Genetics Society Seed Plants Meeting

Durham, UK

8th-9th April 2025

Abstract and information book

Contents

Sponsors

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Please pick up a “passport to prizes” card. Cards stamped by each of the sponsors exhibiting will be put into a draw for a £50 voucher.

Venues

Posters and talks

The scientific sessions will take place at the Teaching and Learning Centre (TLC), which is location 50 on the map below.
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Conference dinner

The conference dinner will be held at Durham Castle, location 36 on the map below. The Castle bar will be open for delegates at 6 pm on the 8th April. Please note the bar is cash free.
(https://www.durham.ac.uk/things-to-do/venues/durham-castle/)

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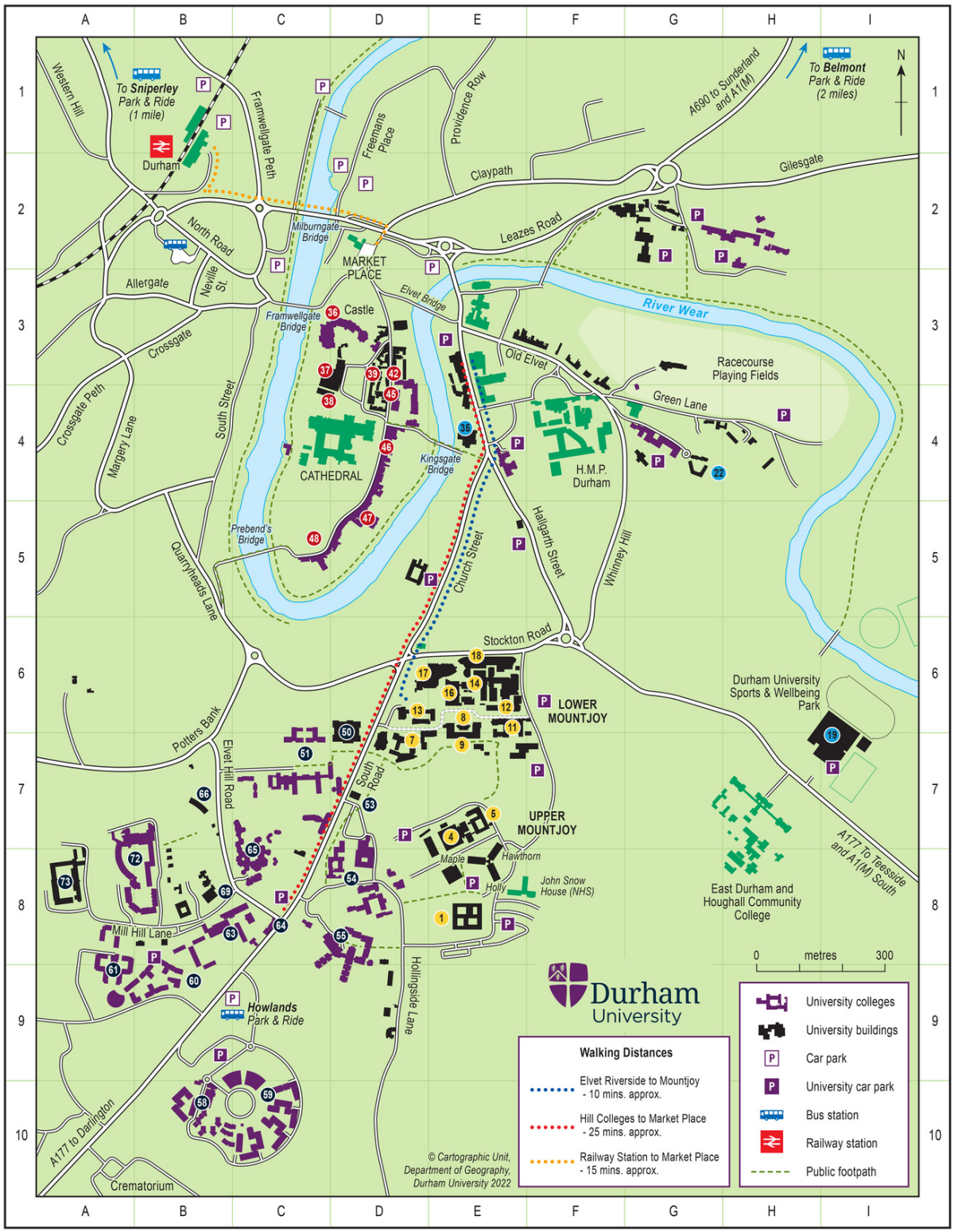
Durham City Map



Meeting Schedule

8th April 2025

Activity	Start	End	
Registration	**08:30**	**10:00**	**Registration, coffee & pastries**
Welcome	10:00	10:05	**Welcome and session introduction**
Session 1	**10:05**	**12:00**	
Signals from within	10:05	10:30	Tony Bishopp
	10:30	10:50	Heena Ambreen
	10:50	11:10	Salma Akter
	11:10	11:35	Elena Baena-Gonzalez
	11:35	12:00	Lisa Smith
Lunch and Posters	**12:00**	**12:55**	**Finger buffet lunch**
Session 2	**12:55**	**14:55**	**Sponsored by New Phytologist**
Responses to the Environment	12:55	13:00	Session Introduction
	13:00	13:25	Beatriz Orosa
	13:25	13:50	Christine Faulkner
	13:50	14:10	Alessandra Devoto
	14:10	14:30	Hee-Kyung Ahn
	14:30	14:55	Miriam Gifford
Tea break and Posters	**14:55**	**15:25**	**Tea, coffee & biscuits**
Session 3	**15:25**	**17:00**	
Education and knowledge exchange	15:25	15:30	Session Introduction
	15:30	15:55	Katharine Hubbard
	15:55	16:20	Dan Jenkins
Keynote	**16:20**	**17:00**	**Yiliang Ding**
Posters	**17:00**	**18:00**	
Castle bar opens	18:00		
Conference dinner	**19:00**	**late**	**at Durham Castle**

9th April 2025

Activity	Start	End	
Session 4	**09:00**	**10:35**	
Interdisciplinary research	09:00	09:05	Session Introduction
	09:05	09:30	Naomi Nakayama
	09:30	09:50	Manoj Kumar
	09:50	10:10	Richard Mott
	10:10	10:35	Nicola Patron
Tea break and Posters	**10:35**	**11:05**	**Tea, coffee & biscuits**
Session 5	**11:05**	**13:15**	
Evolution and Ecology	11:05	11:10	Session Introduction
	11:10	11:35	Ulrike Bauer
	11:35	12:05	Esme Padgett
	12:05	12:25	Saima Shahid
	12:25	12:50	Raj Whitlock
	12:50	13:15	Rafael Gutaker
Lunch and Posters	**13:15**	**14:10**	**Finger buffet lunch**
Session 6	**14:10**	**15:35**	
Development	14:10	14:15	Session Introduction
	14:15	14:40	Sarah McKim
	14:40	15:00	Chris Whitewoods
	15:00	15:20	Kumud Saini
	15:20	15:45	Miguel de Lucas
	15:45	16:10	Peter Etchells
	16:10	16:15	Closing remarks

Abstracts for talks

Signals from within

Keep it simple Spirodela: structural reduction in duckweeds

Anthony Bishopp, Alex Ware, Claire Smith, Rebecca Fairburn, Emily Chan and Rahul Bhosale.

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Duckweeds are free-floating monocots. Evolution has stripped away many features commonplace in flowering plants. Duckweeds exhibit a uniquely simple body plan, variable across five genera. Members of the most ancestral genus, *Spirodela*, have a single frond or thallus producing multiple roots, which lack both branching and root hairs. Members of the most derived genus, *Wolffia*, lack both roots and vascular tissues completely. Using a combination of physiological, ionomic and transcriptomic analyses, we have shown that in duckweed species that produce roots, these roots have lost their salient function of nutrient uptake and can be considered vestigial structures. We believe that duckweeds provide an ideal model for exploring how the molecular mechanisms behind organ loss.

As we follow the trajectory of anatomical reduction, we observe a corresponding decrease in the number of hormone signalling genes, especially for auxin. Through a series of physiological assays and transcriptomic analyses, we see a shift in both the sensitivity of auxin and the classes of genes that auxin targets. This has led us to propose the hypothesesis that streamlining of auxin signalling machinery has led to the anatomical simplifications seen within the duckweed family.

Transposon-mediated somatic mosaicism in Arabidopsis thaliana reveals chromatin-guided de novo integration site preferences

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Transposable elements (TEs) have contributed significantly to crop domestication by generating novel phenotypes and modulating stress response pathways. While transposon movement in germline cells leads to heritable genetic changes, new TE integration events in plant somatic cells can generate cellular diversity and influence regulatory pathways transiently. Comprehending somatic TE movement is thus essential for both fundamental genome biology and the development of effective crop improvement strategies. Nevertheless, the lack of appropriate tools to enrich low-frequency TE integration sites from somatic cells has impeded an in-depth investigation of somatic TE events in plants. Here, we adapt a recent TE high-throughput sequencing technology and establish a new computational pipeline, “deNOVOEnrich” to sensitively identify and analyse de novo TE integrations in the soma of Arabidopsis thaliana. Our work demonstrates a profoundly active and heterogenous mobilome, especially for the heat-responsive ONSEN and epigenetically-induced EVADE and AtCOPIA21 TE families, driving genomic mosaicism in Arabidopsis leaf cells. We identified tens of thousands (~200,000) of somatic transposition events, with rates varying depending on the genotype background and conditions. For example, the abolishment of epigenetic silencing of DNA methylation in a DNA methylation maintenance mutant generates higher TE loads than the loss of polIV/polV polymerases of small RNA-directed silencing pathways. Our findings show that TEs do not randomly integrate into the somatic genome but instead show distinct TE-specific integration preferences. Both ONSEN and EVADE insert in genomic regions enriched in H2A.Z, H3K27me3 and H3K4me1, however, only ONSEN targets active and open chromatin, while EVADE displays depletion in these regions. Furthermore, our data support an insertion bias of these TE families in environmentally-responsive genes, including Resistance (R) genes and metabolic gene clusters. Overall, our study highlights the high mobilization of TEs in Arabidopsis and underscores the key role of epigenetic signatures in shaping TE de novo integration preferences, revealing that somatic genomes are partitioned into regions of variable TE integration likelihood. These findings open a new research field on somatic genome plasticity in plants and its functional impact.

H_2O_2 repurposes the plant oxygen-sensing machinery to control the transcriptional response to oxidative stress

Salma Akter[1†], Monica Perri[2†], Mikel Lavilla-Puerta[2], Beatrice Ferretti[2,3], Sophie Lichtenauer[4], Laura Dalle Carbonare[2], Vinay Shukla[2], Yuri Telara[2], Daai Zhang[2], Dona M. Gunawardana[1], William K. Myers[1], Pedro Barreto[4], Beatrice Giuntoli[5], Markus Schwarzländer[4], Emily Flashman[2*] and Francesco Licausi[2*]

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Understanding plant responses to flooding is crucial for strategies to increase resilience. Plants respond to submergence-induced low oxygen (hypoxia) through decreased Plant Cysteine Oxidase (PCO) activity which stabilises Group VII Ethylene Response Factors (ERFVIIs), master regulators of adaptive metabolic and anatomic responses. Plants must also survive desubmergence stress, which includes a burst of reactive oxygen species (ROS) and rapid metabolic re-acclimation to normoxia. Restoration of PCO function and ERFVII degradation may be expected on reoxygenation. In contrast, we report here that ERFVIIs are important in post-submergence recovery, remaining stable upon reoxygenation through ROS-mediated inactivation of PCOs. Stabilised ERFVIIs are retained at hypoxia-responsive promoters, becoming repressors of typical hypoxia marker genes but upregulators of genes involved in ROS homeostasis and oxidative stress protection. Our findings suggest that PCOs respond to both oxygen and ROS to coordinate ERFVII stability and regulate timely responses to damaging fluctuations in oxygen availability.

Regulation of plant growth by the SnRK1 carbon sensing kinase

Baena-González, E.

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Plant growth and development are largely influenced by environmental conditions. An increasing body of evidence suggests that environmental information is partly conveyed as sugar signals which have accordingly been linked to stress responses, phase transitions such as germination and flowering, and growth control. A central component of the sugar signalling network is the evolutionarily conserved SnRK1 protein kinase. SnRK1 is activated under low carbon conditions often associated with compromised photosynthesis and/or respiration, driving a vast metabolic and transcriptional reprogramming that promotes energy-saving and nutrient remobilization strategies.
In this talk, I will show that several aspects of plant growth and development are regulated by the SnRK1 signalling pathway in response to sugar availability but also in response to the phytohormone abscisic acid that signals water scarcity.

Carbohydrate-binding kinesins in cell division and plant development

Allwood EG[1], Sloan J[1], Thompson EK[1], Lelenaite I[2], Willats W[2], Amsbury S[1], Smith LM[1].

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[2] School of Natural and Environmental Sciences, University of Newcastle, Newcastle, UK

The kinesin family of proteins has expanded in plants to facilitate plant-specific cytoskeletal rearrangements associated with cell division. The precise functions of these kinesins are determined by the protein domains attached to their motor domains, with a plant-specific clade within the kinesin 14 family containing a putative carbohydrate-binding domain. This malectin domain kinesin clade has deep evolutionary deep roots, with family members found in early vascular plants, monocots and dicots.

MALECTIN DOMAIN KINESIN 2 (MDKIN2) knockout lines have stochastic reproductive defects, however vegetative development is only affected under stress conditions. While both Arabidopsis malectin domain kinesins are localised to the phragmoplast during cytokinesis, MDKIN2 is also found at the spindle and in interphase nuclei.

Ashe malectin domain is known to bind cell wall carbohydrates when extracellular as part of a kinase, we tested whether carbohydrate binding had altered since the divergence of malectin domain kinesins from malectin domain kinases. MDKIN2 retains the ability to bind cell wall carbohydrates, however carbohydrate arrays indicate that the malectin domain from kinesins may have altered specificity to reflect its cytoplasmic/nuclear localisation. Our results reveal a non-essential but important role for a carbohydrate-binding kinesin during development and stress responses in plants.

Responses to the environment

Unravelling plant-pathogen interactomes - A Modelling Odyssey into Ubiquitin Targeting

Qiaona Pan[1] , Karolina Brzezinska[1], Zhishuo Wang[1], Steven Spoel[1] and Beatriz Orosa[1,2]

[1]Institute of Molecular Plant Sciences, University of Edinburgh, United Kingdom), [2]Centro Singular de Investigación en Química Biolóxica y Materiais Moleculares (CiQUS), Universidade de Santiago de Compostela, Spain

Plants deploy a sophisticated multi-layered immune system to defend themselves, tailoring responses specifically to encountered attackers. These adaptive responses are primarily regulated by post-translational modifications, with ubiquitin playing a pivotal role. Ubiquitination is a highly versatile protein modification as a variety of different ubiquitin chain linkage types are added to substrates by specific E3 ubiquitin ligases. On the other hand, some pathogens secrete effector proteins that target host ubiquitin signalling to disrupt immune responses. Comprehending this dynamic interplay between host and pathogen is essential for advancing disease resistance. Here, we use barley as a model crop for studying immune-induced ubiquitin signalling in response to the fungal pathogen Puccinia hordei. Additionally, we have identified Puccinia effectors that know down the ubiquitin system, compromising plant immune responses. In summary, this work reveals how the ubiquitin system regulates immunity in crops and how Puccinia pathogens hijack this system to establish disease.

Plasmodesmal regulation of carbon distribution balances defence responses with growth.

Emma C. Raven, Rhea Stringer, Christine Faulkner

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

Plant cells transiently isolate themselves in response to a wide range of immune elicitors by closing their plasmodesmata, the cytoplasmic connections between cells. Plasmodesmal closure is executed independently of other responses to the same elicitors, raising the question of what a cell gains from independent control over plasmodesmal function. We have identified that plasmodesmal closure is differentially executed in leaves of different ages. We determined that this difference is dependent on whether the leaf is a carbon sink or carbon source; sink leaves do not close their plasmodesmata and are more suceptible to a bacterial pathogen. Forcing plasmodesmata closed in young sink leaves imbalanced the normal outputs of growth and defence and revealed that plasmodesmal closure regulates transcriptional immune responses. Therefore, plasmodesmata, as pathways through which soluble sugars travel, balance growth and defence processes to optimise overall physiological success, identifying primary metabolism as a regulator of immune responses.

Analysis of chromatin landscape during drought and jasmonates treatment to increase plant fitness potential

Nancy McMulkin[1], Imma Pérez-Salamó[1], Stacey Vincent[1], Showkat Ganie[1], Colin Turnbull[2] and Alessandra Devoto[*1]
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Stress-sensing signals such as phytohormones trigger large-scale transcriptional changes and it is unclear to what extent chromatin remodelling and chromatin remodelling enzymes play a part in response to stress. Jasmonates (JAs) are key signalling molecules in responding to adverse environmental conditions orchestrating developmental and morphological changes to survive stress. The chromatin modifying HDA6 is intimately linked to JAs signalling and response to flowering, leaf senescence and the acetic-acid-JA pathway conferring drought tolerance.
A key part of the stress response is the growth-defence trade-off, whereby a plant induces the costly expression of genes associated with protection, at the expenses of growth and development. HDA6 acts as a transcriptional repressor of JAs response genes, however its role in the growth-stress response remains unclear.
Recently published from the Devoto laboratory, ChIP-seq analysis revealed over 2000 genes are marked with the chromatin modification Histone 4 acetylation (H4ac) in response to methyl jasmonate (MeJA) treatment, associated with abiotic and biotic stress responses or specialized metabolism, highlighting that extensive chromatin remodelling takes place during JAs signalling. Homologues were then identified in Tomato and investigations are being performed to evaluate their role in the drought response. The findings will clarify the molecular mechanism of the JAs-dependent drought tolerance in both Arabidopsis and Tomato, to identify novel targets for agriculture and industrial applications.
1 Pérez-Salamó, I. et al 2019 APR 2; 2 Howe, G.A. et al 2018 ARPB; 3 Kim, J.M. et al 2017 Nature Plants; 4 Vincent, S.A. et al 2022 BMC Biol

Different roles of TIR domains during immune activation in plants

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2 Department of Plant Soil & Microbial Sciences, Michigan State University, East Lansing, Michigan, USA

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Abstract

Intracellular immune receptors in plants are crucial for rapid detection of pathogens. Some of these NLR (Nucleotide-Binding, Leucine-rich-repeat protein) gene family of plant immune receptors have TIR (Toll-like, Interleukin, Resistance) domain that assembles into NAD+ hydrolysing enzyme upon oligomerization. In Arabidopsis, some of these TIR-NLRs form pairs, whereby two TIR-NLRs share a short transgenic region and divergently transcribed. In the paired NLRs, 'sensor' NLR is evolved to detect pathogen proteins called effectors, whereas 'executor' NLRs are required for activating immune signaling. We identified that in many cases, the 'sensor' TIR-NLR has lost the catalytic residue required for NAD+ hydrolysis, as well as an important motif in the TIR domain required for enzyme activity. Another important feature of these paired TIR-NLRs is their constitutive interaction in both the presence and absence of effector as heteromeric complexes. Furthermore, we found that some TIR-NLRs with two TIR domains have also lost the NADase active site in one of the TIR domains. For example, RPP2A has two TIR domains, and TIR domain near the C-terminus has lost the NADase active site. As these TIR domains are in one protein, these TIR domains are also in close proximity constitutively. It is shown that sensor NLRs can function as repressing activation of executor NLRs alone. However, the function of the sensor NLRs during activation are still unclear. We hypothesize that these TIR domains from the sensor NLRs may have an additional role of providing binding interface for the substrate NAD+. By using various genetic, biochemical, and evolutionary analyses, we aim to investigate the role of sensor TIR-NLRs during activation of paired NLRs.

Nodules and clocks: communication underground

Liam Walker[1], Suzanna H. Dickson[1], Jamie Burgess[1], Clare Hurst[1], Matthew Jolly[1], Monique Rowson[1], Emma Picot[1], Isabelle Carré[1], Miriam L. Gifford[1,2]

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Legumes house nitrogen-fixing endosymbiotic rhizobia in root nodules that are factories of metabolic activity, shaped by the plant, bacteria and environment. Our recent work has approached each of these components to understand how nodulation efficiency is shaped and controlled. We found that symbiotic state and also efficiency can shape endosphere composition and plant nutrition, and have implicated small peptides in plant control. We identified endosphere-located microbes whose abundance is associated with more efficient symbiosis and are characterising their effects. The efficiency of symbiosis is also affected by the plant circadian clock via *LATE ELONGATED HYPOCOTYL* (*LHY*) activity. Rhythmic LHY-regulated transcripts in root nodules include a subset of Nodule-specific Cysteine Rich peptides that may coordinate bacterial activity and the bacterial clock with the rhythms of the plant host to ensure optimal symbiosis.

Education and knowledge exchange

Plant Biology Education for the Future: Building an authentic and inclusive approach

Katherine Hubbard

Buckinghamshire New University

Plant Biology has never been more important for society, whether it is to combat the impact of climate change, create sustainable food security or develop novel plant-based products. If plant biology research and innovation is to make maximum impact, we need to ensure that plant biology education is preparing all students for these challenges. To do this we need to pivot towards a curriculum emphasises technical competency, communication, stakeholder engagement, project management, ethics, sustainability and self-reflection as well as disciplinary knowledge. We also need to ensure that this curriculum is inclusive, ensuring that all plant biologists feel they belong and are able to succeed. This interactive presentation will explore these issues and stimulate discussion around where the future of plant biology education lies.

Gatsby Plant Science Education Programme

Dan Jenkins

Sainsbury Laboratory Cambridge and Cambridge University Botanic Garden.

Funded by the Gatsby Charitable Foundation the Gatsby Plant Science Education Programme works to support a pipeline of plant science enthused future generations in the UK, working with science teachers through the Science and Plants for Schools project (www.saps.org.uk), supporting careers promotion through www.plantsciencefutures.org.uk and running summer schools to engage UK bioscience undergraduates. Against a backdrop of reduced interest in plants by students of all ages, the project adopts a research-informed approach to help engage students, teachers and curriculum designers about the place plants have in solving many of our global challenges, such as climate change, food security and building a sustainable future. More details on the work of the programme at https://www.slcu.cam.ac.uk/gatsby-plant-science-education-programme
In this session we'll explore some of the blockers for young people engaging with plants and the projects we run to try and promote an enthusiastic generation of future plant scientists and plant aware citizens.

www.slcu.cam.ac.uk/gatsby-plant-science-education-programme
@gpsep.bsky.social

www.saps.org.uk
@saps-news.bsky.social

www.plantsciencefutures.org.uk
https://www.instagram.com/plantsciencefutures/

Keynote

RNA structure, an important regulator in living cells

Yiliang Ding

John Innes Centre

RNA structure plays an important role in the post-transcriptional regulations of gene expression. Using in vivo RNA structure profiling methods, we have determined the functional roles of RNA structure in diverse biological processes such as mRNA processing (splicing and polyadenylation), translation and RNA degradation in plants. We also developed a new method to reveal the existence of tertiary RNA G-quadruplex structures in eukaryotes and uncovered that RNA G-quadruplex structure serves as a molecular marker to facilitate plant adaptation to the cold during evolution. Additionally, we have developed the single-molecule RNA structure profiling method and revealed the functional importance of RNA structure in long noncoding RNAs. Recently, we established a powerful RNA foundation model, PlantRNA-FM, that facilitates the explorations of functional RNA structure motifs across transcriptomes.

Interdisciplinary research

Predicting future plant forms with mechano-eco-evo-devo

Naomi Nakayama[12]

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From hairs to worm-like bodies, slender body structures are ubiquitous throughout the Tree of Life. This prevalence may be because such structures can confer various fitness-enhancing functions by interacting with the physical factors in the environment. Small changes in their forms may shift their functions and vice versa; these functional structures are anticipated to evolve in the changing climate. A likely example is the environmentally sensitive flight of the common dandelion – one of Nature's most iconic flyers. The dandelion pappus increases air drag, although the parachute-like structure contains >90% empty space. Through a fluid dynamical characterization, we revealed a previously unseen flow behaviour likely aiding flight. The pappus is sensitive to the moisture level in the air and closes when wet; this morphing tunes the flight capacity. Through an imaging-based deformation analysis, material characterisation, and finite element method mechanical modelling, we gain insights into the mechanisms of the pappus actuator. The dandelion is a pioneer and foundation-building species of an ecosystem that feeds numerous bees and birds. Its dispersal dynamics have deep impacts on ecological geography and agriculture. A future direction will be discussed as to how we could predict and engineer climate-resilient plant forms.

Identifying robust protein interaction networks using multi-bait proximity labelling

Manoj Kumar, Simon Turner

The University of Manchester

Identifying protein interactions is essential for understanding the components of important plant signalling and biochemical pathways. While methods such as immunoprecipitation have successfully identified components of protein complexes, challenges often arise when attempting to detect weak or transient interactions. One way to address this issue is to employ proximity labelling with tags like TurboID. Nevertheless, advancements in mass spectrometers and improved software for peptide identification enhance protein sensitivity, allowing for the identification of a large number of potential candidates. Consequently, interesting proteins can become obscured among the many possible candidates. Our approach involves collecting more data by utilising a greater number of baits and controls, and then meticulously filtering the data to create a network with a manageable and realistic number of interactions.

To illustrate these points, we have utilised the cellulose synthesis pathway in Arabidopsis as a test case. Cellulose is synthesised by a protein complex localised in the plasma membrane, known as the cellulose synthase complex. The core components of this complex are the CESA proteins, which form the catalytic subunits. We conducted proximity labelling experiments by tagging 10 different proteins known to be involved in cellulose synthesis and 7 distinct control baits targeted to various subcellular locations. Our 10 baits initially identified 2,688 interactions from 1,290 proteins enriched against a no-bait control and a soluble control protein (YFP). However, by enriching against our organelle control baits and applying additional criteria, we identified a core network of 115 interactions among 45 proteins, which included previously known proteins associated with cellulose synthesis as well as novel candidates. Subsequently, we employed reverse genetics followed by phenotypic analysis to identify new candidates that affect cellulose synthesis. Among these candidates, we identified a novel protein family that appears to regulate the levels of CESA proteins. Overall, we have demonstrated that a proximity labelling approach involving multiple test baits and various control baits is a powerful and robust tool for identifying protein-protein interactions.

Revisiting the Central Dogma: the distinct roles of genome, methylation, transcription, and translation on protein expression in *Arabidopsis thaliana*

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8. Corresponding author

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¶ Joint senior authors

Background We investigated the flow of information from genome sequence to protein expression implied by the Central Dogma, to determine the impact of intermediate genomic levels in plants.

Results We performed genomic profiling of rosettes in two *Arabidopsis* accessions, Col-0 and Can-0, and assembled their genomes using long reads and chromatin interaction data. We measured gene and protein expression in biological replicates grown in a controlled environment, also measuring CpG methylation, ribosome-associated transcript levels and tRNA abundance. Each omic level is highly reproducible between biological replicates and between accessions despite their 0.5% sequence divergence; the single best predictor of any level in one accession is the corresponding level in the other. Within each accession, gene codon frequencies accurately model both mRNA and protein expression. The effects of a codon on mRNA and protein expression are highly correlated but are unrelated to genome-wide codon frequencies or to tRNA levels which instead match genome-wide amino acid frequencies. Ribosome-associated transcripts closely track mRNA levels.

Conclusions DNA codon frequencies and mRNA expression levels are the main predictors of protein abundance (give value). Neither methylation, tRNA nor ribosome-associated transcript levels add appreciable information. The impact of constitutive gene body methylation is mostly explained by gene codon composition. tRNA abundance tracks overall amino acid demand. However, genetic differences between accessions associate with

differential gbM by inflating differential expression variation. Our data show that the Central Dogma holds only if both sequence and abundance information in mRNA are considered.

Key words
Gene body methylation, mim-tRNAseq, RNAseq, ribosome-associated expression, gene expression, protein expression, data-independent acquisition, genome assembly, chromatin interaction, long reads

Synthetic Expansions of Plant Metabolism

Nicola J. Patron

Department of Plant Sciences, University of Cambridge.

To thrive in environments they cannot escape, plants have evolved intricate regulatory networks and diverse chemical arsenals to fine-tune their metabolism and growth. Our lab employs engineering approaches to uncover how quantitative plant traits emerge from these networks and to harness the vast potential of plant metabolic diversity. Recently, we investigated the widely reported anti-inflammatory properties of the floral extracts of *Calendula officinalis* (pot marigold), an ancient medicinal herb. Our cross-disciplinary approaches uncovered a novel mechanism by which they act in modulating interleukin 6 release in human cells as well as elucidating the genetic basis of the bioactive compounds. By reconstructing the biosynthetic pathway in the heterologous plant chassis *Nicotiana benthamiana*, we provide a platform for production of the anti-inflammatory components. In ongoing work, we are applying synthetic biology approaches to optimise *N. benthamiana* for the production of bioactives for agriculture and medicine.

Evolution and ecology

How to make a springboard trap? Insights from carnivorous pitcher plant in composite trait evolution

Ulrike Bauer

University of Exeter

Composite traits are composed of multiple, independent components that only in combination acquire a novel synergistic function. Their emergence requires the coordinated evolution of multiple independent traits and especially in cases where the component traits on their own do not have an obvious function, this can be difficult to conceptualize. When we discovered that two distantly related species of carnivorous *Nepenthes* pitcher plants use a sophisticated “springboard” trapping mechanism – exploiting the impact of rain drops to catapult insects from the underside of the pitcher lid into the trap – we used this as an opportunity to probe the origin of this composite trait. By combining field experiments with microscopy, biomechanical experiments and biochemical and phylogenetic analyses, we showed that both species independently evolved similar adaptations in three distinct traits to enable this new and unique trapping mechanism. We show that the specific trait combination necessary to enable “springboard” trapping likely arose by a spontaneous coincidence of suitable trait combinations, facilitated by high stochastic phenotypic variation in at least one of these traits. Our results indicate a plausible mechanism for composite trait evolution where high trait plasticity *sensu lato* increases the odds of a beneficial new combination that, once occurred, entails a new beneficial function and is subsequently fixed by strong stabilizing selection. Our findings highlight the importance of stochastic phenotypic variation and random events as facilitators of evolutionary novelty.

Structural variation across the pangenome of flax (*Linum usitatissimum*)

Esme Padgett, Adrian Brennan
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As the performance of modern crop cultivars is losing pace with mounting agricultural demands, crop breeders are seeking strategies that exploit natural genetic variation and fitness within crop wild relatives (CWRs) and landrace varieties. Plant genomes are being mined for the molecular bases of agronomic traits, many of them resulting from human-driven selection pressures. However, technological limitations to complex genome assembly and inferencing currently restrict breeders' ability to engineer superior crop performance and stress resilience.

At the forefront of genomics, 'pangenomic' research has recently identified structural variation (SV) as an underestimated variable in determining functional trait diversity. This project aims to extend genome construction and analysis tools to **explore emerging evidence that variation in the physical structure of genomes influences functional traits**. As part of a morphologically and geographically diverse *Linum* genus, this project assembled the first flax pangenome graph. The pangenome integrated the genome assemblies of several *L. usitatissimum* cultivars and the wild flax *L. bienne*, creating a representative pangenome graph for *L. usitatissimum* cultivars across the Northern Hemisphere.

SV extracted at the genome level were validated by the pangenome, which detected greater variation than genome-to-genome structural comparisons. Within the new SV collection, we have found indels, inversions, and duplications spanning thousands of bases that can be explored for differential, functional variation amongst flax varietals.

The pangenome-validated SV are being used to address the following:

1. Are there SVs associated with (post)-domestication selection amongst flax varieties?
2. Are detected SVs functionally enriched for agronomic or domestication traits?
3. Can the evolutionary history of L. usitatissimum, and the influence of selection pressures be reconstructed from genomic SV?

The mini-pangenome assembly will further characterize the genomic impact of crop cultivation using commercial and wild genomes. This project is also part of a larger trend, using pangenomes to investigate the SV consequences on genome evolution and phenotypic variation.

Dynamic DNA (de)methylation underpins parasitic *Cuscuta*-host plant communication

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Parasitic plants are globally widespread parasites that rely on specialized feeding structures called haustoria to form graft-like connections with diverse hosts and steal water and nutrients. Aggressive parasitic plants feeding on economically important crops cause significant yield loss, but the molecular mechanisms underlying host-parasitic plant communication are poorly understood. Previous work demonstrated that *Cuscuta campestris*, a generalist parasitic vine with a broad host range, uses microRNAs to silence host defence genes via interspecies RNA interference at the haustorial attachment region. Besides microRNAs, *Cuscuta* also induces transposable element (TE)-derived small RNAs at the haustoria, but their function remains unknown. In plants, TE-derived small RNAs establish and reinforce de novo DNA methylation of targeted regions for epigenetic silencing. Using whole-genome enzymatic methylation sequencing, we identified widespread DNA methylation changes in haustorial and nonhaustorial tissues from *Cuscuta-Arabidopsis* associations. Our results demonstrate that *Cuscuta parasitism* triggers global DNA demethylation in the *Arabidopsis* host genome, affecting thousands of TE, genic and intergenic regions. Differentially methylated host targets include several well-characterized defence genes, which is consistent with the known role of DNA (de)methylation in immunity response against other plant pathogens and herbivory damage. Furthermore, the parasite genome also undergoes de novo DNA methylation and demethylation at many regions, suggesting extensive epigenomic reprogramming during parasite establishment. Findings from this work could provide future directions for targeted epigenome editing for broad-spectrum crop resistance to plant parasites.

Parallel genomic responses to moisture availability in the widespread grass *Festuca ovina*

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We know little about the extent to which adaptation by natural selection will allow populations to persist *in situ* through climate change. These adaptive responses are potentially important, as they may play a key role in mediating the resistance of ecological communities to environmental change. Here, we use long-term experimental and natural variation in soil moisture availability (drought) in two geographically separated grassland ecosystems to evaluate the extent and nature of moisture-associated adaptive genomic changes in the grass *Festuca ovina*. Outlier analyses revealed a small fraction of loci whose variants were significantly associated with soil moisture availability—which are likely to be ecologically adaptive—against a background of extremely low genetic differentiation across the rest of the genome. Genomic responses to moisture in the separate populations involved an almost completely non-overlapping set of genes and variants. However, they shared a common functional signature involving variants within metabolic, defence and stress response genes. Our results show that plant populations make functionally consistent genomic responses to soil moisture availability, and suggest that evolution may partially buffer populations from the effects of climate change.

Plant ecology and evolution through the lens of herbarium genomics

Rafal M. Gutaker

Royal Botanic Gardens, Kew

Herbarium collections contain over 400 million specimens, preserving plants and their environments from across the globe. Spanning the last 400 years and virtually any modern plant species, this remarkable resource is useful for addressing a vast range of evolutionary and ecological questions. Herbarium specimens enable comparing plant morphology, classification and is a convenient source of DNA for evolutionary inferences. More recently, a temporal scale of herbarium sampling has been leveraged for genomic studies to track changes in plant diversity and ecology at the population level. In this talk, I will discuss challenges and promises of herbarium genomics, focusing on its potential to investigate human impacts on crops and agricultural ecosystems. I will highlight our research on i) adaptive changes in potato associated with its introduction from South America to Europe, ii) the effects of agrochemical use on rice-associated microbiome, and iii) the lasting impact of chemical warfare on agricultural ecosystems. Overall, this talk aims to emphasize the power of herbarium genomics to elucidate the last centuries of anthropogenic pressure on agricultural and natural ecosystems.

Development

Waxing on about adaptive epidermal features

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Plants develop highly adaptive surfaces to survive on land. Crucial to prevent water loss, the outer epidermal cell layer secretes an external, impermeable, waxy *cuticle*, channeling gas exchange and transpiration instead through dispersed epidermal pores or *stomata* that open and close as needed. As plants colonized diverse terrestrial niches, they evolved further epidermal elaborations including new cell types, stomatal morphologies and cuticular chemistries. We study genetic networks which control distinctive epidermal specialisations in staple cereal grass crop species, barley and wheat. We want to understand not only how cereals arrange multiple epidermal features into coherent, functional surfaces, but also how exploiting epidermal variation in cereals could stabilize yield under changing climates. To improve our understanding of epidermal variation we study epidermal mutants, including the *eceriferum* (*cer*, 'waxless') alleles in barley. In my talk, I will discuss our work revealing a shared genetic network controlling epidermal cell patterning and cuticle properties across multiple tissues in barley and wheat. I will also describe our progress to integrate precise genetic, transcriptomic, chemical and ultrastructural analyses to unravel the hierarchy coordinating cereal epidermal features, including the interplay between cuticle and epidermal cell development, and gene subfunctionalisation. We anticipate that developing a comprehensive genetic and developmental understanding of the cereal epidermis will help us predictively engineer this critical adaptive feature in crops.

Brassinosteroids control leaf air space patterning by promoting epidermal growth

Chris Whitewoods

SLCU

Up to 70% of leaf volume is made of air-filled space between cells. These spaces increase light scattering and gas exchange for efficient photosynthesis. However, despite the ubiquity and adaptive importance of leaf air spaces, the developmental mechanisms that control their formation are poorly understood. I will discuss our recent work using developmental genetics and inducible, layer-specific changes in growth rates to show that Brassinosteroid (BR) signalling is necessary for proper air space patterning in arabidopsis leaves, and that BR acts non-cell autonomously by promoting growth in the epidermis and allowing the expansion of internal air spaces. This work identifies the first molecular mechanism controlling air space patterning in leaves.

Warming up to rush to exit: Leaf growth reprogramming in a fluctuating environment

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Living organisms exhibit maximum growth when they are in optimal conditions. A plastic developmental program allows organisms to sense environmental cues and express phenotypes better fitting their environments. In plants, warm growth temperatures promote cell growth in the hypocotyl, stems, and petioles, which is predicted to aid in cooling and protecting meristems. In contrast, warm temperature restricts leaf growth by suppressing cell number. To explain this opposing effect on basal cell processes and to study how growth temperature fine-tunes the balance between cell division and growth, we combined live cell imaging with Atomic Force Microscopy (AFM) in young proliferating leaves. Through cell-lineage tracking and imaging of cell cycle markers, we show that elevated temperatures affect cell division frequency and alter the timing of cell cycle phases. Analysis of cell shape properties indicated an early onset of cellular differentiation at higher temperatures. AFM measurements showed that the mechanical properties of cells are influenced by growth conditions, where warm temperature softens the wall, potentially explaining the differences in division and growth rates in plants grown under normal and warm-temperature conditions. These results are being integrated into computational models to define the rules that govern cell cycle dynamics and cell wall properties in regulating organ growth in response to environmental changes.

DOF6 Transcription Factor Acts as a Cell Cycle Brake to Regulate Lateral Root Morphogenesis in Arabidopsis

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The precise regulation of cell division and differentiation is crucial for determining organ growth and architecture. In Arabidopsis roots, meristematic cells undergo multiple transit-amplifying divisions before reaching the elongation zone (EZ). Within the EZ, most cells cease division and begin longitudinal expansion, ultimately achieving full differentiation in the differentiation zone (DZ). However, (pro)cambium and pericycle cells retain meristematic potential throughout the EZ and DZ, enabling future radial growth and lateral root (LR) formation respectively. What mechanisms allow (pro)cambium and pericycle cells to preserve their capacity for cell division without actively engaging in the meristematic activity? Our research, combining conditional expression analysis with transcriptional and cell cycle approaches, reveals that the transcription factor DOF6 plays a crucial role in preventing premature meristematic activity in pericycle cells. We discovered that DOF6 acts as a potent repressor of the mitotic machinery; its overexpression produces phenotypes of those observed in mutants with impaired G2-M cell cycle transition. In pericycle cells, a decrease in DOF6 expression precedes LR primordia formation. Notably, auxin, which are key drivers of LR initiation, inhibit DOF6 expression.
Overall, our findings indicate that DOF6 functions as a gatekeeper to control redundant LR development. Therefore, optimising root architecture adaptation to soil conditions.

Receptor kinase heteromers define the cambium stem cell niche

Qing He, Jingyi Han, J. Peter Etchells

Durham University, Department of Biosciences

Wood constitutes the majority of terrestrial biomass and is a globally important carbon sink. Composed of xylem, it arises from one side of the cambium, a meristem that also produces phloem on the opposing side. Cells within the cambium include xylem and phloem initials, and the stem cells from which they are derived. We have recently shown that in Arabidopsis, the cambium stem cell factors are members of the AIL family of transcription factors. AIL expression is controlled by TDIF, a peptide ligand, signalling to PXY, a plasma membrane-localised receptor.

The phenotype of loss-of-function cambium-expressed *ail* mutants is more severe than that of *pxy* lines, which suggests additional factors act redundantly with *PXY* in regulation of the cambium stem cells. We discovered that PXY forms complexes with ER which, like PXY, is a plasma membrane bound receptor kinase. ER is broadly expressed and has pleiotropic functions. Because PXY is only active in the cambium, our results suggests that PXY-ER complex formation defines ER signalling in the cambium. In support of this hypothesis, lines in which PXY signalling was constitutively active had dramatic phenotypic changes that required the presence of ER. Furthermore, combinatorial mutations between *er*, *pxy* and their respective homologues had severe cambial defects, reaching similar levels of cambium loss to those observed in the cambium ail mutants. Thus, we show that coordinated PXY-ER signalling, mediated by heteromer formation, underpins cambium stem cell activity and consequently biomass accumulation in seed plants.

Abstracts for posters

Signals from within

A TOR-NOT4 signalling module coordinates co-translational protein quality control to limit proteotoxicity in plants.

Maximillian A Schwarze and Daniel J Gibbs

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Protein synthesis via mRNA translation is essential for proteome homeostasis (proteostasis) and cellular function. Consequently, co-translational quality control mechanisms have evolved to prevent mistakes from occurring during this process. Remarkably, we know very little about these mechanisms in plants. We have been studying the NOT4-like ubiquitin E3 ligases of Arabidopsis, which contain both a ubiquitylating RING domain and RNA-Recognition motif (RRM), a unique pairing that places their function at the interface of proteolysis and RNA biology. NOT4s are ubiquitously expressed and interact with the TOR kinase, a major regulator of stress- and nutrient-responsive mRNA translation. We show that *not4* mutants are differentially sensitive to chemical inhibitors that target TOR, and that TOR signalling is perturbed in these mutants. Furthermore, an RNA-seq analysis of *not4* mutants reveals roles linked to ribosome function and protein translation. Our data suggest that NOT4s coordinate translation rates via modulated TOR signalling, which led us to hypothesise they may play a role in co-translational protein quality control. In support of this we show that *not4* mutants have increased global translation rates, accumulate polyubiquitylated proteins, and are sensitive to protein-misfolding elicitors, which is indicative of increased error rates during protein biogenesis. We propose that NOT4s are components of a co-translational surveillance mechanism that is directly tuned to TOR function, and which is required for safeguarding protein production under normal and stressed conditions.

Investigating the influence of three-dimensional chromosome topology upon biosynthetic gene clusters in *Arabidopsis thaliana*

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Recent developments in genomics have revealed diverse examples of biosynthetic gene clusters (BGC) in eukaryotes. These are functionally related groups of neighbouring genes which are tightly colocalised and coregulated, that encode a pathway for the production of specialised metabolites

In plants, several BGC's have been identified to play key role in plant development and response to environmental conditions. One of which, the thalianol gene cluster has been identified as responsible for the production of the specialised metabolite thalianol, which has an key role in defining the microbiome of *Arabidopsis thaliana* roots.

Previous studies have shown that the thalianol cluster resides in a dynamic chromosomal domain that undergoes distinct changes in local conformation and nuclear position. These changes are dependent on organ location; the BGC is highly expressed in the roots but not in the leaves. This complex chromosomal architecture appears responsible for constraining co-expression within the cluster.

In this study, we aim to further explore the structural chromatin landscape of the thalianol gene cluster, and how this influences the transcriptional control of the cluster. Specifically, we seek to examine whether the disruption of the chromatin landscape result in changes in transcriptional activity, leading to changes in metabolite production.

Using a targeted genome-editing approach in *Arabidopsis thaliana* combined with conformational chromosome analysis, metabolic profiling and transcriptional assessment, we will manipulate the chromatin landscape and determine how this influences gene expression and metabolite production.

This work will provide valuable insights into the complex chromatin landscape that regulates BGC's, offering a deeper understanding of the role of chromosome organisation in determining gene expression.

CLE sigNal peptides: potential targets for optimising nitrogen efficiency in legumes

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Medicago truncatula is a legume that can form a symbiotic relationship with rhizobial bacteria. The nodule-based rhizobia fix nitrogen for the plant and receive carbon compounds in return. The Autoregulation of Nodulation (AON) mechanism regulates nodule numbers and activity in *Medicago truncatula*, involving local and systemic signalling. CLAVATA3/ESR (CLE) peptides have previously been implicated to have a role as a signal in AON. However, CLE34 has previously not been studied as was first described as a pseudogene without a functional CLE domain in *Medicago truncatula* ecotype A17 due to the presence of a stop codon. However, 99% of other accessions, such as ecotype R108, do not have this stop codon.

The aim of the project is to characterise the CLE34 peptide in *Medicago truncatula* AON, focusing on a hypothesised functional CLE34 in ecotype R108. Alongside this, CLE35 still has yet to be characterised extensively, and is closely related to CLE34, akin to the roles of CLE12 and CLE13, thus may have an overlapping role with CLE34 and is being studied.

A split root method has been adapted to use to study local and systemic signaling during nodulation with high vs. low efficiency strains of rhizobia and in different external nitrate concentrations. Synthesised CLE13, CLE34 and CLE35 peptides are being applied to confirm their function. The key differences in AON between A17 and R108 from a hypothesised functional CLE34 presence are also being explored through RNAseq, use of perturbation mutants and measuring nodule nitrogen content.

Preliminary data in the Gifford lab led to the hypothesis that CLE34 and CLE35 act within a mechanism relaying nitrogen levels. Therefore, targeting these genes for future crop improvement studies may lead to generating plants with altered AON, capable of producing nodules to increase plant yield even in high-nitrogen soils, with broad impact.

Responses to the environment

Harnessing light heterogeneity to optimise controlled environment agriculture

Will Claydon, Ethan J Redmond, Gina YW Vong , Alana Kluczkovski, Alice Thomas, Phoebe Sutton, Katherine Denby, Daphne Ezer

6 Billion people will live in urban areas by 2050. Vertical farming is a means of producing nutritious living greens in these areas. However, operational costs associated with lighting and cooling are substantial. Controlled environmental agriculture provides growers with an opportunity to fine-tune environmental conditions for optimising yield and crop quality. However, space and time constraints will limit the number of experimental conditions that can be tested, in turn limiting the resolution to which environmental conditions can be optimised. Here we present an innovative experimental approach that utilises the existing heterogeneity in light quantity and quality across a vertical farm to evaluate hundreds of environmental conditions concurrently. It proposes a three-phase workflow for identifying critical light variables, which can guide targeted improvements in yield and energy use. Using an observational study design, we identify features in light quality that are most predictive of biomass in different microgreens crops (kale, radish and sunflower) that may inform future iterations of lighting technology development for vertical farms. The findings suggest that light quality, rather than just light intensity, plays a crucial role in uniform crop yields and that light sensitivities are variety-specific, highlighting the importance of tailored light recipes for different crops.

Elucidating the Molecular Mechanism of the Ubiquitin-Proteasome System in Mediating Drought Stress Response in Potato

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This study systematically analyzed the gene families involved in ubiquitination modification in potatoes and their molecular regulatory mechanisms under stress conditions. Using mass spectrometry-based proteomics, 314 ubiquitination-specific protein sites under drought stress were identified for the first time, underscoring the central role of ubiquitination in stress responses. By integrating RNA-seq, qRT-PCR, and transgenic functional validation, the regulatory network of key genes, including *StPUB51*, *StRFP2*, *StUBC13*, *StPUB27*, and *StUBC18*-*StPUB40*, was elucidated. StPUB51 and *StRFP2* positively regulate drought resistance by enhancing antioxidant enzyme activity and reducing malondialdehyde content, whereas *StPUB27* negatively regulates drought resistance by inhibiting stomatal movement. The *StUBC18*-*StPUB40* complex influences the drought resistance signaling pathway by mediating the ubiquitination-driven degradation of the target protein StBZR1, a key regulator in the brassinolide signaling pathway. These findings establish a molecular regulatory framework linking ubiquitination modification, target protein degradation, and stress response in potatoes, providing valuable candidate genes for the development of stress-resistant potato varieties.

Improving crop immunity by deciphering the interplay between pathogens and the plant ubiquitin system

Karolina Brzezinska, Steven H. Spoel and Beatriz Orosa

Barley (*Hordeum vulgare*) is the second most important crop in the UK, but diseases, including those caused by rust pathogens, such as *Puccinia hordei*, are a major threat to UK crop yield and food security. Understanding and enhancing plant immune responses against pests is crucial for ensuring crop production. Despite efforts to increase pest resistance, the demand for innovative strategies remains high. Plants employ a sophisticated, multi-layered immune system in which the post-translational modifier, ubiquitin, plays pivotal roles. Although ubiquitin signalling is known to be particularly important for establishing immunity, the molecular mechanisms by which this is achieved in crops remain largely unexplored.

We utilised a proteomic approach to investigate changes in the barley ubiquitinome during *Puccinia hordei* infection. Our immune-induced ubiquitome dataset enabled the construction of protein networks, highlighting the key role of ubiquitinated immune proteins in regulating immune-associated pathways. Furthermore, we identified immune-induced E3 ligases that govern the immune ubiquitome. These E3 ligases, along with their target proteins, serve as central components of immune regulatory hubs, which could be leveraged to enhance barley resistance.

Our ubiquitome proteomics approach provides a comprehensive view of the vital role of ubiquitin signalling in crop immune responses. Furthermore, our findings offer new insights into the molecular mechanisms underpinning immune-induced protein ubiquitination in barley. This knowledge has the potential to be harnessed to boost crop resilience and ensure sustainable food production.

Harnessing Plant Immunity for Early Pathogen Detection and Sustainable Agriculture

Thomas, Qiaona, Steven, Bea

Plant diseases cause substantial agricultural and economic losses in the UK, threatening global food security. Fungal pathogens, responsible for up to 40% annual yield losses, extensively colonise hosts during early infection with minimal visible symptoms, making timely intervention with fungicides and bioelicitors challenging. The lack of early disease markers allows pathogens to establish before detection, underscoring the urgent need for improved diagnostic tools. Host-pathogen interactions rely on adaptive immune responses, often mediated by posttranslational modifications such as ubiquitination. Our research demonstrates that barley accumulates ubiquitin conjugates upon immune activation, highlighting the role of ubiquitin signalling in plant immunity. My PhD project is focused on developing biosensors that detect ubiquitin conjugates as early infection markers, offering a novel, highly sensitive approach to pathogen detection.

By identifying immune-induced ubiquitination rather than targeting pathogens directly, this technology enables early and broad-spectrum disease detection. This innovation will enhance precision agriculture by reducing prophylactic fungicide applications, minimising environmental impact, and improving disease management strategies.

Evolving Immunity: Unravelling the Role of E3 Ligases in Plant Defence

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Plants are continuously challenged by pathogens and pests, leading to significant agricultural losses, food insecurity, and high economic costs worldwide. To counter infection, plants have evolved sophisticated immune responses in which the post-translational modifier ubiquitin plays a crucial role. Ubiquitination is a highly versatile protein modification that fine-tunes the amplitude and duration of immune responses, contributing to durable resistance. Substrate ubiquitination is mediated by E3 ligases, which function as matchmakers by recruiting specific target proteins.

Despite their essential roles in plant immunity, it remains unclear how E3 ligases achieve such remarkable substrate specificity and how they have evolved to adapt to emerging pathogenic threats. By functionally tracing immune-induced E3 ligases along the evolutionary tree, my research aims to uncover how these enzymes have developed structural features for precise substrate recognition. Ultimately, this knowledge could be leveraged to engineer de novo substrate-binding domains, enabling the targeted ubiquitination of desirable proteins to enhance host immunity.

PDCB4 is a plasmodesmata protein regulating cell walls and symplastic transport in response to water withdrawal

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Among the main mechanisms controlling plant development is the symplastic pathway, which is a cell-to-cell communication mechanism mediated by plasmodesmata (PD). PD are pores in the cell wall that mediate cell-to-cell molecular transport. One of the components regulating PD is callose - β-1,3 glucan that restricts transport via PD. In this work, we characterized the role of PDCB4 (a PD callose-binding protein identified in *A. thaliana*)) in the response to water and osmotic stress conditions. Our investigation into the drought responses of PDCB4OE and *pdcb4* mutants showed that PDCB4OE demonstrated reduced sensitivity to osmotic stress induced by 3% PEG, displaying sustained primary root growth compared to WT and *pdcb4*. Additionally, in response to water withdrawal in soil, PDCB4OE maintained lower leaf temperature and sustained stomatal conductivity for longer than WT. Furthermore, additional drought experiments demonstrated a 3.5x higher survival rate for PDCB4OE, compared to WT and *pdcb4* plants, highlighting the potential role of PDCB4 in increasing drought tolerance. Using immunolocalization, we found increased callose accumulation in PDCB4OE plants grown on MS plates for 6 days at 22°C. Additionally, analysis using ABACUS lines indicated higher basal levels of abscisic acid (ABA) in PDCB4OE roots, with no ABA increase in response to 3% PEG. These results highlight the potential involvement of PDCB4 in callose regulation and ABA signalling, influencing the plant response to osmotic stress. This information could be used in future breeding for more climate-resilient crops.

Investigating the molecular responses of developing *Arabidopsis* seeds to heat stress.

Henry Swandale, second year PhD supervised by Vasilios Andriotis

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Climate change is driving more extreme weather and increasing the frequency of unfavourable growing conditions, with global temperatures projected to rise by nearly 5°C by the end of the century. Plant growth, development, and reproduction are highly sensitive to temperature fluctuations, which can affect plant productivity. Developing seeds are particularly susceptible to heat stress, which can lead to altered seed growth patterns, developmental defects, reduced seed size, and loss of viability. In cereal crops, this can result in significant yield losses following a period of high temperatures. Studies with the model plant *Arabidopsis thaliana* have shown that the embryos of heat-stressed seeds suffer developmental delays and developmental defects. Interestingly, the severity of these perturbations vary depending on the developmental stage of the embryo when the heat stress begins. Earlier stage embryos are delayed more in developmental progression and exhibit more striking defects following heat stress. The early stages of embryogenesis are vital for the future development of the plant, as this is when the basic body plan is established, and key tissue types are specified. Hence, interruptions in the normal sequence of developmental events during this time give severe defects. It is largely unknown why heat stress disrupts early embryo development so strikingly. What are the molecular responses of developing embryos to heat stress? The research will employ developmental studies, microscopic analysis, and tissue specific transcriptomics to reveal how developing embryos are affected by heat stress ascertaining how this translates into the embryo phenotypes observed.

Interdisciplinary research

Method developments for in-situ phenomics of barley plants using X-ray computed tomography

J Riley Snyder, J Peter Etchells, Agnieszka Gladala-Kostarz, Lisa-Marie Shillito, Katy Statham
Durham University

Traditional methods of identifying phenotypes in plants, such as histology, rely upon time-consuming preparation techniques and targeted sampling of tissue of assumed importance. Single plane 2D examination over small profiles of sub-sampled plant tissue provides limited ability for 3D extrapolation of internal anatomies of plant organs. In the study of nodal tissue from wild and mutant barley plants, the use of X-ray computed tomography has provided a means by which specific anatomies are visualised, isolated and compared in-situ using both qualitative observation and quantitative analyses of targeted volumetric structures. This study has developed methods for obtaining samples suitable for XRCT without the need for extended preparation time or harmful contrast agents, while still retaining the morphological characteristics necessary for identifying phenotypes in multi-scalar 3D digital reconstructions. Furthermore, the resulting high-resolution scan data has been used to extract computationally-segmented models of isolated anatomical features where phenotypes are most prevalent, providing a means of statistically comparing targeted internal anatomies of wild and mutant barley plants using resulting volumetric data.

A Novel Plant-Based Model for Human Tumor Suppressor p53 Research

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p53 is a tumour suppressor that prevents the build-up of DNA damage on animal cells through inducing cell cycle arrest, activating DNA repair machinery and, even, by inducing cell death. Mutations on p53 gene occur in more than 50% of human cancers, making its study of great relevance for understanding cancer and identify new ways of preventing and curing it. However traditional p53 research involves the use of patient biopsies, transgenic animal models, cancer cell cultures, or tedious cell transfection experiments have their own practical and ethical considerations that have limited p53 research to a few specialists' groups and countries. To overcome these limitations and broaden cancer research, we generated conditional expression lines for p53 in plant, allowing precise temporal and spatial control of its expression and demonstrated that p53 in plants conserves many of the functions of the human p53 in terms of protein structure, subcellular localisation, activity and sensibility to metal inhibitors. We consider that our plant p53-model can complement the existing methodologies, make p53 research more ethical, sustainable, reproducible and accessible to a wider range of research groups and countries and, importantly, shed light to new functions of p53.

Evolution and Ecology

Clonal propagation of *Parashorea chinensis*

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Reproductive transition from sexual to clonal propagation using seeds is always thought to affect the evolutionary fate of species. For endangered species with both sexual and clonal propagations, theories predict reproductive transition to clones would be favored as inbreeding could decrease the adaptive potential of sexual offspring. Here, we used an endangered tree species (*Parashorea chinensis*, 2n=14) from the north most of tropical Asian rainforests as a model to examine this hypothesis of reproductive transition, employing genomic at the population level. Population genomic analyses provided substantial evidence for current clonal propagation in the north most of *P. chinensis* but also provided evidence for historical recombination, which indicated sexual propagation in the ancestral populations of *P. chinensis*. The loss of genes involved in sexual reproduction might account for this reproductive transition in these *P. chinensis* populations. Our results warn that plant species that can propagate both sexually and clonally may rapidly change reproductive mode and require more aggressive conservation efforts.
Key words: dipterocarps, conservation of endangered tree species, clonal propagation

Herbarium seed embryos as alternative sources of ancient DNA

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Natural history collections, which contain hundreds of millions of specimens classified in terms of time, space, and taxonomy, provide valuable resources for many fields of research. Since the first success of ancient DNA (aDNA) extraction in the 1980s, these repositories, including herbaria for plants, have been intensively used to study microevolutionary processes, especially genetic responses to anthropogenic activities over the past several centuries. Two crucial challenges of aDNA research comprise contamination by environmental DNA, and degradation. Many factors including species, tissue types, and collection and storage environments influence the DNA preservation state. Specifically, aDNA extracted from herbarium leaf specimens are short fragments, ranging from 50 to 100 bp due to a higher breakdown rate than that in bone assemblage. To maximise the amount of data retrieved and minimise destructive sampling, we have compared whole-genome sequencing libraries constructed for leaves and seed embryos in herbarium specimens (rice and barley) collected in tropical and temperate regions, which have been stored in two different herbaria. The results have shown that libraries generated from DNA extracted from rice seed embryos had significantly higher proportions of endogenous DNA and longer average fragment lengths compared to those from rice leaves, however libraries prepared from barley leaves and embryos did not show any considerable differences. Additionally, the damage patterns of DNA extracted from both species showed a correlation with samples' ages but not their tissue types. Accordingly, depending on the collection and storage conditions, and the characteristics of species and their seed properties, seed embryos may offer a promising alternative source of genomic DNA from herbarium specimens.

Carniverous slime: viscoelastic fluid production in *Nepenthes rafflesiana*

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Carnivorous plants from the genus *Nepenthes* grow in nutrient poor soils and rely on capturing and retaining insect prey in pitfall traps to obtain nitrogen from the environment. Abundant niche specialization within the genus has resulted in a variety of trap morphologies and adaptations, including several species which use sticky viscoelastic fluids to aid in insect retention. The viscoelastic fluid of *Nepenthes rafflesiana* is composed of a hemicellulose polymer, along with enzymatic components for prey digestion. RNA-sequencing has provided insight into the synthetic and regulatory mechanisms behind viscoelasticity. It appears that the hemicellulose polymer is synthesized by standard carbohydrate processing machinery, and production is controlled at the post-translational level. The convenient secretory properties of this system may represent a new model for studying synthesis of non-xylan hemicelluloses in plants.

Development

A novel repressor of cambium stem cell division in *Arabidopsis*

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Plants constitute 80% of terrestrial biomass, of which wood makes up a significant proportion. Woody tissue, or xylem, is derived from cell divisions in the cambium, a meristem which also produces phloem tissue on its opposing side. AINTEGUMENTA (ANT) and three ANT-LIKE transcription factors, AIL5, AIL6, and AIL7, define the cambium stem cells. Their expression is promoted by TRACHEARY DIFFERENTIATION INHIBITORY FACTOR (TDIF), a peptide ligand, binding to its cognate receptor, PHLOEM INTERCALATED WITH XYLEM (PXY). However, the cambium AIL's are probably not primary targets of TDIF-PXY signalling, because changes in cambium AIL expression are detectable only several hours of PXY activation, which is slower than would be expected for primary signalling targets. To identify TDIF-PXY-regulated genes that contribute to AIL regulation, genes differentially expressed within an hour of TDIF treatment were identified using RNA-seq. Among the differentially expressed transcription factors, those that bound to the promoter of *AIL5* in enhanced yeast-1-hybrid assays were selected for further analysis. This led to the identification of WRKY49 as a putative cambium stem cell regulator that is repressed by TDIF-PXY. *wrky49* mutants were characterised by increases in radial growth, and WRKY49 itself was found to repress expression of *AIL5* and *AIL6*. Thus, WRKY49 is a novel repressor of cambium stem cell activity. Its repression by TDIF-PXY allows elevated expression of cambium stem cell factors, which in turn promotes cambium activity and increased wood formation.

WUSCHEL-related homeobox (WOX) transcriptianal factors regulate flowering in pepper

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Abstract

Flowering is a pivotal developmental process in plants that determines reproductive success and crop yield, yet the molecular regulation of flowering in pepper (*Capsicum annuum*) remains poorly understood. The WUSCHEL-related homeobox (WOX) family of transcription factors is known to orchestrate plant development, including meristem maintenance and floral morphogenesis, but their specific roles in pepper flowering have been underexplored. In this study, we identified nine CanWOX genes and reflecting their evolutionary conservation and functional diversification through phylogenetic analysis. Transcriptional activity assays revealed that CanWUS, CanWOX1, CanWOX9, and CanWOX13 exhibit strong activation potential, predominantly mediated by their C-terminal regions, while the N-terminal homeodomain shows negligible activity, highlighting functional modularity within the family. Spatiotemporal expression analysis demonstrated that *CanWUS, CanWOX1*, and *CanWOX9* are highly expressed in shoot apical meristems (SAM) and floral buds, suggesting their involvement in flowering regulation. Virus-induced gene silencing (VIGS) further elucidated their distinct roles: *CanWUS* is essential for floral meristem formation, *CanWOX9* regulates flowering time and inflorescence architecture, and *CanWOX1* controls petal size. RNA-seq analysis of CanWOX9-silenced plants revealed its regulatory role in hormone signaling, particularly abscisic acid (ABA) biosynthesis, which may underlie its effects on flowering. Additionally, we identified CanWRKY2 as a key upstream regulator of CanWOX9, binding to its promoter and positively modulating its expression to fine-tune flowering time. Our study highlight the distinct yet complementary roles of WOX transcription factors in regulating meristem activity, flowering time, and floral organ morphology in pepper, and provides novel insights into the molecular mechanisms underlying WOX-mediated developmental processes.

PLINC transcription factors repress vascular cambium activity in Arabidopsis

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Stem cell niches are generally regulated by opposing molecular mechanisms that balance cell division with differentiation. The vascular cambium contains stems cells, the divisions of which provide precursors for of xylem and phloem. In Arabidopsis, auxin, cytokinin, the TDIF-PXY ligand-receptor pair, and their downstream transcription factor targets, which include WOX14, TMO6, and LBD4 promote cambium activity. However, few repressors of cambium activity have been described. In this study a family of three cambium-regulating homeodomain containing Plant Zinc-finger transcription factors (PLINC), was identified which bound to the promoters of WOX14, TMO6, and LBD4 to repress their expression. PLINC mutants demonstrated increases in cambium proliferation, while their over-expression led to early differentiation. Our study demonstrates that the PLINC's are a novel family of transcriptional regulators that dampen expression of key auxin, PXY, and cytokinin signalling targets. We propose that PLINC-mediated opposition to cell division-promoting factors provides balance to the cambium stem cell niche, thus contributing to cambium homeostasis.

Using CRISPR-Cas9 gene editing to explore alterations in seed traits in *Camelina sativa*

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With the global population set to reach 9.7 billion by 2050, maximising crop yields is an important focus for food security. Seed size is a key component of yield in seed-yielding crops. Seeds are derived from ovules in the gynoecium (the female reproductive organ). The transcription factor *AUXIN RESPONSE FACTOR 2 (ARF2)* is known to play a role in negatively regulating seed size in *Arabidopsis thaliana* by altering cell proliferation in the integument (a layer in the ovule that develops into the final seed coat). However, the potential for altering *ARF2* in an oilseed crop species such as *Camelina sativa* and its effect on seed-related traits remains unexplored. We have utilised gene editing (GE) technologies to generate DNA edits in all three *ARF2* homologs in *Camelina sativa* in three independent transgenic lines. Characterisation of these CRISPR-edited lines revealed an increased seed size compared to the WT control, along with phenotypic changes in plant architecture. These lines will be further explored this year within a GE field trial. We hypothesise that edits in all three *ARF2* homologs may have contributed to the pleiotropic architectural effects observed in the transgenic camelina lines and hope that by re-designing the CRISPR guides to target fewer *ARF2* homologs, an increased seed size can be achieved with minimal secondary alterations.

Characterisation of PUCK in *Arabidopsis* Secondary Growth

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Secondary growth is thickening of the plant stem, which provides structural support and increased nutrient transport to the growing plant. The main driver of secondary growth is the vascular cambium, a bifacial stem cell population which gives rise to xylem and phloem via organised cell division and differentiation. The TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) peptide ligand diffuses into the cambium where it signals to the PHLOEM INTERCALATED WITH XYLEM (PXY) receptor-like kinase to promote cell division, vascular organisation and repression of xylem identity. However, several aspects of TDIF and PXY biosynthesis, turnover and signalling are not fully understood, in part because all the factors involved have not been identified. CLE41 is a precursor of TDIF. *35S::CLE41* lines, which are characterised by ectopic cambium, were used in a mutagenesis screen to identify novel components of TDIF-PXY signalling. Here we show that *PUCK,* which contains several WD40 repeat domains that function in protein-protein interactions, was identified as a suppressor of *35S::CLE41*. Transcriptional reporter analysis revealed that *PUCK* is expressed in the phloem parenchyma and differentiating xylem. Analysis of differentiating xylem and phloem in *puck* mutants has demonstrated that PUCK influences vascular organisation. This evidence supports the hypothesis that PUCK may be a scaffold protein involved in regulating vascular differentiation in conjunction with the TDIF-PXY pathway. Woody tissue is made up of lignified xylem, therefore, understanding the mechanism of secondary growth could allow manipulation of wood formation to increase forest productivity.

Biological characterization of flowering and in vitro regeneration of seed embryo of *Chimonobambusa pingshanensis*

Yang Haiyun

Chimonobambusa pingshanensis is an alpine bamboo species with delicious bamboo shoots ,which has great economic and ecological value. However, due to the phenomena of unpredictable flowering period, long flowering interval, collective death after flowering, and low fruiting rate of bamboo, the process of breeding high-quality varieties is slow, which restricts the development of bamboo industry. In this experiment, the sexual flowering process of *Chimonobambusa pingshanensis* was tested by studying the changes in the flowering forest phase, the structure of the floral organs and young seeds. Callus induction, proliferation, and differentiation, a preliminary in vitro rapid propagation system and callus induction and regeneration system of *Chimonobambusa pingshanensis* were established. The main results are as follows:

1. Establishment of callus induction and regeneration system for *Chimonobambusa pingshanensis*. The seed embryos at Ⅱ stage are selected as explants, and inoculated into MS medium with the addition of 2.5 mg/L 2,4-D to significantly promote the induction of callus, with the maximum induction rate of 64.39%. The pH value of 5.8 is favorable for the induction of healing tissues, the addition of Vc is favorable for the inhibition of browning of healing tissues, and the addition of 0.001 mg/L Picloram and 1 mg/L ABA is favorable for the increase of healing tissues' induction rate, which is 65.80% and 70.06%, respectively. Excessive or insufficient concentrations of 2,4-D and ZT are not conducive to the proliferation of callus tissue. Adding 1 mg/L of 2,4-D and 0.2 mg/L of ZT has a significant promoting effect on the proliferation of callus. The Orthogonal experimental showed that the efficiency of the three plant growth regulating substances on the differentiation of healing tissues is in the order of 6-BA＞NAA＞KT, in which 6-BA has a significant effect on the differentiation of callus ($P＜0.05$), and the addition of the appropriate amount of 6-BA is beneficial to the differentiation of callus, with the highest rate of differentiation reaching 30% among the treatments.

2. Establishment of rapid propagation for *Chimonobambusa pingshanensis*. Using seed embryos as explants, the effect of using NaClO as a disinfectant was better than that of ClO2. The germination rate of seed embryos treated with overnight rinsing under running water, then soaking in 75% alcohol for 30 s and disinfecting with 1% NaClO for 10 min reached 63.33%. The orthogonal experiment showed that the efficiency of the four plant growth regulating substances on bud proliferation was ranked as 6-BA＞NAA＞TDZ＞KT. Among them, 6-BA has a highly significant promoting effect on shoot proliferation ($P < 0.01$), with the largest proliferation coefficient of 3.04 among the treatments, while NAA was detrimental to shoot proliferation. Rooting is significantly promoted by using 1/2 MS (macronutrient) as the basic medium or adding NAA and IBA at the same time, with the maximum number of roots reaching 6.43; the transplanting survival rate of the group-cultured seedlings after domestication was 94.44%.

Modified function of a stem cell regulator in monocots and dicots

Gladala-Kostarz, A., Snyder, R., Ridley, R., Statham, K., Baxter, R., Shillito, LM., Harwood, W., Etchells, JP.

The molecular mechanisms regulating stem anatomy of seed plants are best understood in dicotyledonous plants such as Arabidopsis, which contain a vascular cambium that promotes radial growth via formation of vascular tissues. However, monocot plants, including major grains like barley (Hordeum vulgare), lack a vascular cambium and are characterised by scattered vascular tissues. Dicot apical growth is derived from the shoot apical meristem, whereas monocots contain additional intercalary meristems which promote apical growth above nodes. Dicot cambium is regulated by the TDIF-PXY ligand-receptor pair, which maintains the cambium stem cell pool, promoting cell division and repressing differentiation. Despite an absence of cambium, three PXY-like genes in barley (HvPXY, HvPXL1, and HvPXL2) and a TDIF-encoding gene (HvTDIF) were identified. This raises the question of TDIF-PXY function in barley, given the distinct organisation of stems in monocots and dicots. To address this question, barley pxy mutant alleles were generated. When Hvpxy mutants were subjected to RNA-seq, genes associated with promoting cell division demonstrated reduced expression, thus, HvTDIF-HvPXY retains its cell-division-promoting function. Reporter analysis showed that HvPXY, PXL1, and HvTDIF genes are expressed in the intercalary meristem. Analysis of node-adjacent tissue in Hvpxy mutants found these mutants were characterised by fewer longer cells. X-Ray Computer Tomography (XR-CT) analysis demonstrated that tissue density in the Hvpxy mutant was higher than wild type, indicating that the tissue had differentiated prematurely. Thus, HvTDIF-HvPXY represses differentiation and promotes cell division in the intercalary meristem. These data thus suggest that the mechanism which evolved to regulate the cambial meristem in dicots was modified to regulate the intercalary meristem in monocots and therefore underpins part of the dramatic shift in anatomy that occurred upon the separation of these clades.

Genetics and Biochemistry of Sticky Trichomes

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Most plant species have trichomes or epidermal hairs, which can have diverse structures and functions in providing resistance to pests, UV radiation, or drought. The genus Antirrhinum (snapdragon) provides a useful research system in which to identify and study the genetics and biochemistry of trichomes because its species differ in trichome morphology and the presence or absence of a secretory gland in the trichomes.
Antirrhinum hispanicum trichomes release a viscous secretion which exhibits a remarkable stickiness and is able to trap insects. The sticky secretion is insoluble in water and does not dry or harden at ambient temperature. Liquid chromatography coupled to mass spectrometry (LC-MS) analysis identified a mixture of compounds belonging to the same family: Sphingolipids
Comparative transcriptome analysis of sticky *A. hispanicum* and non-sticky *A. majus* epidermis supported this by revealing that expression of genes involved in fatty acid metabolism was enriched in *A. hispanicum*.
To identify the genetic basis of stickiness and its evolution, *A. hispanicum* was crossed with non-sticky *A. majus*. F1 hybrids and three-quarters of F2 plants were not sticky, indicating that stickiness is determined by the recessive allele of a single gene. The sticky gene has been mapped to a short region of the genome by backcrossing the F1 to *A. hispanicum* and sequencing DNA from pools of sticky and non-sticky plants. The sticky will be identified by a combination of expression analysis and virus-induced gene silencing of candidates in the region.

Deciphering Role of TGA Transcription Factor in Regulating Tomato Trichomes

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Most flowering plants possess trichomes, or hair-like structures, on their surfaces, which play a critical role in protecting against pests and producing valuable chemicals such as pharmaceuticals and flavours. This has led to growing interest in enhancing trichome density to improve pest resistance and increase compound yields. However, two significant barriers hinder this approach: first, although several genes known to affect trichome density are now known in tomato, how they control density remains unclear, making it challenging to increase density without triggering undesirable side effects; second, the potential disadvantages of an unusually high trichome density for the plant are not well understood. This project focuses on tomato (*Solanum lycopersicon* L. cv Micro-Tom) to address these gaps by investigating the genetic and functional aspects of trichome regulation. First, the activity of a gene suspected to repress hair formation in snapdragons will be reduced, testing whether it serves a similar role in tomato and whether its reduced activity can increase trichome density without adverse side effects. Second, the interactions between this gene and other trichome-controlling genes in tomato will be explored to determine whether it regulates, or is regulated by, or acts independently of them, particularly those with known side effects. Third, the impact of increased trichome density will be assessed on the plant's resistance to various pests, including chewing and sucking insects and slugs, and the effect of higher trichome density on plant performance will be examined in pest-free environments, particularly whether they alter light absorption or reflection and gas exchange, which could impact photosynthesis. This research aims to identify key genes involved in trichome regulation and assess the broader implications of enhancing trichome density in plants.

Breaking the mould: does pectin methylation control cell growth and cell shape?

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Plants grow in a variety of shapes and sizes, and understanding how they do so is essential for our food security and crop sustainability. Transition from isotropic to anisotropic growth, where cells grow in specific directions, underpins the formation of these varied shapes. This process depends on the mechanical properties of the cell wall, which are determined by the structure, chemistry and interactions of its constituent polysaccharides. Pectin is one of the most abundant polysaccharides in primary cell walls, and its chemical modification, particularly pectin methylation, has been proposed to play an essential role in controlling plant cell growth and shape. However, the precise role of pectin methylation in cell growth and shape is far from being understood.

Pectin methylation begins with the import of S-adenosyl methionine (SAM) into the Golgi lumen, a process facilitated by Golgi SAM transporters (GoSAMTs). Our discovery of GoSAMTs has provided a unique opportunity to explore the fundamental role of pectin methylation in plant growth and development. To investigate this further, we developed an inducible CRISPR-based system called "pectin methylation switch-off" (PMSO), which enables targeted depletion of GoSAMT activity. This allows us to effectively shut down pectin methylation in response to a specific stimulus.

Strikingly, induced PMSO disrupted the transition from isotropic to anisotropic growth across multiple tissues. This effect persisted even under conditions that typically drive anisotropic growth, such as etiolation and exposure to far-red light. Atomic force microscopy (AFM) revealed that plants lacking pectin methylation exhibit stiffer cell walls and fail to establish the characteristic parallel arrangement of cortical microtubules relative to the axis of maximal stress, which is essential for directional growth. Overall, this work shows clear evidence that pectin methylation is an essential requirement for plants to grow and take shape.

Green Perfect – Manipulating the regulation of cellular chloroplast capacity in *Arabidopsis*.

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RHUL

Enhancing photosynthesis has become a promising strategy for boosting crop production to meet the demands of a growing population. One approach to achieve this is the manipulation of the plant's crucial organelle, the chloroplast. Increasing chloroplast 'greening' and occupancy within the plant cell is a promising strategy to optimise the capacity and volume available for photosynthetic activity and improve crop yield. Photosynthesis-associated nuclear genes (PhANGs) are responsive to light, and their expression is regulated by transcription factors such as GLK (GOLDEN2-LIKE) [Fitter et al., 2002, Plant J, 31(6), 713-27]. The *Arabidopsis* double *glk* mutant has been well characterised as a mutant with significantly reduced greening throughout development, due to compromised photosynthetic capacity of the chloroplast. Previous work in the laboratory has utilised ethyl methane sulfonate (EMS) mutagenesis screening to uncover novel genetic regulators involved in the GLK expression pathway. A putative mutant candidate was isolated as a suppressor of the double *glk* mutant phenotype. Preliminary characterisation through chlorophyll quantitation and cellular microscopy, has indicated that this putative mutant has enhanced greening and a greater cellular area occupied by chloroplast material compared to the double *glk* mutant. Bulk segregant analysing using next generation sequencing (mapping by sequencing), will be applied to identify the polymorphism responsible for the suppression. Complementation of the phenotype with a wild type copy in the *glk1 glk2* mutant will be subsequently completed.

The role of the cell wall in plant fertilisation

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Fertilisation in higher plants involves a finely controlled sequence of chemical and physical interactions between male and female gametophytes. The synergid cells at the entrance to the egg sac are crucial for orchestrating this process as they produce diffusible pollen tube attractants and later control pollen tube growth towards the egg cell. Synergids have a distinctive morphology with a thickened cell wall structure called the filiform apparatus providing the first point of physical contact between the pollen tube and female gametophyte. Despite the apparent importance of this structure, the composition of the cell wall from which is formed has not been investigated in detail. We sought to address this knowledge gap, and hypothesised that correct cell wall composition in the synergid cells is required for their proper function.

We probed the cell wall composition of unpollinated Arabidopsis ovules by immunolabeling resin-embedded tissue sections with a panel of monoclonal antibodies, identifying a range of epitopes in the filiform apparatus including an abundance of callose. We hypothesised that the known fertilisation defects of mutants in CrRLK1L genes could be explained by cell wall differences, but our data show that mutant ovules are indistinguishable from wild type, at least at the unfertilised stage.

In our ongoing work we are gathering equivalent data from pollinated ovules to test the hypothesis that synergid cell wall composition changes during pollen tube reception to facilitate pollen tube growth. To obtain ovules at our target stage of fertilisation (when the pollen tube has entered the synergid but not yet burst) we set up semi-in-vitro fertilisation assays with fluorescent markers in the pollen tubes and synergid cells, monitored pollen tube growth by spinning disc microscopy, and fixed samples for immunolabeling at the desired time point. Further to this, we have collected a panel of mutants in cell wall related genes including both readily available and newly generated lines which we will assess for fertility defects. These experiments will provide a detailed picture of if and how the synergid cell wall composition changes to permit pollen tube growth, and will identify key cell wall components potentially involved in this process.

Hypoxic niches restrict a Polycomb protein to facilitate the repression of PIF signalling

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Plants have developed mechanisms to sense and adapt to low oxygen in their environment, such as at high altitudes or during flooding events. Certain plant tissues (e.g. shoot apical meristems, SAM; lateral root primordia, LRP) however, also possess endogenous hypoxic niches. These niches arise from high metabolic activity of dividing cells, where seedling development has been linked to the oxygen-dependent stabilisation of targets of the PRT6 N-degron pathway. Our previous work identified VERNALIZATION2 (VRN2) as a substrate of this degradation pathway in *Arabidopsis*. VRN2 is a flowering plant-specific subunit of the Polycomb Repressive Complex 2 (PRC2), a conserved eukaryotic holoenzyme that represses gene expression by depositing the histone H3K27me3 mark in chromatin. During normoxic conditions, VRN2 protein is restricted to hypoxic niches (SAM, LRP) and emerging leaves in Arabidopsis, but ectopically accumulates throughout the plant in response to exogenous hypoxia.
Under normoxia, both VRN2-GUS and a GUS-GFP reporter driven by a *pHRPE* (Hypoxia Response Promoter Element) promoter were detected in the SAM and leaf primordia of *Arabidopsis* seedlings and rosettes. Phenotypic analysis of *vrn2* mutants against wild type plants showed larger rosettes due to increased cell size, corroborated by an RNA-seq analysis which revealed a global upregulation of genes linked to cell-expansion mediated growth and auxin-responses (e.g. *SAURs*, *HAT4*). These findings suggest that VRN2 acts a negative regulator of growth and development. ChIP-seq analysis of the same mutants showed that VRN2-PRC2 achieves this by establishing a stable and conditionally repressed chromatin state in key PHYTOCHROME INTERACTING FACTOR (PIF)-regulated genes that promote cell expansion. Hence, we show the VRN2-PRC2 module as an important regulator of light-responsive gene expression that may also act to transduce natural hypoxia gradients into the epigenetic control of plant growth. To further investigate the importance of VRN2 accumulation in hypoxic niches of the plant, we created *vrn2* complementation lines expressing *pHRPE::VRN2* and are currently studying the phenotype and molecular impact of this construct on plant growth and development.

DNA topoisomerase I (TOP1) inhibits *Arabidopsis* lateral root primordium initiation via stabilizing G-quadruplex structures

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The chromatin factor DNA topoisomerase I (TOP1) modifies DNA topology by relaxing negative supercoiling, thereby facilitating DNA replication, gene transcription, and the maintenance of genomic stability. Here, we report a novel function of *Arabidopsis* AtTOP1α in inhibiting lateral root primordium (LRP) initiation stem cell formation. In the AtTOP1α loss-of-function mutants *top1α-1* and *top1α-10*, the density of lateral root primordia was significantly increased. Transcriptome analysis combined with TOP1α CUT&Tag sequencing revealed that the *CYP82C4* gene was significantly upregulated in the TOP1α mutants. Our study found that the template strand of *CYP82C4* gene contains a guanine-rich sequence that can fold into a G-quadruplex (G4) structure, which in turn inhibits *CYP82C4* transcription. In the G4 mutant, *CYP82C4* expression was elevated, leading to enhanced lateral root primordium initiation and growth. This is the first demonstration of DNA G4 structures playing a biological role in lateral root development in *Arabidopsis.* By using genetic, biochemical, and biophysical approaches, we demonstrated that AtTOP1α binds the G4 structure on the *CYP82C4* template strand with high affinity and specificity. By stabilizing the G4 folding, AtTOP1α represses *CYP82C4* expression, thereby inhibiting lateral root primordium stem cell formation. Our findings reveal a novel function of AtTOP1α in regulating lateral root primordium stem cell formation and reveal a new mechanism by which TOP1α targets G-quadruplexes to modulate gene expression.