises to endosomes. Virus-induced gene silencing (VIGS) of SWAP70 in N. benthamiana resulted in enhanced P. infestans colonization and compromised INF1-triggered cell death. Overexpression of SWAP70 showed reduced *P.infestans* infection and accelerated INF1-triggered cell death, indicating that this host protein acts as a positive regulator of immunity. Suppression of INF1-triggered cell death by Pi02860 was significantly attenuated by co-expression with SWAP70. Interesting, Co-expression of Pi02860 and NRL1 with SWAP70 reduces the abundance of SWAP70 in an MG132-sensitive manner. NRL1-mut prevents turnover of SWAP70 by Pi02860. VIGS of NRL1 reduces 02860-mediated degradation of SWAP70. Critically, Pi02860 enhances the interaction between NRL1 and SWAP70. We argue that Pi02860 uses host protein NRL1 as an S factor to target and promote turnover of the plant positive immune regulator SWAP70.

<sup>\*</sup> Co-first author

P63: Short distance cell-to-cell communication in response to wound stress in Arabidopsis root

Selected poster talk

<u>PETER MARHAVÝ</u><sup>1</sup>, A. KURENDA<sup>1</sup>, S.M. SIDDIQUE<sup>2</sup>, J. HOLBEIN<sup>2</sup>, F. ZHOU<sup>1</sup>, E.E. FARMER<sup>1</sup>, N. GELDNER<sup>1</sup>

10:10-10:30

<sup>1</sup>Department of Plant Molecular Biology, Biophore, UNIL-Sorge, University of Lausanne, 1015 Lausanne, Switzerland; <sup>2</sup>Rheinische Friedrich-Wilhelms-University of Bonn, Department of Molecular Phytomedicine, D-53115 Bonn, Germany

Plants during their entire lifetime are opposed to various threats resulting in tissue damage, such as physical wounding, herbivore feeding, or crushing by animals. During attack plants adapt to stresses by recognizing biotic, abiotic and physical factors and adequately quickly respond to it, by orchestrating specific signaling pathways. However, the mechanisms by which these signals are perceived by cells and how the signal is further transmitted from one cell to another for local and systemic signaling is still largely unknown. In the aerial tissues, plants evolved long distance communication system, from leafto-leaf as response to wound signaling, which lead to the distal production of jasmonates mediated by electro potential changes (Mousavi et al., 2013). In our work, we focused on short cell-to-cell signal transmission upon nematodes invasion and single cell laser ablation (mechanical wound) in the root of Arabidopsis thaliana. We demonstrate that physical wounding caused by single cell laser ablation, which mimics nematode behavior during feeding, elicit surface potentials changes depending on ion channels and ROS production. These changes turn on the local production of ethylene as a potent regulator of wound responses and deterrence of nematode feeding. Our observations provide insights into the distinct mechanisms of shortdistance cell- to-cell wound signaling in roots, allowing cells to rapidly spread information among neighbors in response to local stressors physical wounding caused by single cell laser ablation and nematode feeding elicit surface potentials changes depending on ion channels and ROS production.

Mousavi S.A. et al. (2013). Nature. 500(7463):422-6.

# P94: The evolution of flammability among grasses with spatially explicit fire spread

Selected poster talk

### **ANN CARLA STAVER, E. SCHERTZER**

11:00-11:20

<sup>1</sup>Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA; <sup>2</sup>Laboratoire de Probabilités et Modèles Aléatoires, Université Pierre et Marie Curie, Paris, France

Whether plants can evolve to promote flammability is controversial. Ecologically, fire only spreads in landscapes when many plants are flammable, but collective behaviors among large groups are difficult to evolve at the individual level. Here, we formulate a model that combines individual flammability with landscape fire spread, in the context of flammability among grasses. In grasses, flammability has absolute fitness payoffs; grasses self-shade when their moribund biomass does not decompose. Dry grasses burn, and moist grasses decompose microbially; individual dryness (flammability) is partially environmental and partially evolvable (some grasses dry more easily). Fire spreads via an infection process, such that fire spreads and individuals burn only when much of the landscape is flammable.

Fire-prone and fire-resistant landscapes, composed of flammable and non-flammable grasses, respectively, were alternatively stable in some environments. However, flammability only evolved *de novo* in arid environments, when fire spread was inevitable. A positive feedback with fire could maintain flammability in an increasingly wet environment, and flammable grasses could invade wet areas after evolving in dry areas in a heterogeneous landscape. Thus, fire probably did not drive the evolution of flammable grasses, but could have promoted their widespread invasion and persistence.

# On becoming (and remaining) a plant scientist in the genomics era

Plenary lecture

IAN T. BALDWIN 11:20–12:10

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena, Germany

This talk will two major objectives: 1) to provide a distillation of what Baldwin thinks is important, unique and demanding about the particular career path of being a scientist and what he thinks it takes to sustain a research career over a professional lifetime; and 2) to provide an example of how the natural history discovery process can make this intellectual marathon easy and enjoyable.

He and his colleagues at the Max Planck Institute for Chemical Ecology have been unraveling, for more than three decades, what a native tobacco plant, *Nicotiana attenuata*, that lives in the Great Basin Desert of the SW USA, is able to do to solve its ecological challenges, and the talk will be peppered with examples from this research program to emphasize the particular challenges and opportunities of being a plant biologists in the genomics era. For Next-Gen enabled background material to this research program, see the following iBiology talks: https://www.ibiology.org/ibioseminars/short-biased-history-interdisciplinary-field.html

### Workshops

Tuesday 25th July

10:45–12:30 How to get published

John Christie (*New Phytologist*), Chris Surridge (*Nature Plants*), Anne Knowlton (Current Biology), Adam Wheeler (Wiley) and Ashlynne Merrifield (Taylor & Francis).

The members of the panel will provide tips on how to get your work published and there will be time for questions – please come prepared!

15:20–17:00 Publishing ethics

Chris Graf (Committee on Publication Ethics)

In this 'Publishing Ethics' workshop, we will tackle some of the key questions that our community faces, including retractions (Can a retraction ever result from good research practice?), authorship and peer review (Are fake peer reviewers really a problem?), reproducibility (How might we approach the reproducibility challenge?), and how to choose journals to publish your work with (What's all this about predatory journals?). In his capacity as Director, Research Integrity and Publishing Ethics at Wiley, and as elected Co-Chair for the charity COPE (Committee on Publication Ethics), Chris will share insights into the challenges facing academic publishing, as well as measures that might help combat them. The session promises to be interactive with opportunities to ask questions, to hear and draw lessons from stimulating stories, and to contribute to discussions with your own cases.

### **Poster abstracts**

P = poster abstract \* = selected talk

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Borna, Rita Sarah	Р9
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Carmona, Carlos Perez	P8, <b>P12</b>
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### Poster abstracts

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

# P1 PP2A-Β'γ controls methylation of indole glucosinolates and modulates methionine metabolism in Arabidopsis

### S. ALEGRE, M. RAHIKAINEN, A. TROTTA, J. PASCUAL, S. KANGASJÄARVI

Department of Biochemistry, Molecular Plant Biology, University of Turku. Tykistökatu 6 A 6th floor, FI- 20520 TURKU, Finland

Plants need to have a rapid and adjustable system for responding to external cues. Stress-induced reprogramming of primary and secondary metabolism is an essential defence mechanism to prevent pathogen infection. Defence response in plants is under the control of the cytoplasmic regulatory network and tightly connected with biosynthesis and recirculation of amino acids, which provide precursors for a variety of secondary compounds. In cruciferous Brassicaceae family plants, like Arabidopsis thaliana, methionine metabolism is tightly linked with the biosynthesis of glucosinolates (GSL), major secondary compounds that confer resistance against specific stressors. In response to biotic stress, GSL undergo hydroxylation and transmethylation reactions, and the resulting modified glucosinolates display diverse biological functions. We demonstrated that regulatory B'y of protein phosphatase 2A (PP2A-B'y) physically interacts with INDOLE GLUCOSINOLATE METHYLTRANSFERASEs and specifically controls the methylation of indole glucosinolates and formation of 4-methoxy-indol-3-yl-methyl glucosinolate in Arabidopsis leaves. Proteomic and metabolomic approaches revealed that PP2A-B'y is required to control the abundance of oligomeric protein complexes functionally linked with activated methyl cycle and the transmethylation capacity of the leaf. Our results highlight a key role for PP2A-B'y in controlling cross-communicating metabolic cycles in methionine metabolism, which has a significant impact on plant resistance to biotic stress.

# Manipulating the leaf microbiome for improved leafy salads

### E.C. ARNOLD, C.W. KEEVIL, M.A. CHAPMAN, H.K. SMITH, G. TAYLOR

Biological Sciences, University of Southampton, University Road, Southampton, Hampshire, SO17 1BJ, UK

Leafy salads have a short shelf-life. Leaf-associated microbes can reduce crop yield, cause human disease and reduce shelf-life. Through 16S and ITS sequencing we have shown that salad leaf variety is responsible for 33% and 85% of bacterial and fungal variation respectively. Identifying important plant host traits responsible for these differences will enable plant breeders to select traits that lead to cultivars with improved microbiological safety and/or shelf-life. We have phenotyped the L. sativa and L. serriola mapping population for traits associated with microbial attachment and/or proliferation. We have then sequenced the leaf microbiome of RILs with extreme stomatal density and epidermal cell area to see what impact these plant traits have on microbial diversity. We have assessed the impact of these extreme phenotypes on Listeria monocytogenes attachment/proliferation. Improving our understanding of the effect of plant genotype on food borne pathogen attachment/survival will help to identify plant traits for improved fresh produce. This will ultimately help to reduce food waste and food borne disease and help to meet the growing food demands of the human population.

# P3 Making space to breathe: the role of the cell wall in determining stomatal and mesophyll conductance

A.L. BAILLIE, S.M.J. AMSBURY, M.R. LUNDGREN, S. CARROLL, L. HUNT, J.E. GRAY, A.J. FLEMING

The Department of Animal and Plant Sciences, The University of Sheffield, Alfred Denny Building, Western Bank, Sheffield, S10 2TN, UK

Photosynthetic carbon uptake is dependent on regulated gas flux through the stomata pores and complex underlying airspace. Cell walls must be important in stomata and mesophyll formation, yet our understanding of their role remains limited. An immunological screen of Arabidopsis leaves indicated that differential methylation of pectic homogalacturonan (HG) is associated with differentiation of mesophyll and stomata. In the mesophyll, esterified HG is abundant at the corners of airspaces, whereas joined cell walls contain mostly de-esterified HG, suggesting a role for pectin modification in regulating cell separation and determining porosity. In stomata, guard cells lack methyl-esterified HG. Our previous work, which identified a pectin methyl-esterase with a specific role in guard cell function, showed that gain of methyl-esterified HG in guard cell walls compromises stomatal function. In addition, we show that stomatal poles have a specific pattern of methyl-esterified HG. Atomic force microscopy suggests that this polar pattern of methyl-esterified HG confers functionally important mechanical properties. Finally this project aims to examine coordination of mesophyll airspace with stomatal positioning to form sub-stomatal cavities. By examining cellular architecture and measuring gas exchange in mutants with altered stomatal density or reduced stomatal function, we will investigate the coupling of these developmental processes.

# P4 Investigation of *Arabidopsis thaliana* glutathione peroxidase-like enzymes

<u>K. BELA</u><sup>1</sup>, E. HORVÁTH<sup>1</sup>, Á. HURTON<sup>1</sup>, RIYAZUDDIN<sup>1</sup>, Z. TAKÁCS<sup>1</sup>, S.A.K. BANGASH<sup>2</sup>, J. CSISZÁR<sup>1</sup>

<sup>1</sup>Department of Plant Biology, University of Szeged, Szeged, Hungary; <sup>2</sup>INRES Chemical Signalling, University of Bonn, Bonn, Germany

Glutathione peroxidases (GPXs) are common enzymes found in animals, fungi and plants. These proteins protect against reactive oxygen species, catalyze reduction of hydrogen peroxide ( $H_2O_2$ ), organic hydroperoxides and lipid peroxides using glutathione or other reducing components, such as thioredoxin. The plant glutathione peroxidase-like enzymes (GPXLs) are mostly similar to animal phospholipid hydroperoxide glutathione peroxidases (PHGPX). These PHGPXs play a very important role in protecting against oxidative damage of membranes. In addition to the possible antioxidant functions, plant GPXLs also participate in redox signaling.

The *Arabidopsis thaliana* contains 8 glutathione peroxidase-like isoemzymes, however their role in plant development and stress responses and their exact mechanisms are not well-known.

Experiments were performed on T-DNA insertion mutants (*Atgpxl1-8*: SALK\_128885C; SALK\_082445C; SALK\_071176C; SAIL\_623\_F09; SALK\_076628C; WiscDsLox321H10; SALK\_072007C; SALK\_127691C, respectively).

Based on our results, in the mutant plants not only the activity of enzymatic antioxidants (glutathione peroxidase, thioredoxin peroxidase, glutathione transferase) changed, but the level and reduction state of non-enzymatic antioxidants, such as glutathione and ascorbate, too.

The drought or osmotic stress experiments conducted on *Atgpxl1–8* mutants demonstrated, that the AtGPXL3 and 5 have important role in the development, while the AtGPXL2, 4, 6, 8 especially in the stress responses.

# P5 Circadian regulation of plant responses to herbicides

### F.E. BELBIN, C. FORMSTONE, G. HALL, K.A. FRANKLIN, A.N. DODD

School of Biological Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK; Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK

The effectiveness of some herbicides depends on the time of day at which the herbicide is applied. Maximising herbicide efficacy through application at specific times of day could reduce the quantity of chemical used, and reduce time and cost to consumers, while minimising potential environmental leaching. Mechanisms underlying the time of day variation in the efficacy of herbicides are unknown. The circadian oscillator regulates vast numbers of plant processes; we hypothesize that circadian regulation has a major role in plant responses to herbicides. Using Arabidopsis thaliana and a broad range of approaches including chlorophyll fluorescence, EM-CCD imaging, RNA sequencing, and developmental phenotyping, the processes underlying time of day variation in herbicide efficacy is being investigated. Results show that glyphosate is more effective at reducing photosynthesis, inhibiting hypocotyl elongation, and altering expression of marker gene transcripts when applied at dawn or dusk, compared with other times of the day. The mechanisms underlying these time of day responses are being investigated in depth, including the extent of the involvement of the circadian clock and phytohormone signalling. I am exploiting these results from Arabidopsis to determine the extent of conservation and homology of responses between Arabidopsis and common agricultural weeds.

## P6 Environmental and epigenetic regulation of transposon activity in tomato

#### M. BENOIT, J. PASZKOWSKI

The Sainsbury Laboratory, University of Cambridge, Cambridge, UK

We are investigating the role of transposons in the regulation of plant development, by monitoring transposon activity during critical developmental processes in tomato. Long terminal repeat (LTR) retrotransposons represent a large fraction of plant genomes. The annotation of LTR retrotransposons in tomato revealed more than 5800 intact elements, some of them suggesting recent transposition events as revealed by sequence identity between LTRs. Therefore, tomato represents a powerful model to study transposon activities and their links to associated environmental responses in crops.

The phytohormone ABA (abscisic acid) plays an important role in mediating plant adaptation to stress, including water, light and temperature-related stresses. We present here evidences of a LTR retrotransposon family called *Rider* taking advantage of increased ABA levels upon drought stress to substantially enhance its transcription level. *Rider* activity is further enhanced in tomato RNA-dependent DNA Methylation (RdDM) mutants showing impaired DNA methylation.

In summary, our work identified a LTR retrotransposon family subjected to environmental regulation through ABA-specific transcriptional activation. Our results reveal a tight interplay between environmental stress and epigenetic control of retrotransposons in tomato.

### P7 Characterisation of acquired thermotolerance in potato

C.E. BITA, L. DUCREUX, R. HANCOCK, P. HEDLEY, J. MORRIS, W. MORRIS, A. TRAPERO-MOZOS, C. WIESE, M. TAYLOR

Cell and Molecular Sciences Group, James Hutton Institute, Dundee, UK

For many commercial potato cultivars, tuber yield is optimal at average daytime temperatures in the range of 14-22°C. Further rises in ambient temperature can reduce or completely inhibit potato tuber production, with damaging consequences for both producer and consumer. Despite centuries of potato breeding, high temperature tolerance has not been significantly improved. In the field, in the major European growing regions, it is more likely that plants will be exposed to short periods of elevated temperature rather than continuous high temperatures and so acquired thermotolerance is likely to be an important trait. Acquired thermolerance is a powerful adaptive response that has been observed in many plant species. Following exposure to sub-lethal heat stress, plants acquire enhanced tolerance to subsequent exposure to more severe levels of heat stress. Many temperature profiles have been reported to induce thermotolerance, with different plant species having different requirements for acquiring heat tolerance. Recent studies have started to address molecular mechanisms that underpin acquired thermotolerance in Arabidopsis. Direct exposure to high temperature elicited a very different transcriptional response compared with plants that are acclimated at a moderately high temperature prior to high temperature treatment. Furthermore, different acclimation treatments resulted in different transcriptional profiles although the plants appeared to have a similar degree of priming to the high temperature condition. Although acquired thermotolerance has been characterizedin Arabidopsis, little detail is available of the process in potato, despite its prospective significance. Here we describe the temperature and light conditions that elicit acquired thermotolerance in potato. Notably we demonstrate the inability of plantsto acquire thermotolerance in the dark, potentially implicating light signaling in the acclimation response. In time course experiments we describe gene expression and metabolite changes associated with acquired thermotolerance. We also define changes in the main cellular redox buffers

associated with thermotolerance and subsequent exposure to severe heat stress. Using an electrolyte leakage test to monitor cell damage, comparison of different potato genotypes indicated considerable variation for this trait. Ultimately, our results will be used to develop genetic screens for heat tolerance identification and to implement novel targeted breeding approaches.

### Nuclear roles for the prefoldin complex in plants

<u>N. BLANCO-TOURIÑÁN</u><sup>1</sup>, D. ESTEVE-BRUNA<sup>1</sup>, C. CARMONA-MARÍ<sup>1</sup>, C. FUENTES CHUST<sup>1</sup>, M. DE LUCAS<sup>2</sup>, M.A. BLÁZQUEZ<sup>1</sup>, D. ALABADÍ<sup>1</sup>

<sup>1</sup>Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), C/ Ingeniero Fausto Elio s/n, 46022 Valencia; <sup>2</sup>School of Biological and Biomedical Sciences, Durham University, UK

PREFOLDIN (PFD) is an evolutionarily conserved heterohexameric complex (PFD1–6) that presents unfolded actin and tubulin to the main cytosolic chaperone CCT for their correct folding. This function takes place in the cytosol, but PFD can also accumulate in the nucleus of Arabidopsis cells through the interaction with the DELLA transcriptional regulators. Although this interaction is meant to impair PFD function in the cytosol, it exists the exciting possibility that PFD may have a DELLA-dependent role in the regulation of transcription in plant cells.

An *in silico* analysis of the predicted interactome in Arabidopsis, which is based in interacting orthologs in yeast, flies, worms and humans, has allowed us to identify numerous putative nuclear interactors for the plant PFD. Many of them have a relevant role in chromatin remodeling, transcription and splicing. Remarkably, we have demonstrated that some interactions are conserved in Arabidopsis, providing hints about new roles for the PFD complex in plants.

# P9 Unravelling the function of the rice orthologues of the F-box gene HAWAIIAN SKIRT (HWS) in plant development

#### R.S. BORNA, J.A. ROBERTS, Z.H. GONZALEZ-CARRANZA

Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK zinnia.qonzalez@nottingham.ac.uk

The F-box gene HAWAIIAN SKIRT (HWS) plays a significant role in overall plant development and timing of floral abscission in Arabidiopsis. Loss of function hws mutants show sepal fusion and other abnormal flower phenotypes in Arabidiopsis; additionally shoot, roots, leaves and seeds are bigger in hws mutants than wildtype. Those promising traits for crops, triggered the idea to investigate the role of HWS orthologues in rice (Oryza sativa).

ERECTA PANNICLE3 (EP3) and Oryza sativa HAWAIIAN SKIRT (OsHWS) genes of rice are the functional orthologues of HWS from Arabidopsis (Gonzalez et al., unpublished data). The loss of function mutant ep3 creates an erect panicle phenotype in rice. The ep3 mutants exhibit decreased leaf photosynthetic capacity; it has been demonstrated that EP3 and plays role in stomatal guard cell development.

To further understand the role of these genes in rice development, the present study involves the generation of a series of transgenic lines including reporter promoter fusions, loss of function mutants, downregulation and ectopic expression.

### P10 Regulation of immunity by subcellular transport processes

<u>G. BOURDAIS</u><sup>1</sup>, M. MBENGUE<sup>2</sup>, D.H. MCLACHLAN<sup>3</sup>, L. RICKETT<sup>1</sup>, J. ZHOU<sup>1,4</sup>, A. SIWOSZEK<sup>1</sup>, H. HÄWEKER<sup>1</sup>, R. MORRIS<sup>5</sup>, D. MACLEAN<sup>1</sup>, S. ROBATZEK<sup>1</sup>

<sup>1</sup>The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK; <sup>2</sup>LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France; <sup>3</sup>School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK; <sup>4</sup>The Earlham Institute, Norwich Research Park, Norwich, NR4 7UH, UK; <sup>5</sup>John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

Pattern recognition receptors (PRRs) are receptor kinases and receptor-like proteins that must be presented at the plasma membrane to recognize invading pathogens. We found that PRRs from different protein families (FLS2, EFR, PEPR1) are internalized in a clathrin-mediated ligand-induced dependent manner via a common endosomal pathway. Furthermore, our studies have revealed that inhibition of FLS2 endocytosis leads to enhancing bacterial infection through differential modulation of flg22-induced defences such as stomatal closure. Indeed, plants evolved stomata pores in the leaf epidermis for photosynthetic gas exchange, but stomata make plants vulnerable to drought and invasion by microbes. To counter this, the guard cells translate signaling cues that result in closure of the stomatal pore. We develop a high throughput quantitative imaging of stomatal apertures and perform a reverse genetic screen in a group of Arabidopsis mutants mainly affected in vesicle trafficking to define the common and specific regulators of stomatal closure induced by biotic and abiotic stress. We find that mutants broadly affected in stomatal closure are mostly in genes encoding SNARE membrane regulators. By contrast, mutants in Rab GTPase genes play a prominent role in the response to biotic stresses highlighting the importance of subcellular transport processes in plant immunity.

## P11 Adaptation of plants to cold temperatures by a chloroplast-based signalling circuit

#### D. CANO-RAMIREZ, B.F. MANLEY, K.A. FRANKLIN, A.N. DODD

School of Biological Sciences, Life Sciences Building, University of Bristol, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK

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

## P12 Functional traits hierarchies rather than trait dissimilarity determine competitive interactions in a pool of Mediterranean annual species

C.P. CARMONA<sup>1</sup>, C. ROTA<sup>2</sup>, F. DE BELLO<sup>3,4</sup>, F.M. AZCÁRATE<sup>2</sup>, B. PECO<sup>2</sup>

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The assumption that interspecific differences in traits promote species coexistence has not been explored in depth. I present an experiment using eight Mediterranean annual species. We sown six replicates of all possible pairwise combinations of these species, including conspecifics, in pots under two levels of fertilization. After two months, we measured above and belowground vegetative traits and total biomass of the focal individuals, also grown with intraspecific competition and alone.

We analysed the relative decrease in biomass of the focal individuals – compared to the average biomass of the conspecifics without competition. We considered alternative models, consistent with two potential mechanisms regulating interspecific competition: competitive hierarchies or trait dissimilarity. Competitive hierarchies occur when the relative effects of the competitor species are directional and proportional to their relative position along the trait axis (e.g. taller plants have competitive advantage). Under the trait dissimilarity hypothesis, competitive effects decrease as trait distance increases, irrespective of the direction. We found a clear evidence of hierarchy in height values: competition increased with the height of the competitors. Moreover, in conditions of low fertility lower SLA also conferred competitive advantages. Interestingly, trait plasticity tended reduce competitive hierarchies, and hence the intensity of competition.

### P13 Measurements of carbon age distribution could revolutionize the terrestrial vegetation models

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The global carbon cycle is strongly controlled by the source/sink strength of vegetation as well as the capacity of terrestrial ecosystems to retain this carbon. These dynamics have been studied using ecosystem models, but different assumptions regarding the carbon allocation strategies and other model structures may result in highly divergent predictions. We modeled three systems of vegetation compartments and assessed their performance by calculating the age of the carbon in the whole systems and in each compartment, and the overall transit time of C in the system. First, we used published measurements of ecosystem C compartments from the Harvard Forest Environmental Measurement Site to find the best set of parameters. Second, we calculated C stocks, release fluxes, radiocarbon values based on the bomb spike, ages, and transit times. We found a good fit of the three models to the available data, but more constraints are required in order to reduce the high parameter collinearity and model equifinality that we observed. Promising candidates for future constraints that emerged from this analysis were the distributions of C age and transit times, since they were the most sensible to the differences in model structure. In particular, the inclusion of two storage compartments resulted in the prediction of a system mean age that was 78-124 years older than in the models with one or no storage compartments, respectively. The age of carbon in the wood compartment was distributed towards older ages, whereas the age distribution of fast cycling compartments did not exceed 10 years. As expected, models with C distributed towards older ages also had longer transit times. These results suggest that ages and transit times, which can be indirectly measured using isotope tracers, serve as important diagnostics of model performance and could largely help to reduce uncertainties in model predictions. Furthermore, by considering age and transit times of C in vegetation as distributions, not only as mean values, we obtain additional insights on the temporal dynamics of carbon use, storage, and allocation to

plant parts, which not only depends on the rate at which this C is transferred in and out of the compartments, but also on the stochastic nature of the process itself.

## P14 Cadmium triggered signaling network in soybean plants

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Plants are sessile organisms unable to actively escape from metal contaminated sites. However, they are not defenseless. Some of the described metal protective mechanisms include stimulation of antioxidant and DNA repairs systems, over-production of metal binding phytochelatines and cell wall thickening. An intriguing questions arises: How do plants sense and transduce metal signal to activate the defense? Presented study describes signaling network triggered by cadmium in soybean plants. The results show that the earliest response to this metal includes induction of signaling associated genes engaged in ethylene biosynthesis, nitric oxide production, mitogen- activated protein kinase cascades and regulation of genes expression. Further research suggests that the expression of Cdinducible genes is modulated by nitrate oxide and reactive oxygen species. Additionally, exposure to short term cadmium stress led to higher level of RNA oxidative modifications. Interestingly, although Cd-stresses plants exhibited symptoms of metal toxicity, they efficiently recovered after the transfer to optimal growth conditions.

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# P15 Nematode infection redirects hormonal homeostasis via Rboh-mediated ROS to facilitate their parasitism of host roots

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Cyst nematodes are obligate parasites that establish syncytial-feeding sites in roots of their host plants. Their invasion and feeding causes tissue damage in the host roots triggering an oxidative burst. In plants, ROS is mainly produced by plasma membrane-bound NADPH oxidases, named respiratory burst oxidase homolog (Rboh). Surprisingly, Arabidopsis plants lacking in ROS production by Rboh (rbohD/F) have been shown to be less susceptible to cyst nematode attack. A comprehensive microscopic, biochemical and molecular analysis has demonstrated that Rboh-dependent ROS are not required for Arabidopsis root invasion by cyst nematodes; however, the absence of Rbohmediated ROS impairs syncytial establishment and development. To understand the underlying mechanistic details of Rboh- mediated ROS in syncytium formation, we performed a genome-wide transcriptome analysis between Col-O and rbohD/F upon nematode infection. Several genes involved in auxin transport, synthesis and/or homeostasis were down regulated in rbohD/F as compared to wild type. Notably, we identified an auxin transporter as one of the downstream targets of ROS. Hormone quantifications, metabolic profiling, genetic complementation and mutant analysis suggest that it regulates the pathways linking Rboh-mediated ROS to downstream responses. In summary, our work provides a first mechanistic understanding of role of ROS in promoting nematode and other pathogen infection.

### P16 Floral heteromorphy in *Primula*: new insights for an old model

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The genetic and evolutionary basis of floral heteromorphy in *Primula* has been debated for 150 years. Darwin demonstrated how reciprocal anther and stigma positions in the two floral morphs, pin and thrum, serve to physically promote insect-mediated outcrossing. This phenomenon evolved independently in over 28 angiosperm families. The *Primula S* locus, which regulates heterostyly and self-incompatibility, is recognised as a "supergene", a cluster of tightly-linked genes inherited as one unit; self-fertile homostyle primroses, with anthers and stigma at the same height, were predicted to arise through rare recombination events in heterozygous thrums. This model underpins 60 years of research into heterostyly. To characterise the S locus we undertook assembly and annotation of the *Primula* vulgaris genome, alongside RNA-Seq and cross-species comparisons. We show the S locus is hemizygous in thrums, not heterozygous, comprising five thrum-specific genes absent from pin; homostyles result from mutation, not recombination. We identify the S locus genes, estimate assembly of the supergene at 51.7 MYA, and reveal conserved genetic architecture across the Primulaceae. These findings represent insight into the structure and origin of the *Primula S* locus, providing a platform for identification and evolutionary analysis of the genes regulating outcrossing in *Primula* and other heterostylous genera.

## P17 Omics analysis of *Arabidopsis halleri* populations with contrasting strategies of Cd hypertolerance

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Arabidopsis halleri is considered as a model species to study adaptation to extreme metallic conditions. This pseudo-metallophyte shows a huge variation in the levels of Cd accumulation and tolerance among populations originated from metalliferous (M, metal-contaminated) and nonmetalliferous (NM) soils from different phylogeographic origin (genetic units, GUs). Some populations are Cd and Zn hyperaccumulators, presenting in situ concentrations above 0.01% or 0.3% of the shoot dry weight without showing toxicity symptoms, respectively. We compared two metallicolous populations from different GUs showing both Cd hypertolerance but contrasting Cd accumulation. Our results provide a detailed picture of plant ionomic, transcriptomic (RNA-Seg) and metabolomic profiles. Several new candidate genes possibly involved in Cd tolerance and hyperaccumulation or exclusion were identified. The study of A. thaliana knockout mutants supported a role for these newly identified *A. halleri* candidate genes. Taken together our results suggest the evolution of two divergent strategies for Cd uptake, transport and detoxification in A. halleri metallicolous populations from different GUs driven by different metabolic pathways.

# P18 Differences in nectar spur length in two sister species of *Linaria* are attributable to cell division rather than cell anisotropy

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Nectar spurs (tubular outgrowths of the petal) are hypothesized to be a 'key innovation' which can lead to rapid speciation within a lineage and are important for pollinator specificity. However, there is still much to learn about nectar spur development. We investigated variation of nectar spur length, in a clade of eight Iberian toadflaxes with varying spur length. Spur length was recorded over thirteen days and it was found that the initiation and end of spur growth was not significantly different in species with the longest and shortest spurs; it was growth rate that differed. Within the clade we then focused on *Linaria salzmannii* and *Linaria clementei*, two closely related species which have extremely long and short spurs respectively. A morphological characterisation was performed across a range of key developmental stages to determine whether the difference is due to cell expansion or cell division. We found that cell number and therefore cell division largely explains the difference in spur length. This contrasts with previous studies in Aquilegia which have found that variation of nectar spur length is due to directed cell expansion (anisotropy) over a longer timeframe. This study adds to knowledge about nectar spur development in a comparative context.

## P19 Evolution of plant breeding systems: Male form and function in the nightshade family

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Solanum is entirely buzz-pollinated; the plant's gametes are used as the pollinator reward. This places special selective pressures on plant and pollinator leading to adaptation in the morphology of the highly diverse Solanum anthers. This project investigates 'How does stamen morphology evolve to improve pollinator efficiency and/or attraction in a buzz pollinated genus?' through identifying key evolutionary transitions in stamen morphology at a macro- and micro-morphological level across the Solanum phylogeny. Once identified, selected evolutionary transitions will be further investigated from a developmental genetic point of view using MYB transcription factor MIXTA in a candidate gene approach to investigate the genetic control and development of transitions through gene over-expression and CRISPR gene knockout. The interactions of pollinators with stamen morphologies will then be examined using sister pairs of plants with contrasting morphologies. Overall this will give greater understanding of the driving forces behind evolution of an economically important genus.

## P20 Natural variation in a rice immune receptor interface extends response to pathogen effectors

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Pathogens deliver an array of molecules, termed effectors, to manipulate host cellular processes for their own benefit. Perception of effectors inside cells largely relies on immune receptors of the NLR (Nucleotide-binding, Leucine-rich Repeat) family, triggering a response to stop infection. This process creates a high selection pressure in the pathogen, driving the emergence of new effector variants that escape recognition. However, plants also evolve receptor alleles with broader recognition for these variants. Here, we unravelled the molecular details of this arms race co-evolution between the rice NLR Pik and the rice blast pathogen effector AVR-Pik. The rice NLR allele Pikp1 recognizes AVR-PikD through direct binding to an unconventional integrated Heavy Metal Associated (HMA) domain, resulting in disease resistance. Polymorphic effector variants have lower binding affinity for Pikp-HMA in vitro, and evade immune recognition in plants. Another rice NLR allele, Pikm1, recognizes these polymorphic variants, and biochemical characterization of the Pikm-HMA domain has revealed a higher binding affinity for these effectors. Crystal structures of Pikm- HMA complexed with different effector variants provides an atomistic explanation for the extended recognition specificity. This study shows the structural basis of natural variation in pathogen recognition specificities by NLRs, and will allow structure-guided protein engineering to improve disease resistance in crops.

## P21 Comprehensive capture-seq (Coca-seq) unravels gene regulation mechanism in plant immune signalling

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Unlike the vertebrate immunity, plants lack a circulatory system. In order to achieve a certain threshold of inducible immunity, plants have evolved intracellular immune receptors to recognize pathogenic effectors deployed by infectious microbes. Here we are using versatile paired nuclei-localized immune receptors RPS4 and RRS1 from Arabidopsis as our model, for they together can confer resistance to multiple pathogens, including bacteria Ralstonia solanacearum, Pseudomonas syringae, Xanthomonas campestris and fungus Colletotrichum higginsianum. Similar to inflammosome signalling in mammalian innate immunity, upon pathogenic ligand recognition, RPS4/RRS1 can trigger cell death and resistance in planta. However how the downstream defence genes are switched on is largely unknown. Here we are reporting two distinct groups of master transcription factors (calmodulinbinding and MYC-like TFs), identified from a high-throughput yeast onehybrid screen, play important but divergent roles in early defence genes activation and cell death. To investigate the molecular details of how these TFs regulate the target defence gene expressions, we generated customized clusters of 120nt RNA probes to capture/enrich the gene-of-interest sequences from ATAC-seq, RNA-seq and ChIP- seq libraries. From this comprehensive capture-seq (Coca-seq) datasets, we aim to identify key components sitting on the target gene bodies in the chromatin context and in relation to the activation of early time-point plant immune signalling.

## P22 Characterization of a pipecolic acid biosynthesis pathway required for systemic acquired resistance

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Systemic acquired resistance (SAR) is an immune response induced in the distal parts of plants following defense activation in local tissue. Pipecolic acid (Pip) accumulation orchestrates SAR and local resistance responses. Here, we report the identification and characterization of *SAR-DEFICIENT4* (*SARD4*), which encodes a critical enzyme for Pip biosynthesis in *Arabidopsis thaliana*. Loss of function of SARD4 leads to reduced Pip levels and accumulation of a Pip precursor,  $\Delta 1$ -piperideine-2-carboxylic acid (P2C). In *Escherichia coli*, expression of the aminotransferase ALD1 leads to production of P2C and addition of SARD4 results in Pip production, suggesting that a Pip biosynthesis pathway can be reconstituted in bacteria by coexpression of ALD1 and SARD4. In vitro experiments showed that ALD1 can use L-lysine as a substrate to produce P2C and P2C is converted to Pip by SARD4. Analysis of *sard4* mutant plants showed that SARD4 is required for SAR as well as enhanced pathogen resistance conditioned by overexpression of the SAR regulator FLAVIN-DEPENDENT MONOOXYGENASE1.

Compared with the wild type, pathogen-induced Pip accumulation is only modestly reduced in the local tissue of *sard4* mutant plants, but it is below detection in distal leaves, suggesting that Pip is synthesized in systemic tissue by SARD4-mediated reduction of P2C and biosynthesis of Pip in systemic tissue contributes to SAR establishment.

## Petal epidermal morphology and plant-pollinator interaction in the genus *Nicotiana*

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Petal epidermal cell shape has been shown to affect pollination success in flowering plants. Conical epidermal cells may increase grip for insect pollinators and enhance flower colouration compared to non-conical cells. The genus *Nicotiana* presents a diverse range of petal cell shapes. Interestingly, sister species in at least two phylogenetically distinct clades of the genus have contrasting petal epidermal cell shapes (conical vs. nonconical). This suggests that independent losses of the conical cells have occurred within the genus. Through sequence comparisons of R2R3 MYB subgroup 9 transcription factors of sister species N. forgetiana and N. bonariensis (Sect. Alatae), and N. cordifolia and N. solanifolia (Sect. Paniculatae), single amino acid changes potentially affecting the control of petal epidermal cell shape have been identified. Future work includes exploring the reversal of molecular evolution of the trait through Agrobacterium tumefaciens mediated plant transformations. Differences in expression patterns between the genes will be assessed by gRT-PCR. Eventually, the tools of behavioural ecology, with bumblebees and/or moths, will be used to explore pollinator responses to perturbed floral morphologies. This work will illuminate our understanding of convergent evolution, and of the diversification of an economically important genus in the hyperdiverse family of flowering plants Solanaceae.

## P24 Manipulating wheat stomatal density for enhanced water-use efficiency

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Stomata are microscopic pores in the aerial epidermis of evolutionarily diverse land plants that facilitate gas exchange across an otherwise impermeable waxy cuticle. Importantly, stomata allow carbon dioxide to enter the plant at the expense of water loss. The size, density and degree of aperture of stomata determine the amount of this gas exchange that happens and are actively regulated in response to environmental conditions. Stomatal density can be directly manipulated in *Arabidopsis* by altering the expression of genes encoding EPIDERMAL PATTERNING FACTOR (EPF) signalling peptides, and this results in altered water-use efficiency and drought tolerance. We show that wheat has homologues of these genes, some of which may share the function of their Arabidopsis homologues. Constitutive overexpression of one of these genes, named TaEPF, results in plants with fewer stomata than wild-type. We are using these overexpressor lines to explore the effects of reduced stomatal density on leaf development and physiology. We demonstrate that wheat lines with reduced stomatal density have enhanced water-use efficiency under growth conditions.

## P25 Varying phenology explains the elevational range limit of yellow rattle in the Canadian Rockies

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It is generally expected that along gradients in seasonality, shorter growing seasons select for earlier reproduction. Yet, early reproduction often necessitates reproducing at a small size, limiting fecundity; a tradeoff that may limit range expansion along elevational or latitudinal seasonality gradients. Despite such well-defined hypotheses, the factors maintaining stable range limits along seasonality gradients remain unclear. We quantified elevational variation in seasonality, phenological and morphological traits, and fitness of ~2000 Rhinanthus minor individuals in each of three generations across ~900m of elevation to the upper range limit in the Rocky Mountains. We predicted morphological and phenological differences favouring rapid development and reproduction with increasing elevation, and a lack of concordance among elevational clines of phenotypes and seasonality towards the upper limit. We found significant differences in phenological, but not morphological traits across elevation. Elevational clines of phenology, when significant, differed from clines in seasonality, suggesting selection for variable phenology across elevation, but that adaptation to seasonality along the elevational gradient may be limited. Moreover, the classic time/size tradeoff does not appear to limit R. minor's elevational distribution despite elevational variation in seasonality, phenology, and selection on R. minor phenological traits.

# P26 Barley transcriptome analyses upon interaction with different aphid species identifies thionins contributing to resistance

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Aphids are phloem-feeding insects that cause yield losses on important crops, including barley. Most aphids feed from one or few host species, but some are able to reproduce in many different plants. While feeding, aphids probe the leave surface secreting proteins regardless of the plant species confronted. This observation points to different molecular events taking place during host and non-host interactions and potentially determine the aphid host range. Here, we investigate barley transcriptional responses with three aphid species: Rhopalosiphum padi, Myzus persicae and Myzus cerasi. Colonization efficiency, probing behaviour and elicitation of barley defences defined three different interactions, namely host, non-host and poor-host. Analyses of barley transcriptome showed the strongest response during the poor-host (M. persicae) versus host (R. padi) interaction, and few genes affected by the non-host interaction. We identified gene specifically upregulated during the poor-host interaction. These include a LEA involved in biotic/abiotic stress and several members of the thionin antimicrobial peptide family. Interestingly, ectopic expression of two thionins in Nicotiana benthamiana reduced host susceptibility to M. persicae, indicating thionins contribute to defences against aphids. Future work will be to implicate LEA and thionin family in aphid host range by using barley CRISPR-Cas9 knockout transgenic plants functional assays.

### P27 Deciphering the *Phytophthora palmivora*-host interaction

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Phytophthora palmivora, a tropical relative of the potato late blight pathogen, causes rotting diseases in many tropical crops including papaya, cocoa, oil palm, black pepper, rubber, coconut, durian and mango. Despite this, genetic resources to allow for disease resistance breeding and identification of *P. palmivora* effectors are scarce. We employed the model plant Nicotiana benthamiana to study P. palmivora root infection at the cellular and molecular levels. Time-resolved dual transcriptomics revealed different pathogen and host transcriptome dynamics. While the pathogen transcriptome undergoes sharp transitions, plant responses were characterized by rapid recognition of the invader and steady activation of defense gene expression. Analysis of the pathogen secretome led to the identification of key conserved, disease-promoting P. palmivora RXLR-dEER effectors. Furthermore, a N. benthamiana gene encoding a secreted peptide with similarities to the IDA-like peptide family was locally induced in root tips at early stages of P. palmivora infection. These results constitute a major advance in our understanding of *P. palmivora* diseases and establish extensive resources for P. palmivora pathogenomics, effector-aided resistance breeding and the generation of induced resistance to *Phytophthora* root infections. Our pipeline to identify infection-relevant secreted genes is transferable to other pathogen-host interactions and is not restricted to plants.

## P28 Are arbuscular mycorrhizal fungi intervening in the facilitation of soybean invasion in Americas?

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Soybean is an annual plant originated in Asia which occupies c. 90% of Argentinean agricultural area. Arbuscular mycorrhizal fungi (AMF) have been reported as strongly synergistic with exotic plants colonizing new regions of the world. The objective of this work was to evaluate mycorrhizal status of soybean fields and the effect of resident AMF on plant growth and development. Samples of 125 soybean fields were taken from geographic and intensity land use gradients. Mycorrhizal status was estimated by mycorrhizal colonization, spore identification, and pyrosequencing. The effect of resident AMF on plants was studied in pot experiments under contrasting conditions. We found that 99% of soybean roots were mycorrhizal with a range of 40-61% per site. A total of 36 AMF species were identified. Pyrosequencing yielded 84 and 74 virtual taxa in soil and root samples, respectively. In spite of the contrasting geographical conditions growth parameters did not differed among fields. Pot experiment revealed that soybean plants improved growth and development under AMF presence whether or not limiting conditions occurred. It is proposed that the spread of this non-native plant species could be facilitated by resident AMF, underlining the need to integrate symbiotic interactions in future work on soybean adaptability processes.

## P29 The bHLH transcription factor Ptf1 in wheat in responses (P) to low phosphorus stress differently from the Ptf1 in lower-ploidy crops

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Increasing evidence has suggested transcription factors (TFs) play crucial roles in plants' responses to environmental stresses. A basic helix-loop-helixbHLH TF-Ptf1 has been shown in rice, maize and soybean to be a P-stress responsive regulator, meanwhile, a part of carbon metabolism. We investigated the overexpression lines of this important TF in bread wheat for its transgenic effects in a greenhouse experiment. We found that several of the traits in responses to P stress differed from those previously studied in rice/maize. In fact, overexpressed wheat Ptf1 (TaPtf1) led to opposite results where reductions in tiller number, seed biomass and seed P accumulation under P deficiency were observed, although shoot biomass and P accumulation seemed not to be affected. More interestingly, the chlorophyll index appeared to be negatively regulated in P defectiveness. We also identified the novel sub-cellular location of TaPtf1 which is not exclusively in the nucleus, tending to be distinct from Ptf1 in rice/soybean. Based on these results, we propose that TaPtf1 from a more complex genetic background of bread wheat may adopt different molecular pathways and engage in more extensive cellular activities; especially a possibly negative regulator for photosynthesis and P transport from shoots to seeds.

### P30 Following *Arabidopsis thaliana* root map to unravel radial ion transport pathways

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Radial ion transport across the root is the rate limiting step in mineral nutrient transport to the shoot and involves the movement of ions from the soil, through the epidermal, cortical and endodermal cell layers and into the stele for loading into the xylem. To date technical limitations have restricted our ability to develop a full understanding of these pathways and the mechanisms that drive them. Therefore, we have developed a new approach based on combining Fluorescence Activated Cell Sorting with multi-element ICP-MS analysis of cell-type specific GFP expressing root protoplasts sorted by cell type. This approach allows purification of the epidermal, cortical and endodermal cells from the root of Arabidopsis thaliana in sufficient quantities to allow the quantification of the elemental content of collected cells by ICP-MS and creating thus a high-resolution and cell-type specific map of the elemental composition of the plant root that can be used to help unravel radial ion transport pathways. We have started to use this approach to better understand the role of endodermal lignin-based extracellular structures - Casparian strips in controlling radial ion transport. Therefore, we are investigating the schengen3 mutant that has disrupted Casparian strips and displays selective nutrient homeostasis defects.

### Photoprotection versus productivity in crops

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Photoprotection describes adaptations that allow plants to remove excess energy that they have gained from absorbing high light levels. Many studies have shown that whilst required to reduce photoinhibition, over-protection may also reduce photosynthesis efficiency. Therefore, it is considered an important characteristic for yield improvement. Photoprotection can be measured as non-photochemical quenching (NPQ); specifically high energy state quenching (qE), qE is the most significant component in vivo, strongly influenced by a Photosystem II (PSII) protein PsbS. PsbS plays a key role in rapid formation and capacity of qE under moderate and high light stress. In this project, transgenic rice and wheat overexpressing PsbS in addition to wheat lines with natural variation in qE are studied at both leaf and canopy levels to determine the role of NPQ in crop productivity. We measure the protective component of NPQ (qPd) to assess the trade-offs between protection and productivity. qPd and other measurements of photosynthesis efficiency will be used to estimate light tolerance level simultaneously in leaves at different states of light saturation within the plant canopy. Overall we aim to determine the possible yield and biomass benefit of manipulating NPQ in wheat and rice.

## P32 S-nitrosothiol impact on hormonal trade-offs in plant immunity

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The bacterial pathogen *Pseudomonas syringae* (Psm) hijacks the plant immune system by secreting the phytotoxin coronatine, a highly active jasmonate (JA) analogue that promotes virulence by counteracting salicylate (SA) signalling. Conspicuous to immune responses, plants trigger marked cellular redox fluctuations that coordinate defence. Particularly, the redox active molecule, nitric oxide (NO), is extensively involved in shaping hormonal signalling during immunity. NO bioactivity is mediated through protein S-nitrosylation, i.e. the covalent attachment of a NO moiety to reactive thiol groups of proteins, forming a protein-SNO. Recently, Thioredoxin-h5 (TRX-h5) was reported to determine fate and amplitude of SA-mediated immune responses by manipulating specific branches of protein-SNO. However, it remains largely unknown if TRX-h5 controls other aspects of plant immune signalling. Here we investigated the role of TRX-h5 in providing specificity to protein-SNO signalling in SA/JA trade-offs during plant immunity. Infiltration of (S)NO mutants with Psm with or without COR revealed an unexpected interplay between protein-SNO and resistance. Additionally, TRX-h5 overexpressing lines in different backgrounds were tested for resistance against Psm +/-COR and transcription of SA- and JAmarker genes analysed. Overall, our data suggests that specific protein-SNO are manipulated by virulent pathogens to assist in the development of disease.

## P33 ANJEA1 and ANJEA2 control pollen tube discharge in the female gametophyte in Arabidopsis

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Fertilisation in angiosperms relies on pollen tube targeting to the fertile ovules and efficient discharge of the immobile sperm cells in the female gametophyte. This process is tightly regulated by multiple species-specific mechanical and chemical signals that are exchanged between the female gametophyte and pollen tubes. The Receptor-like Kinase (RLK) FERONIA, LORELEI and NORTIA regulate pollen tube recognition and burst at the entrance of the ovules through a mechanism that involves the production of reactive oxygen species as well as changes in Ca<sup>2+</sup> dynamics. Here we report the characterisation of two novel RLKs regulating fertilisation in *Arabidopsis* thaliana. Our results indicate that ANJEA1 and ANJEA2 act redundantly in controlling pollen tube burst at the micropylar entrance of ovules. Pollen tubes overgrow when they reach anjea1anjea2 ovules and fail to discharge the sperm cells, resulting in a 70% reduction in seed production per silique. ROS production in the most fertile stage of ovule development is also impaired in anjea1anjea2 plants, suggesting these two RLKs act in the same pathway as FER, LRE and NTA. We are currently investigating the cellular localisation of ANJEA1/2 as well as genetic and direct interactions with previously characterised components of this signalling pathway.

### Wheat roots release a multi-polysaccharide complex that promotes soil aggregation

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Plant roots release a complex mixture of high and low molecular weight molecules into the soil. This exudate serves many roles including plant defense, lubrication of roots, water and nutrient acquisition. Polysaccharide released from roots, forms a high proportion of this exudate. Despite their high proportion very little is known about their composition and function. We have identified which polysaccharides are release by wheat roots using monoclonal antibody probes. Enzyme Linked Immuno-Sorbent Assay (ELISA) revealed that arabinogalactan-protein, extensin, xylan and xyloglucan were released into the hydroponic medium of wheat. Using anion-exchange chromatography combined with ELISA, demonstrated that these polysaccharides could also form a multi-polysaccharide complex, Root Exudate Complex 1 (REC1). Scanning electron microscopy uncovered that the commercial forms of these released polysaccharides, tamarind xyloglucan and birchwood xylan, as well as REC1 rapidly increased the abundance of soil aggregates. Subjecting soil to high water and mechanical pressures through dry dispersion and wet sieving assays demonstrated that these aggregates were highly stabile. Increasing the abundance and stability of soil aggregates increases contact that the roots have with soil. Thus, releasing these components reinforces the root-soil interface, which mediates water and nutrient uptake from the soil used for plant growth.

### Plant lectins: New players in mycorrhiza-induced resistance?

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Plant lectins are a family of proteins with sugar-binding properties that are involved in different cellular processes. Some of these processes are associated to plant immunity such as recognition of pathogen-associated molecular patterns (PAMPs) or causing toxicity to insects by binding to glycoconjugates in the digestive tract of the insect. Recent studies have revealed the importance of different Arabidopsis and tobacco lectins in plant defence, however, there is not much information about the role of tomato lectins in plant immunity. Recently, we have demonstrated that arbuscular mycorrhizal fungi (AMF) can modify defence responses in shoots of tomato plants and become more tolerant to biotic stresses. In the present study, we identify two tomato lectins with higher gene expression levels in tomato plants challenged with the generalist herbivore *Spodoptera exigua*. We also show that tomato plants colonized with the AMF *Funneliformis mosseae* displayed more tolerance to *S. exigua* and AMF modifies lectins expression in response to insect attack via jasmonic acid (JA).

## Genetic diversity and demographic patterns in the orchid *Cypripedium calceolus* L.

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The influence of clonal reproduction on species diversity and differentiation remains controversial, because of the difficulty in discerning the relative contribution of the reproduction mechanism to the patterns of variation. *Cypripedium calceolus* L. offers a case study to understand population dynamics in a mixed clonal-sexual system. We employed eleven nuclear microsatellites to analyse European and Asian demes, including thriving to depauperate populations. We examined different demographic scenarios by employing Approximate Bayesian Computation models. Differentiation in the lady's slipper orchid seems not to follow the isolation by distance model. Genetic admixture in most of the populations suggests persistence of the ancestral variation from the refugia, with occasional long distance dispersal, in agreement with, respectively, the long term stability of the clumps and the dispersal capacity. With these premises, the differential decline or longevity among the populations appear to be predominantly due to extrinsic factors, such as climatic variables or habitat disturbance.

### P37 Re-establishment of cell polarity following cell division

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Local concentration gradients of the phytohormone auxin are crucial for many aspects of plant development, including tropic movements. During the gravitropic response of the root, increased auxin concentration on its lower side locally inhibits cell elongation, causing the root to bend downwards. The auxin accumulation at the lower side of the root depends on the polarized auxin flow mediated by the polarized plasma membrane (PM) distribution of the auxin efflux carrier PIN2 within cells. How exactly cells achieve the remarkable polar localization of PIN2 and other polarized PM proteins is unclear. In particular, how this localization is re-established after cytokinesis remains a mystery.

We employ live-cell imaging of *Arabidopsis* roots to study how apical PIN2 polarity is established specifically in epidermal cells that have recently divided. We show that PIN2 molecules are targeted preferentially to the new PM during cytokinesis and for ~2 hours thereafter, rather than to the apical PM domain. The subsequent process of polarity re-establishment relies mostly on polarized secretion of *de novo* synthesized protein and differential rate of removal of PIN2 from the apical and other PM domains. Currently we are searching for molecular mechanisms regulating these distinct trafficking events.

# 'Blinded by the light' – Darkness and high light inhibit seedling photomorphogenesis via suppression of *GLK1*

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Plants obtain and process information about their light environment to optimally use the available light for growth. Young seedlings in the dark undergo skotomorphogenic development to facilitate emergence from the soil, after which they undergo photomorphogenesis and develop green tissues to live as photoautotrophs. Once exposed to the sun, high light levels damage chloroplasts, which trigger a retrograde signal (RS) to the nucleus and suppress photomorphogenesis. These comparable phenotypes in darkness and high light are regulated via suppression of GOLDEN2-LIKE1 (GLK1). In darkness, this is directly regulated by a group of bHLH transcription factors; the PHYTOCHROME INTERACTING FACTORS (PIFs), which bind to GLK1 promotor. It remains unknown how PIFs, known as transcriptional activators, repress GLK1. During high light stress, when PIFs are degraded by active phytochrome photoreceptors, the chloroplast-localized protein GUN1 induces the RS which leads to GLK1 suppression. Our research focusses on the regulation and biological function of GLK1 transcriptional suppression in these seemingly opposite extreme light environments. We especially investigate the role of chromatin-level regulation, and several known regulators of photomorphogenesis. This study converges plant ecophotobiology, RS and epigenetic methods to study plant development in suboptimal light environments.

# Proteometabolic analysis revealed changes in secondary and lipid metabolism upon ethylene and ABA treatments in *Glycine max* leaves

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Phytohormones play a central role in plant's physiology. Despite the significant understanding in the hormone signaling, a deep understanding of downstream targets is missing, especially at the protein and metabolite levels. In this direction, here we used an integrated physiological, proteomics and metabolomics approach to investigate the ethylene, ABA and combined ABA+ethylene signaling in soybean leaves. Protamine sulfate precipitation (PSP) method was employed to enrich the low- abundance proteins followed by their identification and quantification using label-free quantitative proteomics. This approach allowed the identification of 4129 unique proteins and 1617 differentially modulated in one or more treatments. Functional annotation of the identified proteins showed an increased abundance of proteins related to the flavonoid and isoflavonoids biosynthesis in response to ethylene treatment and a shift in the fatty acid metabolism upon ABA treatment. HPLC analysis showed an accumulation of isoflavones (Genistin, Daidzein, and Genistein) upon ethylene treatment, validating the proteomics results. A LC-MS based metabolomics approach was utilized to further investigate the changes in the secondary metabolites that confirmed

accumulation of flavonoids and isoflavonoids in response to ethylene treatment. Taken together, our results provide a holistic view of ABA and ethylene signaling in soybean leaves.

### **P40**

# Deciphering molecular composition of stress granules in *Arabidopsis thaliana* through isolation of TSN-interacting proteins

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Efficient adaptation to stress depends on the availability of energy resources. Stress drives cells to an energy crisis whereupon they have to reduce energy expenditure in order to survive. To this end, eukaryotic cells compartmentalize specific mRNAs and proteins in cytoplasmic ribonucleoprotein complexes (mRNP) known as stress granules (SGs). In these structures mRNA molecules are stored, degraded or kept silent in order to prevent energy expenditure on producing useless, surplus or even harmful proteins under stress conditions. Molecular composition, structure, and function of SGs in plants are largely unknown. Recently, we have revealed that Tudor staphylococcal nuclease (TSN) is essential for the integrity and function of SGs in Arabidopsis thaliana. Yet, TSN is stably associated with SGs, suggesting that it may serve scaffolding role to recruit other proteins to the mRNP complexes. Therefore we used TSN as bait in tandem affinity purification of SG-associated proteins. Localization of identified proteins to SGs in vivo has been further verified using a combination of biochemical and live imaging techniques. As a result, we have produced a list of SG-associated proteins. Some of these proteins have previously been found in animal and/or yeast stress-induced mRNP complexes, while others appear to be novel or plant-specific SG components.

### P41 Soil salinity limits plant shade-avoidance

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Global food production is set to keep increasing despite a predicted decrease in total arable land. To achieve this, denser planting will be required on increasingly degraded soils. In dense canopies, plants perceive shade through alterations in light quality, which then causes them to elongate until sunlight is reached. Shade-induced elongation is mediated by the bHLH transcription factors, PHYTOCHROME INTERACTING FACTOR4 (PIF4), PIF5 and PIF7. Here we demonstrate that very low levels of NaCl in soil strongly impair the ability of plants to respond to the threat of shade.

Using a combination of phenotypic, genetic and biochemical approaches, we show interaction between salt, abscisic acid (ABA), brassinosteroid (BR) and light signal cascades. The inhibition of shade-avoidance by salt is dependent upon ABA perception and signalling and requires the BR signalling kinase BRASSIONSTEROID INSENSITIVE 2 (BIN2). BIN2 is known to suppress PIF4 function. It is proposed that salt-mediated increases in ABA signalling enhances BIN2 action against the PIFs, thereby limiting shade-avoidance. The results represent a substantial step forward in our understanding of how multiple environmental factors are integrated towards optimal growth in variable environments.

## Phytophthora infestans RXLR effector interacts with S factor NRL1 to promote turnover of SWAP70

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Plant pathogens deliver effectors into plant cells to manipulate host processes. Much attention has been focused on identifying their host targets. Recently, we reported that *Phytophthora infestans* RXLR effector Pi02860 targets NRL1, NPH3/RPT2-like protein, in the host cytoplasm and at the cell plasma membrane. NRL1 is susceptibility factor that suppresses INF1triggered cell death. A dimerization-dead NRL1 mutant attenuates infection and loses its ability to suppress INF1-triggered cell death. NRL1-mut reduces the ability of Pi02860 to attenuate INF1-mediated HR, demonstrating that host NRL1 activity is required for Pi02860 to promote disease. NRL1 interacts with a guanine nucleotide exchange factor (GEF), SWAP70, which localises to endosomes. Virus-induced gene silencing of SWAP70 in N. benthamiana resulted in enhanced P. infestans colonization and compromised INF1triggered cell death. Overexpression of SWAP70 showed reduced P. infestans infection and accelerated INF1-triggered cell death, indicating that this host protein acts as a positive regulator of immunity. Moreover, suppression of INF1-triggered cell death by Pi02860 was significantly attenuated by coexpression with SWAP70. Importantly, Pi02860 enhances the interaction between NRL1 and SWAP70, promoting destabilisation of SWAP70 by proteasome dependent pathway. We argue that Pi02860 uses host protein NRL1 to target SWAP70, potentially blocking host vesicle trafficking to suppress immunity.

# P43 Development of primary genomic resources for securing sustainable hazelnut production in Turkey

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The European hazelnut (Corylus avellana) is Turkey's most valuable agricultural export. 70–80% of the world's hazelnut market is produced in Turkey and much of the rural Black Sea population rely on hazelnut for their primary income. Despite this, little work has been done to understand genomic variation within hazel. Here, we aim to generate genomic resources that will act as an initial step towards securing sustainable hazelnut production. By using high-throughput, reduced-representation sequencing, we will lay the foundations for improving resistance to drought, frosts and disease in this economically important species. We aim to assess the genetic diversity within and among over 100 hazelnut cultivars and wild populations, and to determine whether any diversity uncovered is linked to cold/drought tolerance or resistance to an emerging powdery mildew threat. This disease, caused by a fungus of the genus Erysiphe, is thought to be the most significant immediate threat to hazelnut production today. Initial tests reveal considerable variation within and among different cultivars with multiple origins of disease resistance. This work will provide a platform for more targeted genomics research in the future that can enhance the speed at which trees can be bred, through selection of beneficial gene combinations in seedlings.

## Gene expression data support the lycopsid root, rather than the modified shoot, hypothesis for the evolution of Isoetalean rootlets

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Arborescent clubmosses were the first giant trees to grow on the planet; reaching heights of 50 m, they towered over the surrounding flora in the Carboniferous coal-swamp forests that formed over 300 million years ago. These trees were tethered to the ground by rooting structures termed stigmarian systems.

What makes stigmarian systems so intriguing are the numerous parallels in anatomy and development between these below-ground rooting systems and above-ground shoot systems. These similarities led to the longstanding hypothesis that stigmarian rooting systems represent modified shoots, with rootlets representing modified leaves. This hypothesis is termed the modified shoot hypothesis. The only living relatives of the arborescent lycopsids are species of *Isoetes*. By carrying out a comparative transcriptomic analysis of the rootlets of *Isoetes echinospora* with the related lycopsid *Selaginella moellendorffii*, we can test, for the first time, the predictions of the modified shoot hypothesis using gene expression data. We identified a high similarity between the rootlets of *I. echinospora* and the roots of *S. moellendorffii*. Our findings support the hypothesis that roots of all lycopsids are homologous, and are not modified leaves, as predicted by the modified shoot hypothesis.

## P45 Mechanisms of plant growth responses to potassium

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The potassium ion (K<sup>+</sup>) is essential for plant growth and development. K<sup>+</sup> deficient plants exhibit symptoms typified by chlorosis and necrosis of leaves, as well as an overall reduction in the growth of aboveground tissues, translating to greatly reduced yields. Given the importance of K<sup>+</sup> deficiency in agriculture, it is important to understand the mechanisms by which K<sup>+</sup> is taken into the plant and impacts on the architecture of the root system, affecting the ability of the crop to forage for K<sup>+</sup> in the soil. In response to low K<sup>+</sup> Arabidopsis thaliana accession Col-0 reduces its lateral root growth but maintains primary root growth in order to search for more K<sup>+</sup>. Synergistic or antagonistic interactions between different combinations of plant hormones are known to coordinate processes such as lateral root formation and primary root growth, and therefore are key in defining the root system architecture. The work described here characterizes the architectural response to low K<sup>+</sup> as well as identifying a role for the phytohormone gibberellin and DELLA proteins in modulating lateral root growth in response to K<sup>+</sup> starvation.

# P46 Expression of a mammalian DNA demethylase in tomato activates expression of an endogenous *CETS* gene

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DNA methylation, an epigenetic modification that occurs in both plants and animals, affects the transcription of genes and other genetic elements. The removal of DNA methylation, DNA demethylation, occurs via different pathways in plants and mammals. We have previously shown that the expression of a mammalian demethylase enzyme, TET3, in plants induces changes in DNA methylation. Here, I show that TET3 expression in tomato causes distinct phenotypes including a change in shoot architecture. These phenotypes resemble those observed when members of the tomato CETS gene family are mutated or overexpressed. In TET3 tomato lines, I identified ectopic expression of a putative CETS family member that has not previously been functionally characterised, CEN1.1. I demonstrated that expression of CEN1.1 correlates with upstream hypomethylation both in TET3 plants and wild type tissues. CEN1.1 was overexpressed in tomato and similar phenotypes to those seen in TET3 were observed. Vegetative growth increased in a variety of tissues, resulting in a delay in flowering, instability of the inflorescences and, paradoxically, an increase in the number of fruit. When expressed in Arabidopsis thaliana, CEN1.1 again caused increased vegetative growth and floral repression.

### P47 MPK12 and MPK4 regulate HT1 kinase in stomatal CO<sub>2</sub>-signalling

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Plant gas-exchange with the environment occurs via stomata, small pores on the leaves and stems. Adequate stomatal responses to changes in environmental factors ensure efficient uptake of CO<sub>2</sub> for photosynthesis with minimal water loss. Increase and decrease in CO<sub>2</sub> concentration cause stomatal closure and opening, respectively. The signal transduction events underlying these responses have largely remained elusive. We identified key regulators CO<sub>2</sub>-induced stomatal closure via analysis of ozone-sensitive mutants of Arabidopsis thaliana. A dominant mutation in HIGH LEAF TEMPERATURE1 (HT1) kinase, a regulator of stomatal CO<sub>2</sub> responses, caused high stomatal conductance and complete loss of stomatal CO<sub>2</sub>responsiveness. We also showed that MITOGEN- ACTIVATED PROTEIN KINASE12 (MPK12) is a regulator of stomatal CO<sub>2</sub> signalling as plants deficient in MPK12 had impaired stomatal responses to CO<sub>2</sub>. MPK12 and MPK4 interacted with HT1 and inhibited its activity in vitro. Lack of both MPK12 and MPK4 in guard cells caused complete CO<sub>2</sub>-insensitivity of stomata. These data indicate that MPK12 and MPK4 are negative regulators of HT1 kinase in stomatal CO<sub>2</sub>-signalling. Characterization of HT1-MPK interaction in crop plants will help to further understand how plants respond to the changing atmospheric CO<sub>2</sub> concentration and contributes to breeding of crop plants with higher water use efficiency.

### P48 Resistance against *Phytophthora* in plants and the role of elicitins

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Phytophthora cinnamomi is a destructive oomycete plant pathogen in both natural and agricultural systems worldwide. Resistance to *P. cinnamomi* is rare. We have explored resistance mechanisms in roots of Zea mays and Lomandra longifolia following pathogen inoculation. Inoculated plants developed restricted lesions and lateral roots formed above the inoculation point. Microscopic analysis of lignin, callose and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) formed in inoculated roots indicated their involvement in L. longifolia resistance. The transcriptome of L. longifolia was analysed using RNA-seq and following inoculation of roots resistance-related genes involved in defence signalling, phytohormone biosynthesis and phytoalexin biosynthesis were markedly induced. Elicitins are conserved proteins secreted by many Phytophthora species. We isolated and purified the β-cinnamomin elicitin from P. cinnamomi and used an immunoaffinity purified antibody as a tool to examine loss of virulence. Confocal microscopy was then used to show that β-cinnamomin was produced at different *P. cinnamomi* life stages and in inoculated plant roots. Zoospores pre-treated with antibody and then exposed to susceptible Lupinus angustifolius roots lost virulence suggesting the intrinsic role of the elicitin. Elucidation of resistance- related mechanisms in plants and pathogenicity factors in *P. cinnamomi* opens up opportunities for modification of susceptible plant responses and targets to prevent infection.

## P49 Hormonal interactions in root responses to mechanical impedance

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

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

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

### P50 Doppelgängers or long, lost cousins: Insights into the evolution of Bowman-Birk Inhibitors

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Bowman-Birk Inhibitors (BBIs) are a family of plant protease inhibitors which have thus far only been described in the legume (Fabaceae) and cereal (Poaceae) families. This distant phylogenetic distribution implies BBIs evolved independently by convergence whereas their high structural and sequence similarity suggests they originate from a common ancestor. By targeted bioinformatic searches for the highly conserved BBI inhibitory loop, we discovered BBI-like sequences exist in many angiosperm species including the basal angiosperm Amborella trichopoda. Our findings suggest that BBIs have undergone lineage specific gene expansion as well as lineage specific gene loss. Intriguingly, we also found highly divergent BBI-like sequences in the seedless, vascular spikemoss Selaginella moellendorffii. Selaginella belongs to the lycopod plant lineage that diverged ~200-230 million years before the common ancestor of angiosperms. For one of the S. moellendorffii BBI-like sequences, we showed its encoded protein inhibits trypsin. It was necessary to mutate two lysine residues to abolish trypsin inhibition suggesting its mechanism of inhibition is shared with characterized double-headed BBIs from angiosperms. Identification of BBIs in Selaginella, along with the identification of BBI-like sequences in the basal angiosperm A. trichopoda, implies legume and cereal BBIs share a common ancestor.

# P51 Mechanistic understanding of nutrient limitation on carbon sequestration in two phosphorus-limited ecosystems

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Understanding how plants respond to increasing atmospheric CO<sub>2</sub> concentration under nutrient limited conditions is important for an accurate estimate of the global carbon budget under climate change. It has been well established that elevated CO<sub>2</sub> increases plant growth, but it has also been observed that this CO<sub>2</sub> fertilization effect may not be sustained over time due to progressive nutrient limitation. As such, there is a need to quantify the magnitude of the CO<sub>2</sub> fertilization effect on plant carbon storage through space and time. Field-based observations are accumulating (e.g. Free Atmospheric CO<sub>2</sub> Exchange (FACE) experiments), but data are constrained to a relatively short time period. On the other hand, there has been an increasing momentum to include nitrogen and phosphorus cycles in global vegetation models, but models differ in their structures and levels of complexity. Model-data synthesis provides a unique opportunity for a mechanistic and predictive understanding of the plant-soil interactions in a changing world. In this study, we used the Generic Decomposition and Yield model (GDAY) to estimate how a temperate evergreen broadleaf forest (site Euc-FACE) and a tropical rainforest (site Amazon-FACE) respond to elevated CO<sub>2</sub> under phosphorus limited conditions. We do so by 1) implementing a phosphorus component into the original C-N-H<sub>2</sub>O simulation framework; 2) estimating the effects of various model assumptions on the long-term plantsoil equilibrium behaviours through an analytical approach; and 3) simulating how carbon sequestration in these ecosystems respond to the 21st century climate. This research is significant and novel in that, 1) we explicitly test the effects of phosphorus cycle on carbon sequestration under future climate in two globally important phosphorus-limited ecosystems, and 2) we provide a simple but effective analytical framework to evaluate the long-term equilibrium behaviour in a comprehensive plant-soil model. This framework is generally applicable to other models and model assumptions. Our results highlight that providing a mechanistic understanding of the phosphorus and

nitrogen co-limitation impact on ecosystem productivity is vital for a more accurate estimate of global carbon budget and the provision of ecosystem services under future climate change.

### **P52**

The altered actin dynamics leads to trigger of salicylic acid signalling pathway which outcome in increased Arabidopsis resistance to Pseudomonas syringae

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Actin cytoskeleton is an important platform for signalling involved in plant immunity, a complex process in which phytohormone salicylic acid (SA) plays a key role. We previously described that treatment of Arabidopsis seedlings with SA causes disruption of actin filaments in plant tissue. Additionally, treatment with latrunculin B, a drug depolymerising actin filaments, induces transcription of marker genes of SA signalling. Deeper insight into the mechanism of the connection between SA signalling and actin dynamics is still needed.

Using LC-MS analysis we show that treatment with latrunculin B increases the level of SA in *Arabidopsis thaliana* through isochorismate synthase 1 dependent biosynthetic pathway. Using Arabidopsis mutants (*sid2*, *npr1*, NahG), impaired at different stages of SA signalling pathway, we show that latrunculin B induces transcription of "SA marker genes" both dependent but also independent on the proper function of SA pathway. Additionally, altered actin dynamics modulates callose accumulation and enables *Arabidopsis thaliana* to "prime" against subsequent infection with *Pseudomonas syringae*.

Collectively these results imply that disruption of the actin cytoskeleton triggers processes leading to the induction of plant immunity.

# P53 Flower color polymorphism has cascading effects on plant defence traits in the invasive weed Solanum eleaegnifolium

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Although flower color polymorphism is common in angiosperms, most studies have examined it from a pollinator selection standpoint- where pollinators preferentially choose morphs rich in anthocyanins. Therefore, whether flower color polymorphism can also affect plant defense against herbivores, is poorly understood. Using multiple populations of the invasive weed S. eleaegnifolium, we show that while pollinators do not discriminate between white and blue morphs of S. eleagnifolium in the field, the herbivores prefer the anthocyanin rich blue flowers, causing higher floral damage. Quantification of metabolic intermediates of the anthocyanin pathway underlying flower color revealed that, although the white morphs have lower amounts of total floral pigments, the flavonoid intermediate myricetin, a herbivore feeding deterrent, accumulates in white morph flowers, consequently affecting the feeding of the specialist herbivore Manduca sexta. In addition, white morph plants, which constitute less than 1% in *S. elaeagnifolium* populations, also have an additional line of defense through the constitutive emission of significantly higher amounts of floral volatiles that have herbivore repellant properties.

Based on these findings, we speculate that the presence of two different modes of floral defenses sustain white morphs from being outcompeted by the blue morphs.

### P54 Small RNA analysis for identification of viruses

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Plant viruses cause devastating loss of crop yield. Most of the plant viruses have a RNA genome. To overcome any disease epidemic efficient diagnostic techniques are required. Early detection of virus can help in controlling spread of disease and save crop from further yield losses. Diagnostic techniques for plant viruses include electron microscopy, cell cultures, nucleic acid based methods, i.e., RT-PCR, RT-LAMP gPCR, RFLP, microarrays, fluorescence techniques, immunology-based techniques like ELISA and tissue blot immunoassay (TIBA), and mass spectrometry. All techniques have their own advantages and disadvantages and require prior knowledge regarding the virus species. The emerging viruses evolve in host plant. The evolution of virus occurs due to mutation and adaptation to the host genome. This feature of viruses produces new viral strains. Virus is exposed to new host, which leads to mutation and recombination in the virus. Different types of viruses have different selective requirements for infecting the host. When virus enters into a new host there will be fitness tradeoffs to adapt, so that virus can infect the host. Due to high mutation rate of RNA viruses during replication, and the high capacity of DNA viruses to recombine, there will be mutations which will be fractional, deleterious, neutral, beneficial or lethal making way for the natural selection of viral population. Consequently, viruses can gain compatibility with new hosts by establishing the necessary virus-host interactions or overcome different defense strategies (gene-forgene, systemic acquired resistance, and RNA silencing). We utilize the RNA interference (RNAi) defense mechanism of plants for diagnostic purpose; the mechanism is directed by small interfering RNA (siRNA) to target and inactivate viral RNA. This strategy has been used successfully in our experiment to identify Petunia vein clearing virus in infected plant samples, followed by further characterization of the viral genome.

### **P55**

Multiplex Polymerase Chain Reaction and loopmediated isothermal amplification for the diagnostic of *Pantoea* species: the new threat for rice production in Sub-Saharan Africa

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Members of the genus *Pantoea* are responsible for many diseases of economically important crops worldwide. Emerging diseases of rice due to infection by Pantoea cause significant damage in most rice-growing areas in sub-Saharan Africa. The aim of this study was to develop a diagnostic multiplex polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) assay for rapid, sensitive and simultaneous detection of the Pantoea spp. belonging to three major species of Pantoea, Pantoea ananatis, Pantoea stewartii and Pantoea agglomerans. Genus- and speciesspecific primers targeting four housekeeping genes of *Pantoea* spp were designed and evaluated on a total of 183 Pantoea spp strains. Sensitivity of detection was monitored on isolated DNA, on in vitro-grown bacterial cells, on artificially contaminated rice seeds, on artificially inoculated rice leaves and on symptomatic and asymptomatic leaves collected from affected rice fields. The reaction parameters were optimized for the multiplex PCR and the loop-mediated isothermal amplification (LAMP) scheme which accurately revealed the presence of pathogens on rice seeds and leaves. This is the first report of a method allowing simultaneous detection of three important Pantoea spp., which will be useful in epidemiological surveillance programs.

# P56 Identifying novel, rhythmic interactors of the Arabidopsis clock protein GIGANTEA using a proteomics approach

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The Arabidopsis protein GIGANTEA (GI) has a wide range of functions: it is involved in the circadian clock, flowering time as well as stress responses. Biochemically, it is thought to be a co-chaperone and a scaffold protein that facilitates interaction of various other proteins such as ZTL, FKF1, CDF1 or LKP2. If the latter is GI's main mode of action, and given its wide range of functions, we would expect that it has more direct as well as indirect interactors than are presently known. Therefore, we conducted a mass spectrometry based interaction proteomics time course of GI tandem affinity purification. Verification of known direct and indirect interactors in independent experiments gives confidence in the data and therefore allows us to postulate new directly or indirectly interacting proteins. Many of these have previously been implicated in pathways relating to GI's functions. Using one of the newly identified interactors as an example, we validate its direct interaction with GI and present mechanistic follow-up experiments. This demonstrates that our data can be used to generate hypotheses on the mechanisms of the function of GI interaction with other proteins.

### P57 Linking auxin signalling and chromatin dynamics on the *PID* locus

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In plants, the hormone auxin acts as a key regulator of growth and development at every stage of a plant's life cycle. Auxin signalling occurs through a pathway in which auxin molecules bind to TIR1/AFB F-box proteins, promoting their interaction with Aux/IAA repressor proteins, repressor degradation and subsequently to de-repression of auxin response factors (ARFs). Recently, our group has found an alternative auxin signalling mechanism in which the transcription factors ETTIN (ETT/ARF3) and INDEHISCENT (IND) function as a co-receptor complex. This auxin signalling mechanism is important for coordinated gynoecium development. To investigate how exactly the auxin signal is mediated by this co-receptor complex regulation of PINOID (PID) a target gene of the ETT-IND complex is examined in detail. ChIP-qPCR revealed chromatin dynamics on the PID locus. Additionally, a Yeast-2-Hybrid approach revealed several candidates that are associated with chromatin regulation. Therefore, preliminary results support the hypothesis that the ETT-IND complex controls expression of their target genes through processes of chromatin regulation.

### P58 Macroecological patterns of intraspecific trait variation

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Intraspecific trait variation (ITV) in plants within natural communities can be large and important for community processes and dynamics. Several contradictory hypotheses on ITV as a function of large scale climatic and species richness gradients have been proposed. It has been shown that the ratio between ITV and interspecific variation within a community (i.e. relative extent of ITV) decreases with increasing species richness while climatic variables were not found to be predictive for this ratio. However, how the absolute extent of ITV in plant species (= coefficient of variation (CV)) is related to large scale gradients remains unknown. Currently, we are compiling a dataset from databases and individual studies to evaluate if the absolute extent of ITV (vegetative and floral traits) responds to climate and species richness gradients. The outcomes of our study provide novel insights in the macroecological patterns of ITV as well as in the differences and/or similarities of different trait groups (e.g. vegetative and floral traits). Further, as high ITV increases the ability of plant to withstand environmental change, knowledge about the global distribution of ITV facilitates a detailed knowledge about the global pattern of plants' vulnerability to environmental change.

## A divergent role of *MAX2* in the strigolactone signaling pathway of *Physcomitrella patens*

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Strigolactones can regulate multiple developmental processes and key ecological interactions. Strigolactones are widely found in the green lineage supporting an ancient role of these compounds during plant evolution. Still, knowledge of the strigolactone signaling pathway evolution is limited. In angiosperms, the F-box protein MORE AXILLARY GROWTH2 (MAX2) is a key element of signaling pathways including strigolactones and a currently unknown plant compound. Moreover, MAX2 has been involved in plant photomorphogenesis. We present here the characterization of the phylogenetic moss homolog of MAX2 asking about their role in the strigolactone signaling pathway evolution. We found that *Ppmax2* moss mutant shows distinct phenotypes from the moss SL-deficient mutant respect to plant extension and gametophore branching. Moreover, Ppmax2 mutant shows sensitivity to the strigolactone GR24 and a transcriptional response was observed in dark conditions. The analysis of *Ppmax2* mutant in red light conditions suggest that PpMAX2 is involved in photomorphogenesis as in seed plants. Therefore, our data suggest a divergent evolutionary pathway of PpMAX2 compare to that of vascular plants. In Physcomitrella patens, the primary role for this F-box protein being more related to photomorphogenesis and moss early development, and less into strigolactone response.

### P60 Derepression of the plant immune receptor complex RRS1/RPS4 by distinct effectors

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Central to plant survival is the ability to activate immunity upon pathogen perception. Plants deploy immune receptors (NLRs) to recognize specific pathogen molecules (effectors) and to trigger defence. How receptor complexes convert effector perception into defence activation is poorly understood. The Arabidopsis RPS4/RRS1 receptor pair detects two distinct bacterial effectors (AvrRPs4 and PopP2) that target WRKY transcription factors via an integrated WRKY domain of RRS1. My work aims to unravel the perplexing intra- and inter-molecular reconfigurations that convert effector/WRKY interactions into complex activation.

I found that deletion of RRS1 WRKY domain constitutively activates the RRS1/RPS4 complex, while further deletion of the 4<sup>th</sup> domain (DOM4) of RRS1 abolishes this auto-activity and renders RRS1<sup>D123</sup>/RPS4 non-functional. This indicates that the WRKY domain is negatively regulating the RRS1/RPS4 complex in the absence of any effectors. Effector perception by WRKY is likely to de-repress DOM4 of RRS1, leading to complex activation. Indeed, Co-IP, BIFC and FRET experiments suggest that AvrRps4 can disrupt the closed conformation of DOM456 via interfering with WRKY/DOM4 association. Additional data also suggest that an open DOM456 is important for RRS1 derepression and the subsequent signal transduction to RPS4. Interestingly, PopP2-triggered activation operates differently and involves domain-domain interactions distinct from AvrRps4-triggered activation.

## P61 Mistletoe mitochondria: the evolutionary mystery of the loss of respiratory complex I

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Mitochondrial respiratory complex I (NADH Dehydrogenase, NADH:ubiquinone oxidoreductase) is the largest complex in the electron transport chain, composed of more than 40 subunits, and plays a major role in aerobic respiration. While numerous single celled eukaryotes have in the course of evolution lost complex I, until recently it was thought that no multicellular eukaryotes could lose such an important respiratory enzyme. However, recent mitochondrial genomic studies challenge this assumption and suggest that the hemi-parasitic plant Mistletoe has lost complex I. Here we purify mitochondria from the European mistletoe, Viscum album and use BN-PAGE and in-gel activity assays, along with proteomic approaches, to biochemically confirm the loss of complex I. We also show that the remainder of the respiratory chain remains intact. This represents the first time a multicellular eukaryote has lost complex I. The loss is thought connected to the plant's parasitic lifestyle, given that parasites often display organeller genome reduction. However this raises interesting questions as to how Mistletoes have adapted to a striking alteration to mitochondrial function.

## P62 LjNBCL1: a key developmental gene essential for multiple aspects of plant development in Lotus japonicus

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Legume plants are able to establish symbiosis with nitrogen fixing rhizobia and to develop a new specific underground organ: the nodule, dedicated to host their symbionts. The molecular mechanisms underlying this symbiotic organ identity establishment and maintenance remain poorly understood. Medicago truncatula forms indeterminate nodules with a persistent apical meristem. The identity of this organ is strictly maintained through the MtNODULE-ROOT1 (MtNOOT1) gene (Couzigou et al., 2012, Plant Cell, 24: 4498–4510). A second MtNOOT gene (MtNOOT2) exists and its expression is mainly associated to the nodule central meristem. The double mutant Mtnoot1noot2 displays a severe nodule to root phenotype, does not fix nitrogen and completely loses its symbiotic organ identity (Magne et al., under review). To understand the role of the nodule central meristem in the nodule identity maintenance, the study of LjNOOT-BOP-COCH-LIKE1 gene (LjNBCL1) has been undertaken in Lotus japonicus. This species forms determinate nodules lacking a persistent apical meristem. In the *Linbcl1* LORE1 insertional mutant, the nodules, nectaries, leaves, axillaries and flower organs were drastically affected in their developments. The *LjNBCL1* function is conserved for the maintenance of the L. japonicus determinate nodule identity and appears to be essential for the correct development of the whole plant body.

## P63 Short distance cell-to-cell communication in response to wound stress in Arabidopsis root

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Plants during their entire lifetime are opposed to various threats resulting in tissue damage, such as physical wounding, herbivore feeding, or crushing by animals. During attack plants adapt to stresses by recognizing biotic, abiotic and physical factors and adequately quickly respond to it, by orchestrating specific signaling pathways. However, the mechanisms by which these signals are perceived by cells and how the signal is further transmitted from one cell to another for local and systemic signaling is still largely unknown. In the aerial tissues, plants evolved long distance communication system, from leafto-leaf as response to wound signaling, which lead to the distal production of jasmonates mediated by electro potential changes (Mousavi et al., 2013). In our work, we focused on short cell-to-cell signal transmission upon nematodes invasion and single cell laser ablation (mechanical wound) in the root of Arabidopsis thaliana. We demonstrate that physical wounding caused by single cell laser ablation, which mimics nematode behavior during feeding, elicit surface potentials changes depending on ion channels and ROS production. These changes turn on the local production of ethylene as a potent regulator of wound responses and deterrence of nematode feeding. Our observations provide insights into the distinct mechanisms of shortdistance cell- to-cell wound signaling in roots, allowing cells to rapidly spread information among neighbors in response to local stressors physical wounding caused by single cell laser ablation and nematode feeding elicit surface potentials changes depending on ion channels and ROS production.

Mousavi S.A. et al. (2013). Nature. 500(7463):422-6.

### P64 Importance of fluctuations in light on plant photosynthetic acclimation

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Little is known about the effect of dynamic fluctuations in light on plant phenotype and acclimatory responses. Here we mimicked natural fluctuations in growth light conditions over a diurnal period to examine the effect on photosynthetic processes and growth. Plants subjected to square wave light had thicker leaves and greater photosynthetic capacity compared with fluctuating light-grown plants. This, together with elevated levels of proteins associated with electron transport, indicates greater investment in leaf structural components and photosynthetic processes. In contrast, plants grown under fluctuating light had lower leaf light absorption, but maintained similar photosynthetic rates per unit leaf area. Despite high light use efficiency, plants grown under fluctuating light had a slow growth rate early in development, most likely due to the fact that they were not able to fully utilize the absorbed light energy for carbon fixation. Diurnal measurements revealed a negative feedback control of photosynthesis, resulting in a decrease in total diurnal carbon assimilated of at least 20%. These findings highlight that growing plants under square wave light ultimately fails to predict plant performance in the field, and stresses the importance of considering fluctuations in incident light in experiments that aim to infer plant productivity under natural conditions.

## P65 Ubiquitination in wheat defence against Septoria fungus

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Wheat is a major food crop for much of the world, and with an ever-increasing population there is a rising demand to produce more food in a smaller area. *Zymopseptoria tritici* is a devastating foliar pathogen of wheat, which can lead to a 20% reduction in yield. Plants have had to evolve a multitude of different defence mechanisms due to their sessile nature. Protein modifications, such as ubiquitination, have been shown to be central to plant defence.

In this study Virus Induced Gene Silencing (VIGS) was used to investigate *Triticum aestivum* E2 ubiquitin conjugating (TaU) enzymes. The main focus of this study is TaU4, the E2 function of which has been proven through ubiquitin charging assays and the active site cysteine identified. The possible function of TaU4 in the Septoria-wheat interaction was investigated after silencing TaU4 in wheat and then infecting with Septoria. TaU4 silenced wheat leaves showed a delay in the onset of Septoria infection symptoms and had reduced pycnidia and spore counts when compared to the vector only control. It was concluded that TaU4 acts as a negative regulator of defence in wheat against Septoria fungal infection.

### P66 Functional characterization of a MAP Kinase cascade substrate with a PHD-like domain

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Plants possess a complex system to recognize pathogen attacks and defend themselves. One key cellular signaling mechanism is the mitogen-activated protein kinase (MAPK) cascade, which leads to defense gene or hormone regulation. To learn more about the complex functions of this cascade, we are focusing on the characterization of MAP kinase substrates. Phosphorylation of these substrates by MAP kinases can influence their properties, such as protein stability or enzyme activity. We chose a candidate identified previously in a protein array screen for in vitro MAPK substrates. The presence of a predicted Plant Homeodomain (PHD) domain hints that it may function as a connection between MAPK cascades and histone modifications. Orthologues exist only in the flowering plant kingdom and we therefore named it as plant-specific PHD-like1 (PPL1). Using biochemical methods and microarray analysis, we were able to show altered protein stability after phosphorylation and a role in defense gene regulation, which seems to be mediated by the yet unknown function of the PHD-like domain of PPL1. Notably, PPL1 does not appear to bind histones. These findings point in the direction of a new regulation mechanism in plant defense.

# Reliance shapes the alliance: plant mycorrhizal status modulates correlation between plant and AM fungal communities

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Interactions between communities of plants and arbuscular mycorrhizal (AM) fungi shape fundamental ecosystem properties. Experimental evidence suggest that compositional changes in plant and AM fungal communities should be correlated, but empirical data from natural ecosystems is still scarce.

We aimed to investigate the successional dynamics of covariation between plant and AM fungal communities, and the biotic and abiotic factors shaping these dynamics in biodiverse dry grasslands. Plant communities where characterised using vegetation surveys. AM fungal communities were characterised by 454-sequencing of the SSU rRNA gene and identification against an AM fungal reference database MaarjAM. AM fungal abundance was estimated using neutral-lipid fatty acids (NLFA). Multivariate correlation analysis (Procrustes) revealed a significant relationship between plant and AM fungal community composition. Moreover, the strength of the plant-AM fungal correlation weakened during succession, reflecting changes in the proportion of plants exhibiting different AM status, with a strong plant-AM fungal correlation when the abundance of obligately AM plants is high. We conclude that the extent to which plants rely on AM fungal partners is likely to be an important factor structuring communities of both symbiotic partners. Accounting for symbiotic interactions can thus improve our understanding of how communities of both symbionts assemble.

# P68 The tomato DELLA protein PROCERA promotes stomatal closure and inhibits water loss during drought stress

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We have shown previously that inhibition of gibberellin (GA) activity in tomato reduced transpiration under drought conditions. Here we studied the mechanism by which the tomato GA response inhibitor DELLA protein, PROCERA (PRO), affects plant response to water deficiency. Exposing pro loss-of-function mutant to drought led to rapid wilting. This was caused by higher stomatal conductance, resulted from larger stomatal aperture. Overexpression of constitutively active stable DELLA, on the other hand, reduced stomatal conductance and the transgenic plants maintained higher leaf water status for longer time under drought conditions. These effects of DELLA, were strongly suppressed in the abscisic acid (ABA)-deficient mutant sitiens (sit) background, suggesting functional interaction between DELLA and ABA. While DELLA had no effect on ABA accumulation in leaves, it affected sensitivity to the hormone; stable DELLA increased, and loss of DELLA reduced guard cell sensitivity to the hormone. Expressing stable DELLA only in guard cells was sufficient to increase stomatal sensitivity to ABA and to reduce stomatal conductance and water loss under drought conditions. The loss of DELLA activity reduced ABA-induced H<sub>2</sub>O<sub>2</sub> accumulation in guard cells, suggesting that DELLA affect the activity of component/s acting at the early part of the ABA signaling cascade. We propose that DELLA has a role in both rapid and long-term responses to drought; it increases sensitivity to ABA, leading to faster stomatal closure and suppresses growth to improve plant adaptation to prolong drought.

### P69 Chromatin marks of metabolic gene clusters

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Genetic, genomic and biochemical research has established a new feature of plant genomes – the widespread co-localisation of genes for specialised metabolic pathways. The ability to synthesise a diverse cocktail of lowmolecular weight molecules is essential for all plants to interact and communicate with the environment. The co-localisation of the functionally related biosynthesis genes contrasts the general gene order in eukaryotes and enables the formation of fundamentally different mechanisms of gene regulation in comparison to the control of dispersed genes. Our research shows that specific chromatin marks delineate and control the expression of plant metabolic gene clusters. Chromatin analyses in A. thaliana reveal that silenced and activated gene clusters are closely associated with histone H3 lysine 27 trimethylation and incorporation of the histone variant H2A.Z, respectively. In contrast, non-clustered genes of multi-step metabolic pathways are not associated with these modifications. Analyses of H3K27me3 profiles in oat, rice and maize suggest a conserved function of histone methylation in the repression of plant metabolic gene clusters across plant species. Our work supports the hypothesis of a chromatin code that is associated with clustered eukaryotic genes and affords new opportunities for pathway discovery and manipulation of plant specialised metabolism.

## P70 Antibiotics from pond scum – exploring natural product biosynthesis in algae

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Most of the drugs we use today are derived from natural products, including many of the most critical antibiotics. With the increasing problem of antibiotic resistance, and with no novel antibiotics commercialised for decades, there is a growing imperative to discover new natural products. Various techniques have been developed to speed the discovery pipeline and reduce the rediscovery of previously known compounds. Whilst most of the research is focussed on bacteria, I am using these chemoinformatic techniques to investigate the production of complex natural products by Eukaryotic microalgae.

Initially, I am focussing on Euglenoid algae, which I have shown to encode the biosynthetic capacity to make a variety of drug-like small molecules, unlike algae typically studied in the lab. These are a diverse group of eukaryotic microalgae and are extremely common in fresh water, such as ponds and ditches. By comparing strains from different culture collection, it can be readily seen that there are differences between the compounds produced, with more than 10% of the compounds identified unique to individual strains. This has allowed me to focus on the most novel and unique compounds for further structure elucidation and analysis of antibiotic activity.

<sup>1</sup>Cruesmann M.C. *et al.*, *J. Nat. Prod.* (2016) DOI: 10.1021/acs.jnatprod.6b00722. <sup>2</sup>O'Neill E.C. *et al.*, *Mol. Biosys.* 11, 2808-2820 (2015)

### P71 The exocyst and SNARE proteins cooperate in penetration resistance

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Biotrophic fungal pathogens, which enter the plant surface in an attempt to draw nutrients from the host, develop specialized feeding structure called haustorium. The haustoria are separated from the cytoplasm by the specialized extra-haustorial membrane. To stop the biotroph entry, plants build cell wall deposition called papilla at the first stage of defence. If plant cells fail, they seal the haustoria in an encasement or they undergo programmed cell death to avoid nutrients removal. Both levels of plant immunity rely on the functional secretory pathway of SYP121/PEN1. Exocyst complex is a vesicle tethering complex and is important for polarized secretion. It was shown that the exocyst subunit EXO70B2 is involved in the penetration defence. It has been unknown whether other exocvst subunits were also involved in defensive structures biogenesis. Here we report that several exocyst mutants have diminished penetration resistance similarly to mutant syp121. We show that the EXO70B2 specifically localises to the papillae and haustoria since their early development and directly interacts with the SYP121. Altogether we present the evidence of functional link between SNARE and exocyst complex in plant basal defence.

## P72 Study of pathogenicity determinants in Cassava brown streak virus (CBSV)

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Cassava brown streak virus (CBSV) is a main cause of yield reduction and economic losses for cassava in Africa. CBSV encodes for a polyprotein, cleaved into 11 mature proteins, which functions have only been predicted by comparison with other Potyviruses. CBSV lacks the Helper component proteinase (indispensable multifunctional protein), but encodes for an atypical protein; Ham1-like, only identified in Euphorbia ringspot virus. I conducted a study for the function analysis of CBSV proteins, identifying determinants of pathogenicity during viral infection, analysing their role in viral synergism, silencing suppression, movement and replication. Transgenic N. tabacum were generated, expressing individual CBSV encoded proteins, to identify synergistic effects with Tobacco mosaic virus, Potato virus Y and Pepino mosaic virus. P1 protein was identified as silencing suppressor in CBSV. I identified the proteins that synergised TMV infections, enhancing titre levels and symptoms, suggesting implication in silencing suppression, movement or replication.

Ham1-like protein and NIb displayed cross protection effect during PVY infection. Knockout of Ham1-like protein, demonstrated its importance during infection for induction of necrotic symptoms.

# P13 Plant hosts fine-tune the protein level of a receptor-like kinase to regulate nitrogen-fixing symbiosis

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Plants utilize receptor-like kinases to monitor environment changes and transduce signals into plant cells. The mechanisms regulating protein level and subcellular localization of these kinases have extensively studied but are still unclear. The *Medicago truncatula* DMI2 (Does not Make Infection 2) protein functions as a co-receptor of rhizobia signals to initiate nodule development and rhizobial infection during nitrogen-fixing symbiosis. Here we report that DMI2 exhibits protein abundance dynamics during nitrogenfixing symbiosis. The stability of the DMI2 protein is controlled by the proteasome signalling pathway: in rhizobia-free environments, the DMI2 protein is degraded by the proteasome continuously; during rhizobia infection, the DMI2 protein is protected from the proteasome apparatus, resulting in protein accumulation, and activates downstream signals. Meanwhile DMI2 may interact with two novel E3 ligases, mutants and overexpressing lines of both E3 ligases show altered nodulation phenotypes. Our findings suggest legumes control nodule development through modulating the protein level of DMI2, adding a novel layer of regulation in the interaction between plants and rhizobia in nitrogen-fixing symbiosis. Using DMI2 as an example, this study have broader implications of how the protein level of receptor-like kinases is regulated by plants to respond to ever-changing environments in general.

### P74 Retrograde signalling-mediated regulation of plant cross-tolerance

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Plants have evolved in environments where multiple stress agents can challenge their fitness simultaneously. As a consequence, response mechanisms to different stress factors are partially overlapping, promoting the exposure to a given stress cross-tolerance to a range of external cues. This phenomenon could be exploited to produce more resistant plants for agriculture, where environmental factors cause significant losses every year. Light-stress, including UV-B-stress, has emerged as an important modulator of plant resistance to disease. UV-B has been demonstrated to prime resistance to necrotrophic fungal pathogens or aphids. However, the underlying molecular mechanisms remain largely unknown. In the last decade, chloroplast-to-nucleus or retrograde signalling has been recognized as crucial in mediating the cross-talk between light acclimation and plant defence responses. In this context, SOT12, a UV-B-induced sulfotransferase whose activity yields phosphoadenosinephosphate (PAP), a key chloroplast retrograde signalling molecule that modulates pathogenesis responses and light acclimation in plants, is an important player. The main aim of this research is to reveal how the regulation of SOT12 activity impacts crosstolerance to UV-B and pathogens in plants. Preliminary exciting results show that SOT12 interacts with key protein kinases and phosphatases that modulate light acclimation and immune responses in plants.

# Heavy soil and treated waste water result in reduced hydraulics and reduced levels of plasma membrane aquaporin (PIP) mRNA in citrus trees

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The concept of available soil water and physical strength for root water uptake and growths hypothesizes that heavy clay are less effective than sandy loam soil. Moreover, the concept of sorption hypothesizes that heavy clay adsorbs all the contaminant of applied water resulting in a toxic environment for roots. Irrigation with low quality water, such as, treated waste water (TWW) in heavy clay soil creates therefore unfavourable environment which might greatly reduce plant performance. In in this study, we examine the effects of TWW, heavy clay, and sandy loam soil on growth, and hydraulic conductivity in comparison to fresh water (FW) and further elaborate on their effect on root aquaporin (CvPIP) gene expression of citrus trees. Almost all investigated parameters, photosynthesis rate, leaf transpiration rate, leaf relative water content, leaf water potential, root water uptake and whole plant specific conductance were lower in TWW as compared to FW and in heavy clay as compared to sandy loam, with lowest values under both TWW and heavy clay. The mRNA levels of eight CvPIP genes showed variable trends upon exposure to TWW and heavy clay; while six of them responded to TWW by reduced mRNA levels, two were unaffected in both soil types. Three PIPs (PIP1:2, PIP2:1, PIP2:2) showed significant reductions in their transcripts levels under TWW and heavy clay, with lowest values under both conditions. Moreover, the mRNA levels of these genes were in a good correlation with root hydraulic conductance, pointing their importance for water balance under optimal conditions. Although salt provides major component of TWW, the expression levels of PIP genes suggest that other components also contribute to the negative effect of TWW.

## Plant diversification in the coldest biodiversity hotspot on Earth: the Andean paramo

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The paramo is among the fastest evolving hotspots on Earth and the most biodiverse high mountain ecosystem, with 5000 vascular plant species of which 60% are endemics. This remarkable phytodiversity has been shaped by the recent orogeny of the northern Andes and the Quaternary glaciation dynamics, which created continental biogeographic islands on mountain tops and promoted plant diversification. Phylogeographic studies offer valuable insights on plant evolution but are often limited to specific taxonomic groups, whereas biogeographic studies may encompass many taxa but not necessarily a temporal component. In this study, we propose to identify plant diversification centers in the paramo and retrace taxonomic evolution based on actual plant spatial distribution models (SDMs). We used presenceabsence data from the VegPáramo database and modelled for each species both their potential and realized distributions, using the Generalized Linear Model and Random Forest algorithms, and the Area Under the ROC Curve for model evaluation. We then selected the best modelling approach and stacked the SDMs based on either taxonomy (genus, family) or biogeographic origin (neotropical, northern temperate, southern temperate). The obtained results on plant richness patterns will suggest taxonomic diversification areas and migration routes, thus improving our knowledge on plant evolution in the páramo.

## P77 A peroxisomal protein import mutant defective in stomatal opening

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Stomata are microscopic pores made up of two guard cells, able to regulate their aperture in response to various internal and environmental signals. The signalling pathways controlling stomatal aperture have been widely studied, however the interplay between cellular metabolism and stomatal dynamics is not as well understood. Recently it was shown that triacylglycerols stored in guard cell lipid droplets function as a key energy source, powering stomatal opening. Here, we present an *Arabidopsis thaliana* peroxisomal mutant, pex13-1, that displays a delayed stomatal opening response to light. PEX13 is a peroxisomal membrane protein, conserved across eukaryotes, involved in importing proteins into the peroxisomal matrix. This study uses confocal microscopy to show the pex13-1 stomatal phenotype is not due to reduced lipid droplet breakdown and hypothesises that it is more likely the downstream metabolic processes, in particular  $\beta$ -oxidation, which reduces the guard cells ability to produce ATP and therefore its ability to power stomatal opening.

# Quantification of the arbuscular mycorrhizal fungi composition in synthetic communities through Real Time-qPCR: calibration and validation

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Arbuscular mycorrhizal (AM) symbiosis represents one of the oldest and most widespread plant-microbes interaction described in the soil. The benefits of the AM mutualism for the development of plants have been tested for decades, and the importance for the structuring and maintenance of plant communities is being intensely studied in the last years. Nevertheless, the characterization of the AM fungi diversity and abundance in soils are poorly understood, and several molecular approaches are being carried out for these investigations. In this work, we tested a Real Time qPCR-based analysis for the quantification of two AM fungal species (*Funneliformis mosseae* and *Rhizoglomus irregularis*) in synthetic communities. Through the design of calibration curves using specific primers for the AM genes expression, we determined the relative abundance of each AM fungal species. The effectiveness of this molecular approach was confirmed and the relevance for future investigations in the plant-AM fungi interactions also suggested.

### P79 Characterization of factors involved in barley stamen development

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Little is known about the molecular mechanisms of stamen development in the important temperate crops wheat and barley. Therefore, my work focuses on the characterization of the barley mutants male sterile genetic 32 (msq32) and msq36 to investigate factors controlling stamen development in this crop. The phenotypic characterization of msg32 and msg36 includes an overview of stamen morphogenesis, tests for pollen viability and histological descriptions, to detect in which tissues and at which stages the defect occurs. Initial data indicate that the msg32 mutant displays an early defect in tapetum morphology and degeneration, leading to microspore abortion. In contrast, the msq36 mutant defect appears later in anther development apparently affecting only anther opening. Putative candidate genes underlying the male sterile phenotypes were identified using a combination of genetic mapping and SNP detection via RNAseq. The candidate gene for msg32 encodes a mitochondrial aldehyde dehydrogenase and for msg36 a pectin-lyase like superfamily protein. To determine the specific function of these factors in stamen development, I will test the hypothesis that MSG32 is required to maintain the metabolic homeostasis of the tapetum and that MSG36 is a cell wall-degrading enzyme that guarantees anther opening for timely pollen release.

### P80 Spatiotemporal transcriptomic changes of Ralstonia solanacearum UY031 during different potato infection stages

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Ralstonia solanacearum is a wide-host-range pathogen that causes bacterial wilt, a devastating disease in tropical and subtropical crops, including potato, tomato, banana and eggplant. Despite the efforts done to understand bacterial wilt on different plants, little is known at the transcriptomic level about the mechanisms by which R. solanacearum modulates gene expression along the infection process. Notably, new insights into the Type 3 Secretion System (T3SS) expression, the main virulence determinant, came from in planta transcriptome studies of the interaction between R. solanacearum and tomato at the onset of disease. Contrary to what was believed based on in vitro studies, it was demonstrated that the T3SS is active at later stages of plant infection. In this work, we aim to study R. solanacearum gene expression at different points of the infection process in potato plants using RNA sequencing. For this purpose, we defined a very early stage (apoplast colonization), mid stage (colonization of xylem vessels in asymptomatic plants) and a very late stage (colonization of dead plants). The data obtained from these in planta conditions will be compared to bacteria grown in synthetic rich medium and also to previous studies from bacteria at the beginning of the disease. Key virulence gene expression behaviors and gene expression patterns will be defined in each infection point. With this information, we will provide new insights in the understanding of R. solanacearum mode of infection and lifestyle, and define genes that are necessary and specific in each step of the plant colonization for a successful interaction.

### P81 The genetic architecture of tristyly and its breakdown to self-fertilization

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The floral polymorphism tristyly involves three style morphs with a reciprocal arrangement of stigma and anther heights governed by two diallelic loci (S and M). Tristyly promotes cross-pollination, but modifications to stamen position commonly cause transitions to selfing. We investigate the genetic architecture of the M locus and the genetic basis of independent transitions to selfing in tristylous *Eichhornia paniculata*. We crossed independently derived semi-homostylous selfing variants of the long- and mid-styled morph fixed for alternate alleles at the M locus (ssmm and ssMM, respectively), and backcrossed the F<sub>1</sub> to the parental ssmm genotype. We phenotyped and genotyped 462 backcross progeny using 1450 genotyping-by-sequencing (GBS) markers and performed composite interval mapping to identify QTLs governing style-morph. A QTL associated with style-morph differences (style length and anther height) mapped to linkage group 5 and spanned ~13–27.5 Mbp of assembled sequence. Bulk segregant analysis identified 334 genes containing SNPs potentially linked to the *M* locus. The stamen modifications characterizing each selfing variant were governed by loci on different linkage groups. Our results provide an important step towards identifying the M locus and demonstrate that transitions to selfing have originated by independent sets of mating-system modifier genes unlinked to the M locus.

### P82 Understanding the CCG effector toolbox of white rust oomycete pathogen *Albugo candida*

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Albugo candida is an obligate biotrophic oomycete pathogen that causes white blister rust disease in Brassicaceae. It comprises of many physiological races that infect distinct host species. Some A. candida races also infect various Arabidopsis accessions, thus facilitating the characterization of effectors and resistance genes involved in this patho-system. A. candida induces a potent **immuno-compromised** state following infection of host plants, which enables different pathogens to colonize and reproduce on same host. Co-habitation of different races on colonized host is therefore possible, and could be an important means of generating novel races through exchange of effector repertoires. Our analyses of multiple A. candida genomes revealed the presence of novel class of secreted CX<sub>2</sub>CX<sub>5</sub>G (abbreviated as CCG) effector family. Every race has around 60-80 CCG proteins which show presence/ absence polymorphism. Multiple CCG effectors are recognized by a White Rust Resistance 4 gene (WRR4) from the model Arabidopsis thaliana (Col-0). Some of these candidate CCG proteins also show an enhanced susceptibility to other comycete pathogens in stable transgenic A. thaliana exhibiting their contribution in promoting disease. Current experiments aim to functionally characterize these CCG effectors especially for their potential involvement in A. candida mediated immune suppression. Recent progress on these aspects will be presented.

### Role of energy metabolism in acquired thermotolerance of *Arabidopsis* seedlings

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In the context of global warming, the frequency and severity of heat waves are likely to intensify. To counteract the impact of heat stress on membrane and protein stability, plants exhibit a heat stress response, which includes the induction of heat shock proteins and antioxidant systems. We aim to decipher the contribution of energy metabolism as a component of heat acclimation mechanisms that allow plants to survive otherwise lethal temperatures. Using a robust system, whereby Arabidopsis seedlings are grown in liquid medium under conditions that cause developmental arrest at the cotyledon stage (Benamar et al., 2013), we have shown that a priming treatment (2h at 38°C) enables seedling survival when, 24h later, they are given a 2h noxious heat shock at 43°C. Measurement of respiration and photosynthesis showed that these activities were partly protected by the priming treatment during and after heat shock. Full recovery was observed one day later in primed seedlings, while damage was irreversible in nonprimed seedlings. The preservation of energy transduction thus appears to be essential for heat acclimation. Ongoing experiments compare the multilevel omics (transcriptome, proteome and metabolome) heat stress responses of primed and control seedlings to build an integrative picture of the acclimation process.

Benamar A., Pierart A., Baecker V., Avelange-Macherel M.-H., Rolland A., Gaudichon S., di Gioia L., Macherel D. (2013) Simple system using natural mineral water for high-throughput phenotyping of *Arabidopsis thaliana* seedlings in liquid culture. *Int. J. High Throughput Screen.*, 1–15.

### The phytohormone ABA activates CONSTANS to promote the floral transition

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The reproductive success of plants depends on their ability to adaptively regulate the time to flowering according to the most favourable environmental conditions. Several phytohormonal networks participate in synchronizing the onset of the reproductive phase. In Arabidopsis thaliana the plant hormone abscisic acid (ABA), known to regulate various drought stress responses, promotes flowering under long but not short day conditions, highlighting a link between photoperiod perception and ABA responses. Genetic analyses support a model where ABA signals interact with the photoperiodic pathway upstream of the florigen gene FLOWERING LOCUS T (FT). The objective of my work is to clarify how ABA signalling might be integrated upstream of FT. Using genetics and gene expression approaches, I could demonstrate that the ABA-dependent activation of FT requires CONSTANS (CO). My genetic study further indicates that ABA acts by modulating CO protein activity, rather than its transcriptional regulation. Transgenic plants expressing a tagged version of CO protein allowed me to directly measure variations of CO protein accumulation under different ABA levels. My results indicate that ABA promotes CO function, supporting a direct interaction between ABA signals and the photoperiodic pathway which may allow plants to coordinate flowering time according to the prevailing watering conditions.

### The regulation of stomatal development in Arabidopsis thaliana

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Environmental signals regulate stomatal development, particularly light, which has a major role in regulating these developmental decisions. Photoreceptors, primarily phytochrome B and the cryptochromes are required for light mediated stomatal development. More recently, we have examined the role of other light signalling systems. It is well-documented that signalling occurs between the chloroplast and the nucleus to coordinate gene expression involved in photosynthesis, chloroplast function and chloroplast maintenance. We now have evidence that chloroplast signalling affects stomatal development or cell fate.

Chloroplast function can be examined by utilising specific inhibitors of photosystem function. DCMU treatment prevents the flow of electrons from PSII to plastoquinone and through the electron transport chain. We have used this to examine the impacts on stomatal development. Our data shows that chloroplast signals specifically control major regulators of stomatal development at the transcriptional and protein level and this results in reductions in leaf stomatal index (SI; the proportion of stomata in the epidermis). We also show via confocal microscopy that chloroplasts are present in the young epidermis, including stomatal lineage cells. We've examined several known retrograde signalling pathways to investigate the signal coming out of the chloroplast and how it controls these regulators of stomatal development.

### The effect of modified scent profiles on pest and pollinator choices

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Recent publications have shown the contrasting floral scent profiles of the laboratory inbred line 165E of *Antirrhinum majus* and a complete set of *Antirrhinum* wild species. The molecular phylogenetic markers and the botanical phylogeny coincide with the different scent profiles analysed. We constructed recombinant inbred lines between *A. majus* 165E and *A. linkianum* differing in the emission of methyl benzoate, methyl cinnamate, acetophenone and ocimene.

We have analysed the genetic structure of scent emission in these RILs. We cloned a loss of function allele of BENZOIC ACID CARBOXYMETHYL TRANSFERASE. The recombinant inbred lines have been used to test attraction and repulsion of the pest *Frankliniella occidentalis* and pollinator *Bombus terrestris*. Our results indicate an evolutionary pressure and/or drift towards distinct scent profiles in the different species. Scent profiles display mendelian segregations linked to single scent components. The null allele of *A.linkBAMT* is caused by a major rearrangement in the promoter including an IDLE MITE transposon insertion. Both thrips and bumblebees show distinct preferences for floral scent blends. Complex scent profiles may be achieved by combinations of genes that are dynamically changing as a result of transposons. Changes in scent profiles may have profound effects on pest and pollinator choices.

Multigenerational plant response to rising CO<sub>2</sub> under climate change: Adaptation of the stomatal patterning pathway in a natural CO<sub>2</sub> spring model

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Global atmospheric CO<sub>2</sub> concentrations have been increasing rapidly since the Industrial revolution, reaching an annual global average of 400 ppm in 2015. The IPCC predicts that without mitigation, CO<sub>2</sub> concentrations are likely to reach 720–1200 ppm by the end of century. As a key substrate for one of the most fundamental processes to life on earth; photosynthesis, increased carbon assimilation can have a profound effect on the growth and development of plants and their interactions within an ecosystem. Current predictions of plant responses to elevated CO<sub>2</sub> are generally based on short exposure experiments which neglect the potential role of adaptation. Using seed from *Plantago lanceolata* growing at naturally elevated CO<sub>2</sub> and a nearby ambient CO<sub>2</sub> control site as a model, we explored the basis of the plastic and adaptive responses to elevated CO<sub>2</sub> through a cross-factored chamber experiment. Growth at elevated CO2 was associated with reduced stomatal density, increased epidermal cell size and increased stomatal index. Analysing gene expression with RNA-sequencing, we identified 1205 genes that were differentially expressed between plants originating from spring and control sites grown at ambient and elevated CO<sub>2</sub>. From this we were also able to identify key components of the stomatal patterning pathway that likely underlie this observed plastic and adaptive response.

# P88 Improving crop production under environmental stress by using seed treatments to prime plant defences

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Priming is a state where plant defences are not actively expressed but the plant is able to respond more quickly or strongly to an attack. This can be artificially achieved through the exogenous application of various chemical activators. Seed treatments are an efficient method which deliver treatments directly to the plant and have been shown to confer plant resistance against a range of stresses. This project aims to use tomato (*Solanum lycopersicum*) as a model system to test priming resistance to a range of biotic and abiotic stresses using seed treatments with a range of activator compounds. Using jasmonic acid as a seed priming compound, we have begun to look at the extent to which seed treatments can mitigate the effects of salt stress. In future work, we will include other stresses, and ultimately aim to develop crop management strategies incorporating seed treatments which reduce pesticide inputs.

## P89 Lipids in cold stress: what can we learn from new methodologies?

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Environmental stress is a major constraint to crop productivity and there is great interest in understanding the coping mechanisms that plants use under such stress, in order to apply new and innovative solutions for crop protection. As a major component of cell membranes lipids have been recognized as having a significant role in cold stress, both as a mechanical defence through leaf surface protection and plasma membrane remodelling, and as signal transduction molecules. To better understand the relationship and functional roles of the cell lipidome, the transcriptome of Arabidopsis was analysed under cold stress and responsive lipid related genes were selected for further characterisation. A set of 46 T-DNA insertion Arabidopsis lines was created and a physiological approach to screen for cold resistance in the selected lines was developed. As new reliable methods for cold screening could not only increase the pace that physiological assessments are produced, but can also be potentially commercially applied. The lipid related knockout lines were subject to cold stress whilst their adaptation was monitored by chlorophyll *a* imaging fluorescence technique. The use of high throughput chlorophyll fluorescence screen not only demonstrated utility of this approach for surveying cold tolerance, but also established the relationship with lipidome remodelling.

### P90 How do circadian rhythms increase plant water use efficiency?

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Reduced soil water availability represents a serious threat to modern agriculture, causing significant decreases in crop yield worldwide. Climate change is predicted to exacerbate this situation, with more frequent and longer droughts in future. Therefore, it is a research priority to develop solutions for more sustainable use of water in agriculture. As global agriculture alone represents 70% of human water consumption, one possible solution is to develop plants that lose less, and so use less, water. Circadian rhythms are known to increase plant water use efficiency, but the mechanisms underpinning this phenomenon remain unclear. I am examining the involvement of circadian regulation in the water use efficiency of higher plants, focusing upon the role of the circadian oscillator in stomatal guard cells. I am analysing Arabidopsis plants with altered guard cell circadian clocks to better understand the relationship between the circadian oscillator and stomatal aperture. I am also using screening approaches to further investigate the contribution of circadian regulation to plant water use. I have explored the involvement of circadian regulation in stomatal movements in naturally-occurring populations of Arabidopsis halleri plants. Overall, this work is providing a deeper understanding of how circadian rhythms optimise plant water use efficiency.

# P91 Investigation of HvABI5 regulatory role in barley response to drought using hvabi5.d TILLING mutant

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Abscisic acid (ABA) phytohormone is responsible mainly for plant response to abiotic stress. ABA pathway includes action of numerous receptors, kinases, protein phosphatases and transcription factors. ABA INSENSITIVE 5 (ABI5) is a basic leucine zipper (bZIP) transcription factor which regulates expression of ABA-responsive genes ensuring plants stress adaptation. We identified a new allele in barley ABI5 gene using TILLING strategy. hvabi5.d showed a higher Relative Water Content (RWC), changed photosynthesis efficiency and reduced stomatal conductance under drought stress. The analysis of the global profile of expression after 10 days of drought allowed to identify 641 genes upregulated and 1665 genes downregulated specifically in hvabi5.d mutant compared to WT. The analysis of the promoter sequences of differentially expressed genes enabled identification of the potential HvABI5 target genes. Our results can be helpful in understanding HvABI5 action in barley adaptation to abiotic stress. We plan to confirm the interactions between HvABI5 and its potential targets using Yeast One Hybrid System.

## Adaptation to drought in *Populus*: is leaf growth a key trait?

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Water scarcity limits plant productivity and consequently threatens food, fuel and fibre production. Although food production remains high on the research agenda, ensuring energy security is becoming increasingly important. This research focuses on delivering new germplasm of *Populus nigra* for sustainable bioenergy. Leaf growth is a critical trait influencing plant productivity, due to the nature of leaves as the photosynthetic organs of the plant. The development of leaves, both in terms of size and cell/stomatal patterning enables plants to maximise light capture while minimising water loss. Crucially, leaf growth is responsive to water availability and is a sensitive indicator of genotypic adaptation to drought.

Here, three morphologically and physiologically diverse genotypes were subjected to intensive leaf phenotyping under control and moderate drought conditions in a fully automated glasshouse. RNA-seq elucidated the transcriptomic basis of the drought response with 2700 genes found to be involved and the reconstruction of gene-phenotype networks allowed five key genes to be identified as central to the drought response. These microtubule-related genes act as hubs in young leaves, whereas, as leaves mature, they are reduced in their connectivity and move to peripheral parts

of the networks. These genes represent candidates for future breeding programs.

# P93 Genotypic differences in environmental effects on photosynthetic isotope discrimination and mesophyll conductance in barley

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Mesophyll conductance (gm) is considered as one of the three major physiological processes limiting photosynthetic rate. Recent studies have proposed g<sub>m</sub> as a trait that may be incorporated into crop breeding programmes for photosynthesis and water use efficiency improvement purposes. However, a prerequisite for utilizing g<sub>m</sub> as a selectable trait is to understand its genotypic and environmental variability. We quantified g<sub>m</sub> using combined measurement of gas exchange and online carbon-isotope discrimination for fifteen barley genotypes grown under non-limiting conditions. The measurements revealed that g<sub>m</sub> displayed significant amonggenotype variation, ranging from 0.36 mol m<sup>-2</sup> s<sup>-1</sup> for the genotype "Tantangara" to 0.49 mol m<sup>-2</sup> s<sup>-1</sup> for "Tallon". Based on the characterized genotypic range, we selected three genotypes that had low, moderate and high g<sub>m</sub> values, to investigate how g<sub>m</sub> responds to short-term variation in leaf temperature. We found that the responses varied from a 2.2-fold increase in g<sub>m</sub> between 15°C and 35°C for "Dash", and a 1.5-fold increase for "Vb1904", to almost no change in "Tantangara". As such, our observed variable temperature response patterns of g<sub>m</sub> at the within-species level extends the results of a recent study that documented significant variations at the among-species level.

## The evolution of flammability among grasses with spatially explicit fire spread

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Whether plants can evolve to promote flammability is controversial. Ecologically, fire only spreads in landscapes when many plants are flammable, but collective behaviors among large groups are difficult to evolve at the individual level. Here, we formulate a model that combines individual flammability with landscape fire spread, in the context of flammability among grasses. In grasses, flammability has absolute fitness payoffs; grasses self-shade when their moribund biomass does not decompose. Dry grasses burn, and moist grasses decompose microbially; individual dryness (flammability) is partially environmental and partially evolvable (some grasses dry more easily). Fire spreads via an infection process, such that fire spreads and individuals burn only when much of the landscape is flammable.

Fire-prone and fire-resistant landscapes, composed of flammable and non-flammable grasses, respectively, were alternatively stable in some environments. However, flammability only evolved de novo in arid environments, when fire spread was inevitable. A positive feedback with fire could maintain flammability in an increasingly wet environment, and flammable grasses could invade wet areas after evolving in dry areas in a heterogeneous landscape. Thus, fire probably did not drive the evolution of flammable grasses, but could have promoted their widespread invasion and persistence.

### P95 Barley physiology and phenotype in circadian clock mutants

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Increasing photosynthetic efficiency combined with managing water use could improve crop yields. Stomata are critical to this process. Circadian clock mutants of the spring barley, Bowman, have an early flowering phenotype, yet photosynthetic capacity and stomatal conductance have not been closely studied. In plants with intact clocks, chloroplast movement and stomatal opening have previously been linked to time of day. To assess the impact of stomatal behaviour on carbon assimilation, we characterised stomatal conductance and CO<sub>2</sub> assimilation during tillering (GS2) in Bowman and two clock mutants over a 10 hour diurnal period using infra-red gas analysis, and the data were linked to phenotype. At GS2, clock mutants had higher assimilation in the morning, although this difference was less apparent later in the photoperiod. The effect of the mutation on stomatal conductance was mixed, but by the afternoon was significantly higher in WT. Consequently, WT had lower WUE for much of the day. The WT also had larger leaves and more tillers than the mutants while the mutants flowered earlier, as expected. The mutants' efficient use of water could be an important breeding characteristic in regions subject to drought.

## P96 Stomatal immunity is cell autonomous and uncoupled from guard cell ABA signalling

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Stomata are formed by a pair of guard cells that actively control the size of the pore aperture. By dynamically increasing or decreasing their volume, these cells act as gateways to the leaf interior for bacterial pathogens. Recognition of invading pathogens by plasma membrane-localized immune receptors i.e. FLAGELLIN SENSING 2 (FLS2) and EF-Tu RECEPTOR (EFR) induces the closure of stomata. Yet, it remains controversial to what extent immune signalling in guard cells converges with guard cell signalling cues for ABAinduced stomatal closure. To address this, we first examined the stomatal closure in response to bacterial flagellin (flg22) in a number of ABA biosynthesis mutants. We found that all mutants exhibited wild type-like flg22-induced stomatal closure showing that stomatal immunity is independent of ABA biosynthesis. We next focussed on the three closely related SnRK kinases, of which OST1 (SnRK2.6) is required for ABA-induced stomatal closure. Analysis of single knock-out mutants revealed that flg22induced stomatal closure required SnRK2.3 but not OST1. These data suggest that flg22-induced stomatal closure is uncoupled from ABA guard cell signalling. We then investigated whether stomatal immunity occurs in a cellautonomous manner as known for ABA. Using virus-induced gene silencing and guard cell-specific promoters to restrict FLS2 signalling to guard cells we observed closure of stomata upon flg22 stimulation. Thus, flg22-induced stomatal closure is cell-autonomous. In a complementary approach, we excluded the expression of the reactive oxygen species (ROS) producing NADPH oxidase and EFR from guard cells. Interestingly, these stomata closed normally in response to bacterial EF-Tu suggesting signalling events from the surrounding ROS-producing and EFR-expressing cells. Taken together, our data suggest that stomatal immunity is uncoupled from ABA guard cell signalling and can be induced by cell-to-cell signals, but also occurs in cellautonomous manner.

## Genes involved in gynoecium patterning recruit cell cycle machinery

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The establishment of symmetry during organogenesis requires tight spatial and temporal coordination of cell division and hormonal control. A rare symmetry transition, from bilateral to radial, takes place during the development of the Arabidopsis female reproductive structure, the gynoecium, which develops into the fruit after fertilisation. Two bHLH transcription factors, INDEHISCENT (IND) and SPATULA (SPT), control auxin dynamics and recruit cell cycle machinery for this transition to take place. The mechanism of controlling cell division in order to achieve organ patterning is still unknown. Microtubules play an important role in the cell cycle: for instance, in plant cells the preprophase band (PPB), a microtubule array, predicts the cell division plane. The gynoecium in the spt ind double mutant fails to achieve the correct symmetry transition resulting in a cleft or split in the style. SEM analysis suggests that misorientated cell divisions are responsible for this phenotype. Our hypothesis is that SPT and IND direct cell division plane orientation. To address this hypothesis, I am studying microtubule dynamics during gynoecium development and downstream targets of SPT and IND using RNA-seg and ChIP-seg.

# Manipulation of *Cassava brown streak virus* infectious clones to gain understanding of viral replication and symptomatology

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Cassava is a staple food crop for approximately 300 million people in sub-Saharan Africa. Tubers are used as a fresh carbohydrate source and can also be processed into a wide range of industrial food products. Unfortunately, production is severely constrained by the Cassava brown streak disease (CBSD), which causes tuber necrosis and can reduce yields by up to 100%. CBSD is now pandemic across East and Central African countries, where it is having devastating impact on the food security of subsistence farmers and results in annual economic losses of more than US\$75 million. Current understanding of CBSD viral gene function is based on homology to related viruses alone, with very little insights into how CBSD replicate, move and cause symptoms. This work shows how CBSD viral infectious clones developed during my PhD have been manipulated through the insertion of the maker gene: GFP to visualise viral movement and replication in planta. This may be used to test potential resistant cassava varieties in the future. This work also involves identifying sequences involved with disease symptomology through mutations and chimera gene swaps. These insights into the molecular biology of CBSD viruses will help to gain understanding that will hopefully lead to control methods in the fight against CBSD.

## Ectomycorrhizal fungi and soil enzymes across elevation gradients in Southern Patagonia – a view from the bottom of the world

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Ectomycorrhizal (ECM) fungi facilitate the degradation of organic matter and mobilize essential nutrients to the plant in nutrient-poor soils. The response of ECM communities to temperature will therefore strongly influence forest dynamics in the context of climate change. Mountain ecosystems have been widely used to study the effects of climate over short distances. However, because vegetation usually varies with elevation, it is difficult to differentiate between abiotic (i.e. edaphic) and biotic (host plant) effects on plant-fungi interactions along elevation gradients. In southern Patagonia, the ECM tree Nothofagus pumilio forms continuous mono-specific forests along mountain slopes (ca. 150–750 m elevation), representing a unique opportunity to study how elevation impacts ECM and soil fungi communities without interference from other ECM tree species. We identified fungal communities by ITS1 Illumina sequencing and detected strong shifts along elevation gradients associated with variation of pH, soil moisture and organic content. In contrast, fungal enzyme activity mostly varied according to local nutrient conditions and was minimally influenced by elevation. The high turnover of ECM fungal communities across elevation gradients coupled with low variation in enzyme activity suggests that *N. pumilio* maintains high fitness across environments and may be resilient to impending climatic changes.

# Comparative genomics reveals strains-specific variations in the genome of the plant pathogenic fungus *Colletotrichum higginsianum*

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Genomic analyses of fungal phytopathogens have revealed that fast-evolving genomic compartments are often enriched in effector genes encoding diverse small secreted proteins involved in pathogenicity. Colletotrichum higginsianum has been a model phytopathogen to study fungal hemibiotrophic infection of plants because it infects Arabidopsis thaliana. In order to investigate genomic variations of this pathogen, we sequenced the genome of strain MAFF305635-RFP from Japan with PacBio RSII, yielding an assembly of 49.8 Mb consisting of 28 contigs. Then, it was compared to the genome of strain IMI349063 from Trinidad and Tobago (Zampounis et al., 2016), revealing that the two strains are closely related, sharing 88.2% of sequence (≥ 99% identity, ≥ 15 kb). However, this analysis also identified extensive genomic rearrangements and strain-specific regions. These largescale structural variations were found to associate with transposable elements. Strain-specific effector candidate genes were also identified from each strain. These results suggest that C. higginsianum has experienced dynamic changes in its genome and then gained strain-specific features which may contribute to pathogenicity.

Phytochrome detection of far-red light enrichment in the shoot regulates lateral root emergence through control of polar auxin transport

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At high plant densities reflection of far-red (FR) light by surrounding plants is detected by the phytochrome photoreceptors as a reduced red (R):FR ratio. Low R:FR induces shade avoidance responses, including elongation of hypocotyls, stems and petioles. In addition, low R:FR perception also leads to changes in the root system, a phenomenon that has received little attention. We adopted an agar-plate system that kept light sources away from the roots to investigate how light quality perception in the shoot, controls root system development. We show that low R:FR causes a strong decrease in lateral root (LR) emergence and a marginal reduction of primary root elongation. This decrease in LR emergence is initiated through phytochrome signaling and involves shoot-to-root communication of a mobile factor, which is induced by low R:FR. Also the amount of auxin in the cortex layer above the LR primordium is reduced during low R:FR exposure of the shoot. This correlates with a lowering of the abundance of the auxin transport proteins PIN3 and LAX3 on the plasma membrane in this cortex layer. Together this forms a model where phytochromes in the shoot activate signaling that negatively affects auxin transport in LR primordia, thus blocking LR emergence.

## P102 Proteasomal protein turnover during defense priming in *Arabidopsis*

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Ubiquitin proteasome system (UPS) mediated protein turnover is involved in local and systemic immunity. The specific role of the UPS in systemic acquired resistance (SAR) and priming is largely unknown. The Arabidopsis mutants rtp2a-2 and rpn12a-1 are impaired in proteasome function and have been subjected to detailed analysis during priming. Compared to Wildtype Col-0, these mutants show higher bacterial multiplication in systemic leaves after a primary local bacterial infection. In accordance rpnt2a-2 and rpn12a-1 show significantly weaker expression of SAR marker genes e.g. PR1 and FMO1 which are involved in salicylic acid (SA) and Pipecolic acid (Pip) signaling and biosynthesis. The proteasome mutants accumulate less SA in infected and/or primed local and systemic tissue compared to wildtype. Interestingly, rpt2a-2 and rpn12a-1 are capable of synthesizing the defense metabolite camalexin to concentrations comparable to wildtype. By infiltration of SA, the SAR-phenotype of both proteasome mutants is cured. It is therefore feasible to postulate that the defense phenotype of rpt2a-2 and rpn12a-1 is caused by a defect upstream of SA biosynthesis. To unravel the link between proteasomal turnover and early SA signaling we are currently analyzing global transcriptional changes via RNAseg analysis and identifying ubiquitinylated proteins addressed by the UPS during priming.

## P103 Gene duplications associated with phytochemistry in *Cannabis sativa*

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Gene copy number variation has been reported in multiple plant species, particularly in genes related to stress response. This fast way of acquiring variation assures rapid diversity under strong selective pressures. One of the main characteristics of *Cannabis sativa* is the production of secondary metabolites -cannabinoids- many of them with medicinal importance, like THCA ( $\Delta$ -9-Tetrahydrocannabinolic acid) and CBDA (Cannabidiolic acid). By using a genomic assembly and the genomes of 67 Cannabis varieties from different groups within the species, we found that the genes that codify for THCA and CBDA vary in copy number. We found copy number variation in these genes both within and between groups, and within varieties. Additionally, using transcriptomic data, we found that major lineages within Cannabis differ in which genes are functional and expressed. The ecological function of cannabinoids is unknown, but it has been suggested that they are produced against predation, or as UV light protection. Copy number variation in these synthases support the hypothesis of the evolution of cannabinoids as stress response. We established that copy number partially explains the amount of cannabinoid produced, supporting that cannabinoid production and amount are an outcome of sequence, copy number variation, and expression levels in these genes.

# Exosome-mediated delivery of cytoplasmic effectors by the potato late blight pathogen, *Phytophthora infestans*

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Potato blight, a ravaging disease caused by the oomycete *Phytophthora* infestans, is a major threat to global food security. P. infestans secretes effector proteins that are delivered inside or outside plant cells to neutralise host immunity. Knowledge of effector delivery mechanisms is limited. Recently, there has been increasing interest in the study of pathogen-derived exosomes. Exosomes are tiny extracellular vesicles (EVs) secreted to facilitate intercellular and extracellular communication, potentially to promote infection. EVs are not known from P. infestans, so this work tests the hypothesis that exosomes may play a key role in the delivery of pathogen molecules during infection. First, P. infestans effectors Pi04314 and EPIC1 were localised to the same infection structures, but determined to be secreted via different pathways. Second, EVs were identified using transmission electron microscopy. Third, proteomic analysis was used to analyse the contents of EVs, showing that they can mediate delivery of cytoplasmic effectors and other infection-related proteins from *P. infestans*. This is a major breakthrough in the understanding of how pathogens and plants communicate during infection development, providing a deeper understanding of the weapons of *P. infestans*. This knowledge may be exploited to develop a new avenue for inhibiting pathogen infection.

# P105 Investigation and description of a new class of SUMO proteases in plants, and their roles in pathogen perception

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Modification of proteins in eukaryotic organisms has been shown to be a major regulator in almost all processes within the cell. The Small Ubiquitin-like Modifiers (SUMO) have been linked to development, stress, growth, defense and more. We have identified several new putative SUMO proteases by bioinformatics using previously described SUMO proteases from mammalian systems. This is the first time this class of proteases has been identified and described in plants.

Using GFP fusion proteins, we have found these different proteases localizing to the tonoplast and cell membrane as well as the nucleus. Further investigation revealed strong roles in plants defense against both biotrophic and necrotrophic pathogens. The specific roles of one of these SUMO proteases has shown a significant involvement in pathogen perception. Using these and other approaches we are further elucidating the function and the roles of the Arabidopsis SUMO proteases.

# P106 Arabidopsis MS1 functions as a hub in the transcriptional regulatory network of late tapetum development

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The development of the pollen grains relies upon the nourishing and secretory properties of tapetum. The transition of the post-meiotic phase of tapetum development depends on the *MALE STERILITY 1* gene. *MS1* has a unique and transient expression pattern, which is tightly regulated and critical for tapetum development and viable pollen formation. Therefore, understanding the genetic control of *MS1* is key to uncover the regulation network for post-meiotic tapetum development.

Here in this project, three regulation levels of MS1 were studied: (i) transcriptional activation, (ii) auto-repression and (iii) post-translational proteolysis. Phylogenetic footprinting analysis and molecular promoter dissection, was used to investigate the transcriptional control of expression and a distal upstream sequence (-2900 to -2066 bp) was found to be essential for the activation of MS1. Three evolutionarily conserved noncoding sequences (CNS), enriched with unusually long consensus motifs, and binding site combinations of MS1 upstream transcription factors (TFs) were found within the -2 kb MS1 upstream sequence. These may serve as essential cis-regulatory elements (CREs) for MS1 expression. ChIP experiments were used to investigate MS1 autorepression; the MS1 protein was shown to bind to the second exon of its genomic locus and to repress its own expression. Post-translational proteolysis was investigated using a triple mutant of LRB1/2/3 that encode the MS1 interacting E3 ubiquitin ligases. The knockout of LRB1/2/3 caused a novel tapetum phenotype, which may be due to altered removal of MS1 protein in the Irb1/2/3 tapetum.

## P107 Transcriptional regulation of proline biosynthetic genes in rice: *In silico* promoter analysis

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Proline accumulation occurs in plants following the exposure to a wide array of stress conditions, as well as during numerous physiological and adaptive processes. This requires the biosynthetic pathway to be regulated by members of numerous and different signal transduction chains. Proline synthesis proceeds mainly from glutamate, which is converted to P5C by P5C synthetase 1 (P5CS1) or P5C synthetase 2 (P5CS2); the latter is then reduced by P5C reductase (P5CR). The regulatory patterns underlying P5CS1, P5CS2 and P5CR gene induction are not fully understood, yet. On the other hand, it is generally believed that genes having common regulatory motifs in their promoters should have similar expression patterns and vice versa. In this work the region comprising 1000 bp upstream the translation start site of OsP5CS1, OsP5CS2 and OsP5CR genes was analyzed in silico for the presence of putative *cis*-regulatory elements (CRE) and a detailed analysis is reported. Numerous CREs have been identified that in turn are recognized by different putative transcription factor (TF) families. Overall, we show that proline biosynthesis in rice could be regulated by a complex network of TFs which are related to almost all plant hormones.

### Purification and characterization of a symbiosisinduced endocellulase from the ectomycorrhizal symbiont *Laccaria bicolor*

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In forest soils, ectomycorrhizal fungi establish a mutualistic symbiosis with tree roots. The mutualistic fungi trade host photoassimilates against soil nitrogen and phosphorus. Differentiation of symbiotic roots induces extensive cell wall architectural modifications in the host apoplastic space. The origin of enzymes involved in these cell wall modifications has been the subject of debate for several decades.

The ectomycorrhizal basidiomycete *Laccaria bicolor* has a restricted set of carbohydrate-active enzymes (CAZymes) degrading plant cell wall polysaccharides. However, several of those genes are upregulated upon symbiosis. We speculate that several of the symbiosis-induced CAZymes are involved in the remodeling of the host apoplastic space. Here, we characterize the sole GH5 endoglucanase with a cellulose-binding motif (CBM1) domain (LbGH5) identified in the genome of *L. bicolor*. We showed that the *LbGH5* gene is induced five folds in ectomycorrhizal roots using qPCR and RNA-Seq. RNAi mutants with a decreased expression of *LbGH5* have a lower ability to form ectomycorrhizal roots. Yeast secretion trap (YST) functional screen confirmed that LbGH5 is a secreted protein. We then produced and purified the recombinant protein LbGH5 with and without its CBM1 domain in *Pichia pastoris*. The recombinant LbGH5 displayed highest activities towards carboxymethyl cellulose (CMC) and cellulose extracted

from aspen roots. In contrast, LbGH5 displayed no activities toward *L. bicolor* cell walls or aspen hemicellulose. *In situ* localization of LbGH5 in ectomycorrhizal roots by indirect immunofluorescence confocal microscopy demonstrated that the enzyme accumulates in hyphal cell walls forming the mantle and Hartig net. These data suggest that cell wall modifications within ectomycorrhizal roots arise from cell wall—modifying enzymes of fungal origin.

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### P109 Mechanistic insights into light regulation of stomatal development

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As sessile organisms, plants need to adapt and coordinate their intrinsic developmental programmes with environmental signals such as light, CO<sub>2</sub> and temperature. Light has a critical role in regulating plant growth and development and photoreceptors play a key role in perceiving the light environment and initiating signalling events that control multiple developmental pathways. The phytochrome red/far-red photoreceptor systems play a major role in controlling flowering, stem and petiole elongation, freezing tolerance, entrainment of the circadian clock, as well as stomatal movement and development. PhytochromeB (phyB) has been found to be the main photoreceptor required for controlling light mediated changes in stomatal density and index. Previously it has been demonstrated that phyB acts both locally and via inter-tissue signalling, with both epidermal and non-epidermal expression of phyB complimenting stomatal defects in the phyB mutant. Using tissue specific phyB expression we have investigated the differential role of phyB signalling in the mesophyll and epidermal tissue and determined how this impacts on stomatal patterning, development and water use.