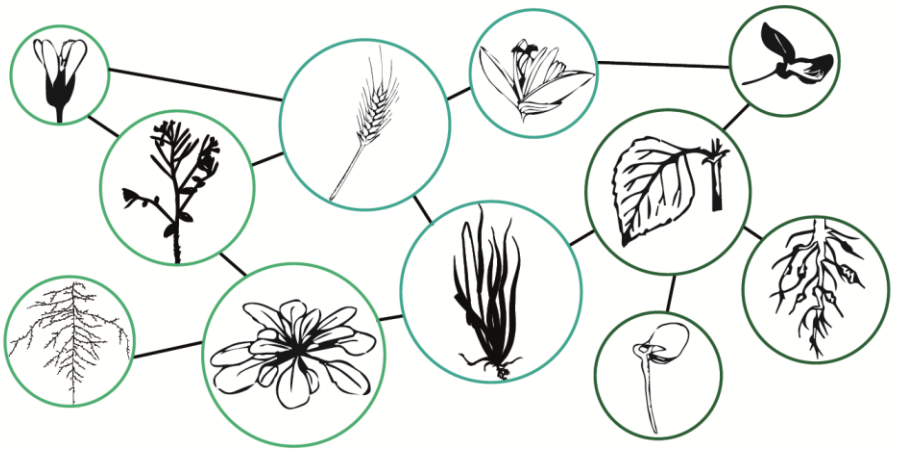
ASPS 2019

Bringing Plant Science Together



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Australian Society of Plant Scientists Conference I 26-29 November 2019 I Melbourne

Abstract Booklet

2019

Session 1: Students

Speaker 1: James Fazzino

Perspectives from a Career in Agriculture

Fazzino, J.[1]

[1]*La Trobe University*

James is Chair of Osteon Medical, a leading digital health business. James is also Chair of Manufacturing Australia, a CEO-led coalition of Australia's largest manufacturers who work with all sides of government, business and community to help the sector realise its full potential. James was also appointed as a Non-Executive Director of Australia Pipeline Limited (the APA Group). APA is an ASX 50 company that owns and operates circa $20bn of energy assets in Australia.

James is Vice-Chancellors Fellow at La Trobe University and in this capacity provides advice to the Vice-Chancellor and Senior Management on strategy, culture and operational excellence. James is also an Adjunct Professor at La Trobe Business School specialising in management, international business and digital. James passionately believes in diversity and is a member of the Melbourne Male Champions of Change group.

James was formerly the Managing Director & CEO of Incitec Pivot. During his 14 year tenure at Incitec, first as CFO and then as CEO, the company increased in size 6-fold to an enterprise value of $8bn. Highlights during his time at IPL included overseeing construction of two new $1bn world scale manufacturing plants (one at Moranbah, Australia and the other at Louisianna, USA), successfully integrating the $3.6bn Dyno-Nobel acquisition (which took IPL global) and restructuring the group to become a global industrial chemical company with operations in 13 countries around the world.

Session 1: Students

Speaker 2: Caitlyn Burt

Plant cell dialysis using vesicles and aquaporins

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Regulation of water and solute transport in plants is indissoluble from survival and productivity. One of the mechanisms plants use to control water and solute transport involves regulating the function of channels called plasma membrane intrinsic proteins (PIPs). In addition to being localised in the plasma membrane and transporting water, some PIPs can also be found in small vesicle, pre-vacuolar, autophagosome and extracellular vesicle membranes and a subset of PIPs can transport monovalent cations. PIP localisation has been observed to change in response to salt and osmotic stress treatments and this is associated with changes in PIP phosphorylation. We observed that the phosphorylation state of water and ion channel PIPs can change the relative permeability of these channels to water, sodium and potassium. This means that plants could use PIP phosphorylation to control both when and where water and monovalent cations are being transported within compartments inside cells and between cells. As root cells take up soil solution the water, nutrient and salt ions within the soil solution can be partitioned into different sub-cellular compartments, such as vesicles, vacuole compartments and autophagosomes. We are exploring whether changes in PIP phosphorylation may be part of the process to control the relative distribution of water, sodium and potassium within different membrane bound compartments inside cells, in a system analogous to dialysis. Sub-cellular control of the transport and compartmentation of water, salt and nutrient ions via aquaporin containing membranous compartments is likely to be an important contributor to the whole plant regulation of hydraulic conductance, nutrient and salt transport.

Session 1: Students

Speaker 3: Maketalena Aleamotu'a

PHI thickenings in Brassica roots – an adaptation to water stress?

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Phi thickenings (PTs) are secondary cell wall bands found in the radial walls of root cortical cells. These bands occur in diverse angiosperms and gymnosperms, and although first described in the 19th century, little is known about their induction and functions. We investigated PTs in young, primary roots of *Brassica oleracea* and *B. napus*. PTs are rapidly induced in 4 day-old seedlings transferred from control plates to plates containing salt. Confocal microscopy demonstrated that PTs form a continuous, lignified ring around the inner cortex, immediately outside the endodermis, and that a delicate, reticulate network of lignified secondary walls developed along the inner face of these cells adjacent to the endodermis after thickening formation. Thickening induction is not specific to salt, with levels of induction generated by salt, mannitol and sucrose equally dependent on the osmotic strength of the media. Glycinebetaine (GB) did not, however, induce thickenings in an osmolarity-dependent fashion. When tested in combination with other osmotica that induce thickenings, GB inhibited induction, suggesting that it acts as an osmoprotectant. Gibberellin was identified as a key hormone regulating PT formation. Time course experiments demonstrated that salt-induced induction occurred in a narrow region within the differentiation zone of the root within 12-24 h after transfer from control to salt plates, with cellulose deposition starting after 12 h followed by lignification from 15 h. As thickening induction in primary roots of *Brassica* is cultivar-dependent, we are now testing a diversity set of ~450 *B. napus* cultivars. We anticipate that quantification of PT induction across this collection may allow discovery of genetic loci linked to water stress-induced PT development in the Brassicaceae.

Session 1: Students

Speaker 4: Deepak Baranwal

Genome-wide association analysis of stripe rust resistance among a wheat diversity panel

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), was estimated to cause A$127 worth of losses in Australia. Virulence for stripe rust resistance genes deployed in current wheat cultivars is carried by derivatives of Pst pathotype 134 E16A+ and another pathotype, 239 E237A-Yr17+Yr33+, detected in 2017. The new pathotype 239 E237A-Yr17+Yr33+ carries virulence for *Yr1*, *Yr33*, *Yr57*, *Yr58*, *Yr72* and *Yr75* that were effective against derivatives of pathotype 134 E16A+. These examples of pathotypic introduction and evolution stressed the need to identify diverse sources of resistance. A wheat diversity panel of 293 wheat accessions including synthetic hexaploid wheat genotypes and progenies derived from landraces selected by scientists of International Maize and Wheat Improvement Centre, Mexico was tested with pre-2002 and post-2002 Pst pathotypes in the greenhouse and field. This panel was genotyped using the Illumina iSelect 90K Infinium wheat SNP array and markers linked with *Yr15, Yr18, Yr29, Yr34* and *Yr46*. Stripe rust response and genotypic data were used for genome-wide association study (GWAS) to detect genomic regions controlling resistance to stripe rust. The GWAS analysis identified 20 new marker-trait associations on chromosomes 1A, 1B, 1D, 2A, 2B, 3D, 6A, 7A and 7D in addition to the previously reported QTL on chromosomes 1A, 1B, 2B, 3B, 3D, 4A, 5A and 7B. The newly identified associations will be validated.

Session 1: Students

Speaker 5: Maja Arsic

Bio-imaging reveals foliar phosphate photosynthetic restoration and entry pathways in P-deficient barley

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Improving phosphorus (P) fertilizer efficiency is a critical challenge due to declining global mineral P supplies, a growing global population and environmental degradation associated with excess soil nutrients. Foliar sprays are a potential alternative to soil-based fertilizers, as they could supplement targeted fertilizer delivery to crops when needed. Foliar P sprays have been investigated in terms of boosting plant biomass or grain yield. However, a quick and reliable assay for screening the effect of liquid P fertilizer solutions on plant physiological parameters determining yield responses is currently lacking. Furthermore, the foliar pathways across the leaf surface are unclear, yet they are important to understand in order to optimize liquid fertilizer solutions. Here we describe the development and application of chlorophyll-a fluorescence and mass spectrometry bio-imaging techniques to investigate restoration of P-limited photosynthetic machinery by foliar P, and the pathways by which the phosphate ions enter across the leaf surface. The development of the PAM (Pulse Amplitude Modulation) imaging system for phosphorus deficiency allowed the observation of transient restoration of P limited photosynthesis in fresh leaves. Further, elemental imaging using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) confirmed that vanadate is a useful short-term phosphate proxy for tracing ionic entry pathways across the leaf surface. Results revealed that foliar phosphate sprays were able to restore photosynthetic function within three days and restoration of the newly emerging leaf one week after spraying. Based on strong co-localization of phosphate and vanadate in LA-ICP-MS elemental images, ion entry points appear to be located above vascular bundles in the fibre cells associated with trichome ridges. The combination of these two complementary bio-imaging techniques provides a promising approach for investigating the performance of foliar P applications in important crop species.

Session 2: Breeding for Human Nutrition

Speaker 1: Jennie Brand-Miller

Why carbohydrate quality trumps quantity

Brand-Miller, J.[1]

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Controversies surround the amount and type of carbohydrate needed for optimal health, longevity, and sustainability. Although human populations have thrived on diets with widely varying macronutrient ratios, a strong case can be made that high consumption of carbohydrate from certain sources is causally related to obesity, diabetes, cardiovascular disease, and some cancers. In contrast, non-starchy vegetables, whole fruits, legumes, and whole kernel grains appear protective. This distinction may be explained partly by differences in how specific carbohydrates affect postprandial hyperglycaemia and hyperinsulinaema. Two empirical metrics have been introduced to rank foods according to effects on blood glucose: glycaemic index (GI) and glycaemic load (GL). The GI compares foods based on a standardised amount of available carbohydrate. Glycaemic load (GI multiplied by the amount of carbohydrate in a typical serving) allows the glycaemic effect of foods, meals, and whole diets to be compared as realistically consumed. Carbohydrate quality (GI and wholegrain vs refined grain) appears to have a more important role in population health than carbohydrate amount. However, the metabolic effects of total and high GI carbohydrate vary among individuals, particularly during pregnancy, because of increasing overweight/obesity, insulin resistance and glucose intolerance. Milling and refining of grains not only increases GI and GL but also results in loss of important micronutrients that influence fetal growth in utero, contributing to increased inter-generational obesity and type 2 diabetes. Breeding grains for both low GI and for higher micronutrient density in the endosperm would be one giant step for mankind!

Session 2: Breeding for Human Nutrition

Speaker 2: Greg Tanner

Creation of the first ultra-low gluten barley, cv Kebari®, (Hordeum vulgare L.) for coeliac and gluten-intolerant populations.

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Coeliac disease is a well-defined condition that is estimated to affect approximately 1% of the population worldwide. Non-coeliac gluten sensitivity is a condition less well defined, but estimated to affect up to 10% of the population, and often self-diagnosed. At present, the only remedy for both conditions is a lifelong gluten-free diet. A gluten-free diet is often expensive, high in fat and low in fibre, which in themselves can lead to adverse health outcomes. Thus there is an opportunity to use novel plant breeding strategies to develop alternative gluten free grains. We describe the breeding and characterisation of a novel ultra-low gluten barley variety, Kebari®, in which the hordein (gluten) content was reduced to below 5 ppm. This was achieved using conventional breeding strategies to combine 3 recessive alleles, which act independently of each other to lower the hordein content in the parental varieties. The grain of the initial variety was shrunken compared to wild type barleys. We implemented a breeding strategy to improve the grain size to near wild type levels and demonstrated that the grains can be malted and brewed successfully. This ultra-low gluten character has been introduced into hull-less lines for use as a whole grain and flour in the food industry. The breeding and commercialisation of these lines is discussed. Barley is not a large component of the diet – however products from barley including malt, and beer are common. The problem for coeliacs and gluten intolerants is these products are often included as “hidden ingredients”, and although they should be completely eliminated from the coeliac diet, in practice this is often difficult. Kebari® has the potential to provide novel healthy foods and beverages for those who require a gluten free diet.

Session 2: Breeding for Human Nutrition

Speaker 3: James Stangoulis

Nicotianamine synthase 3: a molecular marker for breeding zinc biofortified rice

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[1]Flinders University, Bedford Park, Australia

Approximately 30% of the global population suffers from dietary zinc (Zn) deficiency, many of whom rely on rice to meet their daily calorific requirements. Biofortification represents an effective approach to improving nutritional and health outcomes. The PRAY Indica diversity panel, consisting of 300 genotypes, was developed at IRRI for the GRiSP Global Rice Phenotyping Network (http://ricephenonetwork.irri.org) and grown for 4 seasons across 2 years in the Philippines. Genotypic data describing 5.2 million biallelic SNPs was used in a GWAS study of the ionome of rice seed. A significant QTL for Zn was identified on Chromosome 7 and this was stable over three dry and one wet season. Candidate gene analysis revealed the presence of the NAS3 gene within this QTL, whose product synthesises the metal chelator, nicotianamine (NA). Previous studies have identified NA-Zn complexes in the rice phloem, implicating NA in long distance micronutrient transport. To follow-on from this study, comparative transcriptomics was used to uncover the differential expression of NAS3 and other metal homeostasis genes. Different patterns of Zn accumulation were observed throughout the seed filling period. From gene expression patterns and logistic modelling of grain growth and Zn accumulation, the genotypes with a higher level of seed Zn have a longer seed-filling period when compared to genotypes with low seed Zn. Furthermore, many genes previously known to influence Zn and metal homeostasis were upregulated in the high Zn genotypes. NAS3 has a higher expression in the high Zn genotype at 7, 11 and 15 days after anthesis. Interestingly the whole nicotianamine synthesis pathway shows greater expression in the high Zn genotypes. Our results suggest a major role for nicotianamine in translocating Zn into the high Zn rice genotype and NAS3 as a suitable marker gene for improving rice Zn in conventional plant breeding programs.

Session 2: Breeding for Human Nutrition

Speaker 4: Priyakshee Borpatragohain

Resolving genetic components for sulphur pools in the seeds of mustard (*Brassica juncea L.*)

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The production of sulphur (S)-rich glucosinolates (GSLs) that provide distinctive flavour and pungency is characteristic for the family of *Brassicaceae*. *Brassicaceae* includes a range important oil and vegetable crops, where GSL contents and composition have been under strong artificial selection. High levels of GSL are desired for providing the pungency to condiment mustard, while low levels have been selected for canola oil production. S-containing amino acids produced in the S assimilation process act as precursor molecules and S donors in the biosynthesis of GSL and seed storage proteins (SSP), which together act as secondary S sinks in mature seeds. Molecular interactions affecting S assimilation and S fate in the seed of *B. juncea* (Indian mustard) have been partially dissected using quantitative genetic analysis and gene discovery approaches. A high-density genetic linkage map of the *B. juncea* genome based on a doubled haploid mapping population derived from high-GSL (O1493) and low-GSL (C671) parental lines were used to identify quantitative trait loci for seed GSL, seed S and SSP contents. Six overlapping QTL regions for seed GSL, SSP and S content were identified on chromosome A02, A03, A08 and A09. This finding will be further explored to modify the previously developed complex molecular interaction model between S supply and seed S sinks (GSL and SSP) in brassicas.

Session 3: Reproductive and Seed Biology

Speaker 1: Roger Parish

Climate stress and pollen development: the tapetum running hot and cold.

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The layer of anther tapetal cells provides the developing microspores with nutrients, enzymes, carbohydrates and compounds required for the synthesis of the outer pollen walls (exine). Tapetal degradation via PCD occurs shortly after the microspores are released from the tetrad. The timely degeneration of the tapetum is critical for pollen development. Environmental stress can interfere with tapetal development and degeneration. The transcription factor MYB80 is required for pollen development and the regulation of tapetal PCD. MYB80 directly activates transcription of the UNDEAD gene which encodes an A1 aspartic protease. Silencing UNDEAD results in premature tapetal PCD. We have identified four UNDEADLIKE genes and are examining their roles in anther development. UNDL3 and UNDL4 together were found to protect the tapetum against heat stress. Dehydrins protect cells against environmental stress. The XERO2/Lti30 dehydrin gene, induced by cold, ABA and dehydration, is constitutively expressed in anthers, particularly in the tapetum. The UNDEADL and XERO2 proteins may participate in mechanisms protecting tapetal cells against environmental stresses.

Session 3: Reproductive and Seed Biology

Speaker 2: George Bassel

Information processing and distributed computation in plant organs

Bassel, G.[1]

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Plant growth and development is tightly coupled to the environment. These external inputs are processed within organs in order to optimize the timing of key decisions, such as the termination of dormancy or commencement of flowering. In order to better understand how collections of cells in plants process information, parallels and differences between these naturally evolved organisms and engineered computational systems are being examined. Specifically, whether the control principles of distributed computation also apply to information processing in plants. By viewing plant organs as integrated systems of interacting cells, we are mapping intercellular connectivity into networks to reveal the multicellular “circuitry” plants use to compute. Integrating these topological templates with mathematical models capturing the genetic programs that operate within individual cells enables the impact of each cell organization and communication rate on the timing of emergent decision-making to be examined. The development of further theory to identify the bounds of information processing in plants will enable their transformation into rational distributed computing devices.

Session 3: Reproductive and Seed Biology

Speaker 3: Elizabeth Dun

Long-distance flowering signals regulate shoot branching in pea

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The timing and patterning of shoot branching are important determinants of crop yield, and the outgrowth of buds is significantly altered by changes in photoperiod and in photoperiod-insensitive and other flowering mutants. Interestingly, photoperiod is still able to regulate bud outgrowth in mutant plants deficient in the shoot branching hormone strigolactone. However, the mechanism by which photoperiod regulates bud outgrowth is unknown. Here, we show that the photoperiod response pathway regulates bud outgrowth via long-distance signalling, and activates the expression of genes involved in the strigolactone response and downstream pathway. Grafting and phenotypic studies using the photoperiod insensitive mutants *die neutralis* and *late bloomer1* of *Pisum sativum* (garden pea) indicate that the flowering signal, FLOWERING LOCUS T, acts as a long-distance, photoperiod-regulated signal to regulate bud outgrowth. Axillary buds of photoperiod-insensitive mutants showed altered responses to auxin and cytokinin, but not in a manner that could account for their altered branching responses. Photoperiod-insensitive mutants did not respond differently to strigolactone; however, experiments with double mutants showed that strigolactone response, and not strigolactone synthesis, was required for photoperiod regulation of shoot branching patterns. Analyses of bud gene expression suggest that the photoperiod response pathway pre-conditions axillary buds to have differing growth response capacities to endogenous signals, by modulating expression of *RAMOSUS4* and *BRANCHED1*, a known integrator of multiple signals regulating bud outgrowth. These insights into how photoperiod regulates bud outgrowth will help future endeavours to predict and optimise flowering and branching responses, ultimately improving crop yield and reproductive success.

Session 3: Reproductive and Seed Biology

Speaker 4: Yong-Ling Ruan

Sweet initiation of seed precursor: CWIN promotes ovule formation through sugar signalling rather than provision of carbon nutrient

Liao, S.[1], Li, J.[1], Eamens, A.[1], Wang, L.[1] and Ruan, Y.[1]

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Ovule formation is essential for realizing crop yield as it determines seed number. The underlying molecular basis, however, remains elusive. Cell wall invertase (CWIN) plays essential roles in seed development where phloem unloading or post-phloem transport occurs apoplasmically . Indeed, our recent evolutionary analyses established that CWIN was co-evolved with seed plants, further pointing to its role in plant reproductive development. It remains unknown, however, what roles CWIN may play, if any, in scenarios where assimilate import follows a symplasmic pathway and the amount of carbon required for organ-genesis is minimal. Here, we addressed this issue by focusing on Arabidopsis ovule formation where unloading takes place symplasmically.

Our analyses showed that CWIN functions as a major positive regulator for ovule initiation in Arabidopsis via sugar signalling. In situ hybridization revealed that CWIN2 and CWIN4, the two major CWIN paralogs expressed in Arabidopsis reproductive organs, were highly expressed at the placenta region where ovule primordia initiate. Specific silencing of CWIN2 and 4 by using artificial microRNA driven by an ovule-specific SEEDSTICK promoter (pSTK) reduced CWIN transcript and activity, leading to the blockage of ovule initiation and increased ovule abortion. Surprisingly, there was no induction of carbon starvation genes in the transgenic lines and supplement of extra carbon to the newly forming floral buds via manipulating source-to sink ratio failed to recover the ovule phenotype. The findings show that suppression of CWIN did not affect carbon supply to the ovule primordia. Interestingly, a group of hexose transporters was down-regulated in the pSTK::amiRCWIN2,4 transgenic plants. Among them, STP9, SWEET8 were spatially co-localized with CWIN2 and CWIN4, indicating a coupling between CWIN and hexose transporters for ovule initiation. RNA-Seq analysis identified a cohort of putative extracellular receptor-like-kinases (RLKs) and intracellular small GTPases (Rops) that were also altered in their mRNA levels in response to CWIN- silencing. Together, the findings show that CWIN play an essential role in ovule initiation through sugar signalling instead of provision of carbon nutrient. Our data support a model that CWIN- mediated sugar signalling may be perceived by, and transmitted through, plasma membrane hexose transporters or RLKs to regulate ovule formation by modulating the expression of downstream transcription factor genes including STK and AGL66 and early auxin response and signalling genes, such as SAURs and IAAs.

Session 3: Reproductive and Seed Biology

Speaker 5: Xiujuan Yang

Defining a germline niche during ovule development in barley

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In seed plants, the ovule is a multifunctional organ that supports development of the embryo sac and its constituent germ cells, in addition to regulating maternal resource flow into the developing seed. These functions are attributed to development of three main tissues; the nucellus, chalaza (integuments) and funiculus. In Arabidopsis, the nucellus is small and contains few cells, while in cereal crops such as barley (Hordeum vulgare), the embryo sac is embedded in a large multicellular nucellus. The role of a large nucellus is not fully understood, but must somehow balance germline differentiation with nutrient accumulation and subsequent programed cell death to facilitate grain development. We have identified a unique feature of the nucellus in barley, whereby cells adjacent to the germline exhibit a specific cell wall profile. Differences are first observed as the germline precursor expands, when adjacent nucellar cells start to accumulate unesterified pectin, and eventually form a defined niche surrounding the embryo sac. Correspondingly, the division of nucellar cells within this niche is less active compared to nucellar cells with low level of unesterified pectin, possibly due to limitations imposed by cell wall rigidity. To investigate the underlying molecular mechanisms, a tissue-specific transcriptome was generated from different barley ovule tissues. This revealed a number of candidates potentially involved in nucellus differentiation including several pectin methylesterases (PMEs) and MADS-box transcription factors. Promoter analysis suggested that CArG motifs in promoters of PME genes expressed in the nucellus may represent potential MADS-box protein binding sites. Dual-Luciferase assays showed that several MADS-box proteins from the B-sister, C, D, and E class are possible regulators of PME genes in the nucellus. To functionally verify the roles of candidate genes, CRISPR-Cas9 genomic editing has been applied to create mutants. Our research will provide novel insights for ovule and grain development in cereals.

Session 4: Plant Nutrition

Speaker 1: Jianping Hu

Peroxisomes, Photorespiration and Energy Balancing in a Dynamic Environment

Hu, J.[1]

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Peroxisomes are dynamic and essential organelles involved in various physiological processes, including the metabolism of lipids through beta-oxidation and salvage of phosphoglycolate through the photorespiratory pathway, both of which are key to energy metabolism and balancing. Many of these metabolic pathways are accomplished through collaboration between peroxisomes and other subcellular organelles such as mitochondria and chloroplasts. I will present our recent work aimed at understanding the interaction between peroxisomal metabolism/photorespiration and photosynthesis/carbon metabolism under dynamic environmental conditions. Our study may have important implications for future agricultural efforts to improve the efficiency of bioenergy production in crops under stress conditions.

Session 4: Plant Nutrition

Speaker 2: Nicolaus von Wirén

Impact of nitrogen signals on the phytohormonal regulation of root architectural traits

Von Wirén, N.[1]

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Plants adapt root system architecture to the availability of mineral elements. When grown under nitrogen (N)-limiting soils, plants develop longer primary or seminal and lateral roots, but so far it has remained unclear how this N foraging response is coordinated. By exploiting the natural variation followed by a genome-wide association study in 200 Arabidopsis natural accessions, we mapped the brassinosteriod (BR) signaling kinase BSK3 that contributes to natural variation in primary root length under mild N deficiency. Notably, a proline to leucine substitution in the kinase domain of BSK3 determines BR sensitivity and root responsiveness to mild N deficiency. In addition, we found that N deficiency signals enter the BR signaling cascade upstream of BSK3. Thus, BSK3 takes over a novel role in BR signaling by determining root foraging under mild N deficiency.

When plant roots grow under localized supply nitrogen, it has been observed previously that nitrate stimulates lateral root elongation, while local ammonium promotes lateral root branching. Now, we found that local ammonium supply promotes auxin accumulation in the vascular system, generating a source for lateral auxin transport. Based on auxin and pH reporters as well as by pharmacological approaches and mutant analysis, we found that acidification of the root apoplast follows AMT-mediated ammonium uptake to drive pH-dependent radial auxin transport to the cortex and epidermis, thereby circumventing LAX3-dependent auxin import. This study provides a show-case of how nitrogen forms shape root system architecture by modulating phytohormone transport.

Session 4: Plant Nutrition

Speaker 3: Rainer Hoefgen

Control of plant sulphur nutrition through SDI proteins

Hoefgen, R.[1]

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The genes SDI1 and SDI2 have been identified in early transcriptomics studies as being highly expressed in response to sulphate depletion in Arabidopsis and wheat. We linked their induction to the accumulation of O-acetyl-serine (OAS) which highly accumulates in response to reduced sulphate availability, but also in response to other stresses. Both genes belong to a cluster of OAS responsive genes. We identified that in Arabidopsis SDI1 (At5g48850) and SDI2 (At1g04770) are involved in down-regulating glucosinolate biosynthesis. Overexpression of both, SDI1 and SDI2, resulted in a reduced accumulation of aliphatic and to a lesser extent indolic glucosinolates. This is achieved through a direct protein-protein interaction of SDI1 negatively affecting the functionality of the transcription factor MYB28, previously identified to control glucosinolate biosynthesis. As glucosinolates provide a substantial sink for sulphate this regulatory step allows brassicaceae plants under sulphate starvation conditions to reduce de novo glucosinolate biosynthesis in favour of plant primary metabolism. We postulate further functions of SDI on seed development and control of root architecture for which we will present recent evidence.

Session 4: Plant Nutrition

Speaker 4: Oscar Fung

Investigating the Role of the Iron-Deficiency Inducible Transcription Factor OsIRO3 in Rice Using CRISPR-Cas9 Genome Editing

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Iron (Fe) is an essential micronutrient for all life. Plants use Fe for DNA and chlorophyll biosynthesis, cellular respiration and other metabolic processes. However, Fe-toxicity and Fe-deficiency can negatively impact on plant growth and physiology through oxidative damage, chlorosis and other factors. It is therefore crucial that Fe uptake, transport and storage processes are tightly regulated. In rice (*Oryza sativa* L.), several transcription factors have been identified as regulators of genes involved in Fe-homeostasis, but this network is poorly understood. The transcription factor OsIRO3 is a negative regulator of genes involved in Fe uptake, transport and storage. Its expression is induced during the Fe-deficiency response and follows a similar pattern to genes responsible for Fe uptake and remobilization, albeit at a lower expression level. Here, we report on expression of the *OsIRO3* gene under Fe-sufficient and Fe-deficient conditions in wild-type rice plants. Rice genes with putative OsIRO3 *cis-element* binding targets are also identified. Furthermore, we describe *Agrobacterium*-mediated transformation of rice with CRISPR-Cas9 plasmids containing two guide RNAs targeting the OsIRO3 gene to generate *osiro3* loss-of-function mutants. Thirty-six independent mutant alleles representing a range of indels were identified in 42 transgenic rice plants. Mutations included large deletions spanning the two guide RNAs and single or double nucleotide indels at the guide 1 and/or guide 2 positions. Notably, 27 T_0 plants contained indels at guide 1 resulting in premature stop codons and losses of up to 177 amino acid residues. Characterization of the OsIRO3 transcription factor will broaden our knowledge of the Fe regulatory network in rice.

Session 4: Plant Nutrition

Speaker 5: Changyu Yi

Natural variation in the response of Arabidopsis thaliana to a low phosphorus environment

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Phosphorus (P) is an essential macronutrient for plant growth and productivity. The availability of phosphate (Pi), which is the form of phosphorus taken up by plants, is very low in most soils and nearly 70% of the global cultivated land suffers from Pi deficiency, resulting in considerable crop yield reduction. Thus, understanding how plants respond to a low Pi environment will assist in developing crops with higher Pi acquisition and phosphate use efficiency. This work characterizes the Pi starvation response (PSR) of 200 Arabidopsis accessions. Genome-wide association studies (GWAS) for the six measured traits identified 35 distinct genomic regions contributing to the great phenotypic variation observed between these accessions. From the candidate genes, *PHOSPHATE TRANSPORTER 1;2* (*PHT1;2*) and *PIN-LIKES 7* (*PILS7*) were chosen for down-stream validation. In contrast to other *pht1* mutants, the *pht1;2* mutant showed higher biomass accumulation under Pi-sufficient condition. Two *pils7* alleles showed growth arrest irrespective of Pi supply, with significantly lower Pi uptake capacity under low Pi supply. Haplotype analysis of these two candidate genes revealed two contrasting genotype groups with respect to the measured traits. In-depth functional analyses of the underlying alleles in the Col-0 genetic background will be presented. Taken together, this study investigates the molecular basis underlying natural variation in the PSR in Arabidopsis by GWAS.

Jan Anderson Award

Speaker: Monika Murcha

Mitochondrial machineries for import, assembly and proteolysis

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Mitochondrial biogenesis is an intricate process involving the co-ordination of both nuclear and mitochondrial gene expression, protein synthesis, targeting, co-factor incorporation and finally, the assembly of 1000's of proteins. Key processes tightly regulated to allow the mitochondria to respond to its cellular environment and thus meet the energetic demands of the plant. I will present studies in which we have characterised specific receptors and transporters on the mitochondrial membranes that control the rate of protein and tRNA import. Present how a mitochondrial matrix assembly factor is involved in the assembly of Complex I, and in what manner protein degrading machineries can maintain complex homeostasis and turnover of damaged/misassembled subunits to ensure optimal mitochondrial activity. Together, these studies provide a glimpse on how plants are able to co-ordinate mitochondrial protein content and activity.

Session 5: Genome Regulation (tissues & cells)

Speaker 1: Julie Law

Locus- and tissue-specific control of DNA methylation in *Arabidopsis thaliana*

Law, J.[1]

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Cytosine DNA methylation plays crucial roles in gene regulation, transposon silencing, and diverse developmental processes. While DNA methylation patterns are known to differ between tissues, cell-types, and individual loci, how these differences arise remains poorly understood. In plants, DNA methylation is established via the RNA-directed DNA methylation (RdDM) pathway. In this pathway, RNA POLYMERASE-IV (Pol-IV) plays a critical role in specifying DNA methylation patterns as it initiates the biogenesis of 24-nucleotide small interfering RNAs (24nt-siRNAs) that guide methylation at cognate genomic loci. Thus, understanding Pol-IV regulation is key for determining how DNA methylation patterns are generated. Recently we demonstrated that four Pol-IV-associated factors, CLASSY (CLSY) 1-4, act individually as locus-specific regulators of RdDM and together control the production of nearly all 24nt-siRNAs, shaping global DNA methylation patterns. Extending upon these findings, we are now assessing the roles of the CLSY proteins in regulating DNA methylation patterns in a tissue-specific manner. Through these analyses we hope begin assigning specific methylation patterns to subsets of CLSY factors to reveal mechanistic insights into how the epigenome is regulated during plant development.

Session 5: Genome Regulation (tissues & cells)

Speaker 2: Ute Roessner

Tissue-specific effects of osmotic stress on the lipidome of cereal roots

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Lipids occur throughout the living world and are important molecules found in microbes, higher plants, animals and in all cell types. The main biological functions of lipids include storing energy, signalling, and acting as structural components of cell membranes. Lipids are a large and vastly varied group of organic compounds built from a limited number of building blocks resulting in an enormous chemical structural diversity. This diversity is necessary to provide the many roles different lipids provide in cell development and metabolism. The function of many lipid species is yet to be established in mammalian systems, and in plants we have even less understanding of the roles and importance of the more diverse set of plant lipids in development, growth and stress response.

The field of lipidomics, which aims to analyse the entire lipidome of an organism, is now well established and has provided substantial breakthroughs in biomedical research. In our research programs, these methodologies are now being deployed to investigate tissue-specific lipidome changes of cereal roots following exposure to environmental stress. Here we show that the plant lipidome is significantly more complex compared to mammals, is highly defined by tissue type and is highly responsive to abiotic stress, including salinity and temperature stresses. I will present our efforts in the development of comprehensive plant lipidomics methods using orthogonal and imaging mass spectrometry approaches and their application to understand plant root responses to salt and cold stress.

Session 5: Genome Regulation (tissues & cells)

Speaker 3: Ryan Lister

Dissecting plant development at single cell resolution

Lister, R.[1,2]

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Challenges in the isolation of specific plant cell types and the inability to effectively culture plant cells of distinct defined identities have impeded the deep molecular characterization of the diversity of plant cell identities and developmental transitions. Consequently, little is known about the transcriptional state of very rare or transient cell populations that arise during plant development. We have undertaken deep characterization of plant transcriptomes through development at single cell resolution through a combination of genomic and spatial imaging techniques. Improved experimental and computational methodologies for cell discrimination enabled sensitive detection and mapping of transcriptional states of complex tissues, including rare, transient, and new cell states. Reconstruction of developmental trajectories for distinct cell lineages revealed previously uncharacterized and complex topologies and putative governing regulatory pathways. Finally, through co-expression analysis and spatial mapping we have identified superposition of distinct cell types and transcriptional states, and unexpected spatial coordination of functionally specialized cell states within a complex tissue during plant cell differentiation and maturation.

Session 5: Genome Regulation (tissues & cells)

Speaker 4: Buddhini Ranawaka

Effect of histone modifications on differential expression of polyploid *Nicotiana benthamiana* homoeologs

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Nicotiana benthamiana is an important research tool, a vaccine biofactory and a model plant for the economically important crop family Solanaceae. It is an ancient allotetraploid arising from interspecific hybridization events about 5 million years ago. Intergenomic interactions between the subgenomes of polyploids are predicted to induce epigenetic modifications. Epigenetic changes regulate gene expression however, their roles in differential gene expression in ancient polyploids are poorly understood. To study the chromatin basis of biased homoeologous gene expression in *N. benthamiana*, we have performed genome-wide chromatin immunoprecipitation-sequencing (ChIP-seq) using antibodies against a range of histone modifications. The average distribution of "active" and "repressive" epigenetic marks appears to be similar between the two sub-genomes but there are significant differences between some specific homeologous loci. High levels of the active mark H3K4me2 were observed at loci corresponding to highly expressed homoeologs, whilst repressive marks such as H3K9me2 were either low or absent in these regions. The results indicate that variation in epigenetic status can be associated with differential homoeolog expression, and may provide a guide for manipulating the expression of important genes in polyploid crops.

Session 5: Genome Regulation (tissues & cells)

Speaker 5: Shivani Bhatia

Gene regulatory network of epidermal and sub-epidermal cell layers enriched transcription factors and their expression patterns revealed mechanisms involved in cell fate specification in Arabidopsis thaliana.

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Plant development relies on the coordinated expression of hundreds of genes. The transcription factors (TFs) and the regulatory networks that regulate epidermal and sub-epidermal cell layers' specification in Arabidopsis shoot apical meristem (SAM) have not been studied. In this study, we identified 65 TFs enriched in the epidermal and sub-epidermal cell layers of SAM. To study their spatio-temporal expression patterns from early embryo to adult shoot, promoter reporter constructs were made for 44 TFs. Ten major patterns of expression were found to be associated with these TFs in the adult shoot. And in ~50% of the cases, 3kb promoter fragment was found to be sufficient to capture the native expression pattern. Our findings also revealed that the epidermal cell layer identity is established by transcriptional repression of epidermis specific genes in the inner cell layers of globular stage embryo. Interestingly, TFs linked to sub-epidermal cell layer identity were found to express only in post-embryonic stages of development. To further delineate the regulation of these TFs, we mapped their interactions with shoot enriched TF proteins using yeast-one-hybrid assay. A total of 16,023 interactions were set up between 49 DNA baits and 327 prey proteins and a network of 165 interactions was concluded. The regulatory relationships were established using genetic analysis between upstream regulator and target genes. This analysis revealed that *GROWTH REGULATING FACTORS* (*GRFs*) *1*, *2*, and *3*, that express broadly in the leaves and shoot, regulates epidermal cell layer enriched gene *HOMEODOMAIN GLABROUS 12* (*HDG12*). *GRFs* are known to regulate cell proliferation during leaf development. However, loss of function mutants of *hdg12* exhibit reduced shoot and pavement cell size, suggesting its role in controlling cell expansion. Taken together, these results shed light on a transcriptional regulatory mechanism by GRFs that control shoot and leaf size by regulating expression of *HDG12*.

Session 6: Medicinal Agriculture

Speaker 1: Jonathan Page

Cannabis genomics: from biochemistry to crop innovation

Page, J. [1,2]

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[2]*Botany Department, The University of British Columbia, Vancouver, BC, Canada*

Cannabis sativa L. ("marijuana", hemp; Cannabaceae) is an ancient crop plant that produces nutritious seeds, high-quality fibre and bioactive cannabinoids such as THC and CBD. We are using genomics to elucidate the cannabinoid metabolic pathway and to better understand the genetic organization of the genus *Cannabis*. A major experimental approach has been the use of transcriptome data derived from glandular trichomes, the specialized epidermal structures that synthesize cannabinoids. We have successfully applied trichome-focused analysis in combination with classical biochemistry to identify three enzymes of the cannabinoid pathway: hexanoyl-CoA synthetase, olivetolic acid cyclase and an aromatic prenyltransferase. A draft assembly of the ~820 Mbp genome from the drug-type strain Purple Kush has opened up new avenues for gene discovery as shown by the identification of a novel cannabinoid synthase enzyme, cannabichromenic acid synthase. We have recently used genotyping-by-sequencing (GBS) to analyze the genetic variation in 43 hemp and 81 marijuana accessions. GBS shows that hemp and marijuana are genetically distinct, and provides insight into the differentiation of marijuana into "Indica" and "Sativa" groups. The genome is also allowing development of genetic markers for key, high-value traits. As cannabis emerges from the shadow of prohibition, genomics promises both to clarify its evolutionary history and to accelerate the development of this valuable, multi-use crop.

Session 6: Medicinal Agriculture

Speaker 2: Simone Rochfort

The Cannabis Metabolome

Rochfort, S.[1], Elinks, A.[1], Krill, C.[1], Ezernieks, V.[1] and Spangenberg, G.[1]

[1]Agriculture Victoria Research

Cannabis is an herbaceous flowering plant of the *Cannabis* genus (Rosale) that has been used for its fibre and medicinal properties for thousands of years. In recent decades medicinal cannabis has become legal in several jurisdictions and the possibility of legalisation is being explored in many more. This legalisation has opened the field of medicinal cannabis research. The biochemistry of cannabis is rich and varied including phytocannabinoids, terpenes and phenolics. Each of these metabolite classes contains individual compounds with biological activity. This chemical diversity and the interaction between molecules may underpin the 'entourage effect' that is believed to contribute to the medical efficacy of cannabis. In order to fully explore this biochemistry we have undertaken both targeted and untargeted metabolomic and volatolomic analysis of diverse cannabis strains. 70 diverse strains have been characterised using liquid chromatography mass spectrometry (LCMS), nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GCMS). The chemotaxonomic relationship between the strains will be discussed. In combination with genomics these analyses offer the potential for 'designer strains' to be developed for particular medical applications.

Session 6: Medicinal Agriculture

Speaker 3: Matthew Welling

Genetic characterisation of cannabinoid alkyl side-chain length in Cannabis

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The plant genus *Cannabis* produces a class of medically important secondary metabolites known as cannabinoids. The alkyl side-chain is a critical structural feature of cannabinoids which determines pharmacological potency. Despite potential for restructuring of metabolic networks so that novel recombinant chemotypes can be developed for biopharmaceutical end-use, the genetic basis of alkyl side-chain length is largely undefined. To investigate the inheritance of alkyl cannabinoid composition in *Cannabis*, chemotypic segregation was analysed in F2 progeny derived from a cross between two plants divergent for alkyl side-chain length. Segregation patterns were non-Gaussian and consistent with digenic as well as epistatic modes of inheritance. Cannabinoid synthase Sequence characterised amplified region (SCAR) marker analysis of the F2 progeny suggested linkage between cannabinoid synthesis pathway loci. This study provides the basis for more advanced genetic analyses aimed at unravelling the activity of cannabinoid genes and associated regulatory networks governing alkyl side-chain length.

Session 6: Medicinal Agriculture

Speaker 4: Lee Conneely

Cannabis sativa glandular trichomes: Isolation, purification, and proteomics

Conneely, L.[1], Kretzschmar, T.[1] and Barkla, B.J.[1]

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Cannabis sativa is a versatile fast-growing, herbaceous, annual plant that has been domesticated and cultivated since at least 8000 BC for food, fibre and medicine. Cannabis produces a unique class of chemically diverse and therapeutically valuable secondary metabolites that interact with the human endocannabinoid system, referred to as cannabinoids.

Cannabinoids are largely produced in floral capitate stalked glandular trichomes, multicellular epidermal protrusions that act as efficient bio-factories. Cannabis glandular trichomes are capable of producing up to 20% of floral dry weight of specific cannabinoids, yet little is known how this production is achieved, regulated and partitioned.

We developed a method for isolation of intact trichomes from Cannabis floral tissues and downstream purification into head and stalk enriched fractions to serve as a tool in Cannabis trichome biology. Preliminary data suggest that these fractions are suitable for transcript, protein and metabolite studies. A proteomic analyses of a glandular trichome fractions sheds new light on the location of cannabinoid biosynthesis.

Session 7: Plant Development

Speaker 1: Janet Wheeler

An Arabidopsis Natriuretic Peptide can directly modulate Catalase 2 activity and cellular redox homeostasis

Turek, I.[1,2], Wheeler, J.[3,4], Bartels, S.[5], Szczurek, J.[6], Wang, Y.[3,4], Taylor, P.[8], Gehring, C.[1,7] and Irving, H.[2,3]

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H_2O_2 is a key component of abiotic and biotic stress responses in plants and since the systemically mobile Plant Natriuretic Peptides (PNPs) modulate many physiological responses, including responses to plant stress, we set out to investigate if the *Arabidopsis thaliana* PNP (AtPNP-A) can directly or indirectly affect H_2O_2 homeostasis. Firstly, we observed that AtPNP-A enhances flg22-induced oxidative burst and when we screened for AtPNP-A interacting partners we identified proteins capable of modulating cellular redox homeostasis, and in particular Catalase 2 (CAT2). Surface plasmon resonance (SPR) analyses revealed that the full-length AtPNP-A recombinant protein as well as a biologically active fragment of AtPNP-A bind specifically to CAT2 *in vitro*, while a biologically inactive scrambled peptide does not. *In vivo* bimolecular fluorescence complementation (BiFC) revealed that CAT2 interacts with AtPNP-A in chloroplasts. We also noted that CAT2 activity is lower in homozygous *atpnp-a* knockdown plants. Furthermore, *atpnp-a* knockdown plants phenocopy *CAT2*-deficient plants in their sensitivity to elevated H_2O_2 compared to the wild type plants, and this is consistent with a direct modulatory effect of the natriuretic peptide on the activity of the enzyme and hence H_2O_2 homeostasis. We propose that the interaction between AtPNP-A and CAT2 is critical for plant responses to abiotic and biotic stresses.

Session 7: Plant Development

Speaker 2: Dan Syzmanski

A computational and systems-level analysis of epidermal morphogenesis

Syzmanski, D.[1]

[1]*Department of Agronomy, Purdue University*

The actin and microtubule cytoskeletons rearrange dynamically in the cytoplasm to control the material properties of extracellular polysaccharides. It is the properties of the tough outer cell wall that define the geometric paths of morphogenesis. Forward genetic screens and biochemical reconstitution have identified the protein complexes and genetic circuitries that pattern the cytoskeleton. However, the quantitative mechanistic linkages among the cytoskeleton, cell wall properties, and cell shape change remain unknown. This seminar will describe the combined use of genetics, multi-variate live cell imaging, and computational modeling to analyze the how complex cellular systems interact to control cell morphogenesis. Finite element modeling treats the plant cell wall as a thin-walled pressurized shell, and makes testable predictions about what wall properties are needed to generate specific growth outputs. The method also provides a new way to analyze morphogenesis across wide spatial scales, and discover how cell geometry and cell wall stress feedback on cytoskeletal systems during morphogenesis.

Session 7: Plant Development

Speaker 3: Kimitsune Ishizaki

An evolutionary conserved mechanism for production of secondary meristems in land plants

Ishizaki, K.[1]

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Vegetative reproduction, a form of asexual reproduction in plants, is a developmental process by which clonal progeny arise directly from parental tissues. This process is based on the remarkable potential of plants to proliferate meristems, which develop whole plantlets from differentiated tissues. A basal land plant, the liverwort *Marchantia polymorpha L*., as well as certain related species in the class Marchantiopsida, develops specialized organs for vegetative reproduction, termed the gemma cup or cupule. Clonal propagules, called gemmae, develop from single epidermal cells at the base, or floor, of gemma cups. Thus, the floor cells have the capability to produce clonal plantlets. Gemma cups are formed periodically along the dorsal midrib of a thallus. Over 100 gemmae can develop in a single gemma cup. The development of gemma cups has been well described on the basis of a series of histological observations on *M. polymorpha*. However, the molecular mechanisms underlying gemma and gemma cup development are largely unknown. Here we report an R2R3-MYB transcription factor, designated GEMMA CUP-ASSOCIATED MYB 1 (GCAM1), which is an essential regulator of gemma cup development in *M. polymorpha*. Targeted disruption of *GCAM1* conferred a complete loss of gemma cup formation and gemmae generation. Cell proliferation without tissue differentiation was observed in plants ectopically expressing *GCAM1*. Although gemma cups are a characteristic gametophyte organ for vegetative reproduction in a taxonomically restricted group of liverwort species, phylogenetic and interspecific complementation analyses supported the orthologous relationship of *GCAM1* to regulatory factors for axillary meristem formation, *e.g.* Arabidopsis RAXs and tomato Blind, in angiosperm sporophytes. The present findings in *M. polymorpha* suggest an ancient acquisition of a regulatory mechanism for production of new meristems, and the use of the mechanism for diverse developmental programs during land plant evolution.

Session 7: Plant Development

Speaker 4: Xiang Li

Exploring sugar signalling by chemical biology in Arabidopsis

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Sugars are crucial primary metabolites and signalling molecules that contribute to growth and development in plants. Sugar signals regulate developmental transitions, circadian rhythms and interactions with the biotic and abiotic environment. Getting a better understanding of plant sugar signalling helps to improve plant adaptability to different energy situations, which would potentially contribute to agriculture. However, the central role of sugars in cell function has made traditional genetic approaches problematic to define the molecular pathways of sugar signalling, either due to genetic lethality or gene redundancy. We have used a chemical genetic approach to overcome these problems and uncover new components of these pathways. From a high-throughput luciferase-reporter screen of a bioactive small molecule library, we identified chemicals which alter a transcriptional sugar response and inhibit sugar-promoted growth in Arabidopsis seedlings. One of these chemicals is a calcium channel antagonist in animals but its target in plant cells is unknown. We have confirmed the chemical's effect on cytosolic calcium concentration and calcium fluxes triggered by H2O2 and NaCl using AEQUORIN, a luminescent calcium reporter. We are now using both genetic and proteomic approaches to identify the protein target(s) of the compound. The identification of the target protein(s) is expected to reveal a new component of plant sugar signalling and implicate a specific role for calcium fluxes.

Session 7: Plant Development

Speaker 5: Marta Peirats-Llobet

The ABA signalosome interacts with BRAHMA to regulate ABI4 and early seedling establishment.

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Abscisic acid (ABA) plays a key role in regulating seed germination and post-germination growth. In particular, the AP2- and bZIP-type transcription factors (TFs) ABI4 and ABI5, respectively are required for ABA-mediated inhibition of post-germination growth when the embryo encounters water stress. The post-germination growth arrest mechanism induced by ABI4 and ABI5 involves ABA signalling. ABA inhibits the ATPase BRAHMA (BRM) to induce ABI5 transcription. ABI4 is a versatile TF that is highly expressed in seeds. However, its mRNA levels drop a few days after germination, suggesting that a mechanism operates to repress ABI4 expression during germination and to prevent ABI4-mediated post-germination growth arrest under favourable conditions. Since BRM activity is required to repress ABA signalling during seedling establishment, we investigated a possible connection between BRM and ABI4 using abi4 brm-3 double mutant in Arabidopsis. We found that in addition to ABI5, ABI4 is responsible for the brm-3 ABA-hypersensitive phenotype in seed germination and root response during early seedling growth. Our results also suggested that the expression level of ABI4 is regulated by BRM possibly through direct interaction with the promoter. We further found new evidence indicating to the whole ABA core elements, interacting with BRM to control its chromatin remodelling activity. Accordingly, we propose a mechanism where the environmental conditions will regulate the ABA signalosome and BRM to finely tune ABI4 expression by directly interacting with its promoter.

Teaching Award:

Speaker: Sigfredo Fuente

Application of new and emerging technologies for digital teaching and learning in plant physiology

Fuentes, S.[1]

[1]*University of Melbourne*

Nowadays, students and the majority of young people are heavily engaged and reliant on smart devices as part of daily routines. It is therefore wise to take advantage of this avenue to deliver learning tools right into the hands of students. Advances in digital technologies in the form of portable smart devices and app-based sensor technologies, such as infrared thermal imaging and near-infrared spectroscopy handheld devices, have made the application of these novel tools for teaching and learning in plant physiology readily accessible. Students nowadays being tech-savvy, enabled these tools to be easily implemented, with high reception, engagement, and interest. The development of teaching and learning tools based on new and emerging technologies is a critical step to increase engagement of students in complicated physiological processes of plants, such as transpiration rate, stomatal conductance, nutrient levels, and photosynthesis, among others, that can be assessed in real time. A. Prof. Sigfredo Fuentes has been actively creating and writing computer codes, programs, and apps for Plant Science teaching and research. Some of these tools have been adopted commercially and are applied in tertiary teaching and research institutions worldwide. Furthermore, most of these tools have been published in peer-reviewed journals and have been used by several undergraduate/postgraduate students for their Bachelors, Master, and PhD degrees. These tools have also helped to generate new ideas on the implementation of computer and sensor applications using the Internet of Things in Plant Science and Agriculture and have been used to support lectures in Plant Science, Food Science, Sensory Science, Animal Science, Engineering, Viticulture and Oenology subjects.

Session 8: Communication and Education

Speaker 1: TJ Higgins

Communicating about GMOs: Are we there yet?

Higgins, T.J.[1]

[1]*CSIRO Agriculture and Food*

Discussion around genetically modified (GM) plants and other organisms has been full of claims and counterclaims for about three decades. The evidence base is very strong that foods made from GM products are as safe as any other food. However, the opinion-based world is much less clear-cut. Everyone is entitled to an opinion and that's where the trouble starts. Many negative opinions on GM are based on little or no evidence and are frequently very strongly held. Opinions gain a lot of traction in the media especially when there is a sense of doom in the message.

Correcting the scary messages in Australia (as elsewhere) has proved to be difficult especially with a certain proportion of the population (the 13% that are strongly opposed-defined in OGTR-sponsored surveys) and it is likely that they will not ever be convinced by any evidence-based information. It is still surprising that over 70% of the population are positive about biotechnology's role in improving our way of life although only about half were in support of GM.

I have spent over two decades informing decision-makers about the technical aspects of GM and while I feel I have had a fair hearing from that group and that the effort was worthwhile, the overall reputation of GM is still extremely disappointing. It is possible that medical advances such as gene therapy and GM crops such as omega-3 canola will slowly raise the profile of GM in the minds of the unsure. GM crops like virus-resistant papaya, blight-resistant potato, golden rice and pod borer resistant cowpea may also provide some reassurance since they have all been developed in the public sector. That is, they are not products of large multinational companies.

Session 8: Communication and Education

Speaker 2: Beth Loveys

Flipping the Cellar Floor: Instructional Videos to Support Student Learning

Bauer, J.[1], McNamara, I.[1], Huckel, M.[1], Jansz, J.[1], Wang, H.[1] and Li, L.[1]

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As higher education becomes increasingly accessible to students from a variety of backgrounds with diverse pre-existing knowledge it is critical for educators to respond to the challenges this presents with regard to providing resources to support all students in their learning. The University of Adelaide offers degree and post-graduate qualifications in oenology and viticulture where students are required to work authentically on the winery cellar floor, demonstrating practical competencies in techniques and processes which are required when they enter industry. For students with little or no background in winemaking the prospect of undertaking procedures in a semi-industrial setting can be extremely challenging. Increasing international enrolments also present challenges for educators in terms of safety and comprehension.

This case study describes how a team of staff and senior students addressed the challenges of creating curriculum for such a diverse student community by production of subtitled, instructional videos demonstrating essential cellar and laboratory skills. These videos were made accessible to students via the Learning Management System. They were accompanied by quizzes for knowledge checking and transcripts in English and Chinese to increase accessibility for the diverse student cohort.

The video resources were created with the assistance of senior students which increased their authenticity and resonance with the new student cohort. This case study will describe and analyse the process of planning, making and deploying video resources and wider applicability to a variety of learning contexts. Preliminary evidence of the impact of the video resources on student learning will also be discussed.

Session 8: Communication and Education

Speaker 3: Thomas Shafee

Science outreach at the >1 million reader scale via Wikipedia

Shafee, T.[1,2]

[1]*La Trobe University*
[2]*WikiJournal of Science*

When looking to improve access to plant science information, there are few better media than Wikipedia. The encyclopedia is the most-accessed source of scientific information and so the accuracy and completeness of its plant science content has a direct effect on the scientific literacy of the general public - including journalists, policymakers and scientists from other fields. The English-language version has global reach, and there are an additional 300 other language editions (many of which are directly translated from English). Facilitating more direct contribution to the encyclopedia by scientists is therefore one of the most effective outreach routes. Additionally, the rise of Wikipedia-integrated academic journals, such as the WikiJournals of Science is enabling contribution via the more familiar academic journal format.

Such Wikipedia-integrated journals couple the rigour of academic peer review with the extreme reach of the encyclopedia. Firstly, it rewards authors with citable, indexed review publications to incentivise contribution. It is able to publish fully open access and without any author fees since it is hosted on Wikimedia Foundation servers and runs with a volunteer staff. Secondly, by subjecting submissions to rigorous external peer review, it ensures that the information is accurate and up to date. In addition to adding new articles to the encyclopedia, existing Wikipedia articles can also be expanded or overhauled and submitted for the same peer review and publication process. Articles are each commonly read >100,000 times *per annum* with many above a million reads.

In this way WikiJSci and its sister journals improve the accuracy of the encyclopedia with high-quality, peer-reviewed content, and reward authors with publications that achieve far greater reach than any traditional scholarly publishing. Developing and expanding these journals is vital mechanism for ensuring the accuracy of scientific information on a platform that is read millions of times per day.

Session 9: Phenotyping Technologies

Speaker 1: Fabian Pfrengle

A synthetic glycan microarray enables epitope mapping of cell wall glycan-directed antibodies and characterization of biosynthetic enzymes

Pfrengle, F.[1]

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Plant cells are surrounded by a polysaccharide-rich matrix that constitutes the cell wall of all higher plants. These structurally complex polysaccharides provide an important resource for food, renewable materials, and the generation of bioenergy. We have chemically synthesized oligosaccharides derived from different classes of plant cell wall polysaccharides using the new technology automated glycan assembly (AGA). Similar to solid-phase peptide synthesis, in AGA suitably protected monosaccharide building blocks are iteratively added to a linker-functionalized resin. After assembly, the linker is cleaved and all protecting groups are removed. The assembled glycans were subsequently printed as microarray to investigate carbohydrate-protein interactions. Using our synthetic plant glycan array, we were able to determine the epitopes of 81 antibodies that recognize plant cell wall glycans, including arabinogalactan, rhamnogalacturonan, xylan, and xyloglucan. These antibodies represent important molecular tools for analyzing cell wall glycans in mutants, organ and tissue types, and developmental stages. We demonstrate that, with knowledge of the exact epitopes recognized by individual antibodies, specific glycosyl hydrolases can be implemented into immunological cell wall analyses to obtain structural information on plant cell wall glycans with very high molecular precision. Finally, we also determined the acceptor substrate specificities of several glycosyltransferases that are involved in plant cell wall biosynthesis.

Session 9: Phenotyping Technologies

Speaker 2: Viridiana Silva Pérez

The power and limitations of hyperspectral reflectance: A wheat case

Silva-Perez, V.[1]

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Cutting edge technology such as hyperspectral reflectance, is increasing in popularity. A few decades ago, numerous indices based on wavelengths in the visible and infrared ranges of the electromagnetic spectrum were developed in remote sensing to predict vegetation biomass, biochemical and some physiological traits. Interest in the infrared (IR) part of the spectrum has increased because hyperspectral sensors now enable the measurement of a wider spectrum (i.e. 350-2500 nm). Research is exploring what information can be extracted from the extended spectrum. In the case of wheat, we predicted photosynthetic traits and plant structural traits based on a calibration from multiple experiments in Mexico and Australia with measurements on glasshouse and field-grown plants. Leaf reflectance spectra were measured on intact plants to avoid environmental noise. Models were built using partial least square regression (PLSR). Different machine learning methods can be explored where there is a limitation on gaining more observed data to develop the models. The main power of this technology is that allows the prediction of multiple traits from one leaf reflectance measurement that takes 30 seconds to collect. The method can be scaled up to a canopy measurement with hyperspectral imaging. Multiple measurements could be made at different crop stages, which would help to increase repeatability and heritability for pre-breeding programs.

Session 9: Phenotyping Technologies

Speaker 3: Harvey Millar

What drives the phenotype of plant respiratory rate: mapping out the details of mitochondrial metabolic function

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Mitochondrial respiration in plants provides energy for biosynthesis and its balance with photosynthesis determines the rate of plant biomass accumulation under optimal and limiting conditions. Because of this the importance of respiration rate as a plant phenotype is longstanding, but the ability to assess it and understand 'respirotypes' has been limited. Much has been learnt from functional analysis of isolated organelles provided with exogenous substrates and the rates and dynamics of single enzymes or biochemical pathways. This work has built our understanding of plant mitochondrial enzymology, designed diagrams in textbooks of plant metabolism and has identified what make plant mitochondria unique to mitochondria from other eukaryotes. Separately the study of CO_2 production, O_2 consumption and metabolite levels and fluxes *in vivo* has built a complex and at times perplexing presentation of what plant mitochondria consume *in vivo*. Combining these two perspectives through detailed longer term study of respiratory rates and responses *in vivo*, large scale phenotyping of respiratory rates and correlation to substrates and respiratory machinery, and analysis of respiratory phenotypes using reverse genetics of mitochondrial components is now providing new insights into what drives respiratory rate changes in plants. This talk will outline new research on high-throughput screens of respiration rate, the role of transport processes, and responses of respiration to harsh environments by adoption of new respirotypes.

Session 9: Phenotyping Technologies

Speaker 4: Yuval Sadeh

Estimating Leaf Area Index from space at 3 m

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Monitoring the dynamic of Leaf Area Index (LAI) from space is a key attribute to estimate crop types and their phenology over large areas, as well as to characterise spatial variations within grower's fields. Until recently, satellite-based monitoring of crops could either be done at high temporal (<5 days) or spatial resolution (<5 m), which limited remotely sensed application. Improving spatial and temporal quantification of LAI and developmental stage using remotely sensed imagery could assist to improve products and services to growers and enable more profitable decision making.

Over the last decade, the number of companies developing nano-satellites (also known as CubeSats) has increased. These new satellites that can be in a size of a shoe box, are relatively inexpensive to build, thereby enabling the creation at low cost of large image collections with a high spatial and temporal resolution. However, contrary to larger and expansive satellites such as Landsat or Sentinel-2, the images obtainable from nano-satellites constellations frequently suffer from inconsistency in the data collected by different satellites in the constellation.

This study aimed to develop a technique to fuse PlanetScope images (with a spatial resolution of ~3 m, and a daily revisit time) and Sentinel-2 images (10 m and five-day revisit time) to create daily images of crop LAI with a 3 m resolution. This was achieved by creating time-series of 13 different remotely sensed vegetation indices (e.g. NDVI) based on the radiation reflected from the crops in the visible and near-infrared spectrum, which was then converted into LAI. The results show that our approach succeeded to estimate LAI with less than 0.5 RMSE throughout the growing season without using any ground calibration. This method is useful for daily high-resolution monitoring of crops over large scales and can be used to improve decision making tools and yield forecasting.

Session 9: Phenotyping Technologies

Speaker 5: Rosemary White

Second harmonic imaging of developing cotton cell walls - clues in crystallinity

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Plant cell walls regulate plant cell development and function. The major structural elements are cellulose microfibrils, whose orientation in the expanding cell wall dictates the direction of expansion and hence cell shape, contributing largely to the shape of the plant organ assembled from these cells.

Cotton fibres are remarkable single-celled trichomes that develop about from about 30% of cotton seed coat epidermal cells. The fibres emerge as small balloons at the day of flowering, and once the flowers open, begin rapid elongation to a final length of up to 4 cm. Transversely-oriented cytoplasmic microtubules ensure that the cellulose microfibrils are also transversely-oriented during this period of rapid expansion. Once the fibres cease elongating, about 15-18 days after flower opening, there is considerable additional deposition of cellulose to form a thick cellulosic secondary cell wall, and the predominant microfibril orientation becomes more oblique to the long axis of the fibre.

We are interested in new methods to examine cellulose microfibril orientation during fibre development. Here, we used second harmonic imaging from a multiphoton confocal to reveal the orientation of crystalline structures within the walls of mature cotton fibres.

Strong second harmonic signals were obtained from mature cotton fibres, in both the forward and reverse directions. The subtle differences between these two signals may indicate details of inter-microfibril organisation, similar to the interpretation of signals from collagen assemblages in animal cells. Varieties of cotton producing different types of fibre also generated different second harmonic signals. However, the nature of the crystalline structures generating these signals is unclear, and we are currently exploring additional structural analyses of fibre walls, as well as following the development of second harmonic signals over time. These and additional data will be discussed with reference to the use and interpretation of second harmonic signals from plant cell walls.

Session 10: Abiotic Stress

Speaker 1: Richard Threthowan

Pushing the boundaries of high-temperature tolerance in wheat

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Australia's variable climate exposes wheat crops to high-temperature stress and wheat breeders have historically improved temperature tolerance through empirical selection in the cropping environment. However, if higher levels of tolerance to heat stress are sought, a combination of relevant germplasm, effective screening methodologies and optimized recombination and selection strategies are required. We used a three-tiered field-based phenotyping approach that allowed large numbers of fixed lines to be evaluated. In the first stage, a large training population comprising thousands of genotypes was evaluated for a suite of traits in the field in times-of-sowing experiments. The heat tolerance of selected materials was then confirmed using field-based heat chambers placed on optimally sown plots to induce a heat shock between meiosis and anthesis. A subset of materials was then evaluated under controlled conditions in the greenhouse to confirm the expression of key traits. All new materials entering the training population were genotyped using a 90K SNP platform. Lines with high genomic estimated breeding values (GEBVs) were retained for evaluation in the following year and genotypes with low GEBV were removed. Narrabri was considered the 'mother' site nationally where all materials were evaluated. Subsets of 200 genotypes, based on diversity and high GEBV, were evaluated concurrently in times-of-sowing experiments in Victoria and Western Australia. Progeny derived from recombination among polymorphic genotypes with high GEBVs entered the training population for further testing and recombination. Genotypes with significantly improved heat tolerance compared to local wheat cultivars were identified using this approach.

Session 10: Abiotic Stress

Speaker 2: Megan Shelden

Comparative spatial ionomics in the roots of two barley genotypes in response to salt stress

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Abiotic stresses are major causes of crop yield losses in agriculture significantly impacting on sustainability. Salinity results in a reduction in root growth, however, some species can maintain root elongation at salt concentrations that inhibit root growth; an adaptive mechanism to ensure seedling establishment and maintain water and nutrient uptake. Barley cv. Clipper (malting barley) and Sahara (North African landrace 3771), have previously been shown to have a contrasting root growth phenotype and metabolic profile in response to salinity stress. In saline soils, sodium (Na) and chloride (Cl) ions are taken up by the root influencing the uptake and retention of other important ions required for growth. We conducted spatially-resolved elemental analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) on the roots of Clipper and Sahara to understand changes in the root *ionome* in response to salinity. High concentrations of Na^+ and Cl^- in the soil can lead to Na^+ toxicity of plant cells, however, measuring soluble ions (ie. Na^+) in their native, cellular environment is inherently difficult. We developed a method to measure Na^+ at high spatial resolution in barley roots to determine if Na^+ toxicity in the root growth zone directly inhibits root growth, and influences the distribution of other elements (potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), phosphorus (P), iron (Fe)) in response to salinity. In both Clipper and Sahara, Na^+ was excluded from the meristem and cell division zone indicating that Na^+ toxicity is not directly impacting on cell division. K^+ was strongly correlated with Na^+ concentration in both genotypes. We have demonstrated that LA-ICP-MS can be used for quantifying the soluble ions Na and K in plant tissues, providing insight into the link between Na^+ toxicity and root growth responses to salt stress.

Session 10: Abiotic Stress

Speaker 3: Max Cowan

Crop wild relatives as a genetic resource for generating low-cyanide, drought-tolerant sorghum

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Sorghum (*Sorghum bicolor*) is an important food and forage crop that can accumulate high concentrations of the cyanogenic glucoside dhurrin in all vegetative tissues, particularly leaves and sheaths. Cyanogenic glucosides are chemically inert nitrogenous compounds, but upon plant tissue disruption they are hydrolysed by specific degradative enzymes to release hydrogen cyanide. Severe abiotic stress such as chronic drought is known to induce higher concentrations of dhurrin in crop sorghum. This is problematic for farmers relying on sorghum forage, as hydrogen cyanide can cause respiratory poisoning in feeding livestock. The wild species of the *Sorghum* genus are an untapped genetic resource that may offer a solution to the drought-linked hydrogen cyanide issues in crop sorghum. This study compared general plant performance, hydrogen cyanide potential and nitrogen management of an *S. bicolor* cultivar and seven endemic Australian wild *Sorghum* species grown under controlled well-watered or water-limited conditions. The biomass of *S. bicolor* was affected most severely by water limitation, while the shoot hydrogen cyanide potential, already 2-3 orders of magnitude higher than in the wild species, doubled under water deficit. In contrast, the growth of the wild species was less affected by water limitation, and shoot hydrogen cyanide potential was essentially unchanged. Two wild species, *S. brachypodum* and *S. macrospermum*, displayed a much higher tolerance to drought and a lower hydrogen cyanide potential in aboveground tissues under stress. These species would be of particular interest in *S. bicolor* crop improvement programs. The high tissue-specific polymorphism of hydrogen cyanide potential and variable drought response in the wild *Sorghum* species also implies additional non-defensive functions for cyanogenic glucosides.

Session 10: Abiotic Stress

Speaker 4: Brett Williams

Metabolic priming of the Australian resurrection plant, Tripogon loliiformis, in the hydrated state allows the early onset of long-term drought responses and desiccation tolerance

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Conventional breeding programmes have produced crops that metabolise rapidly and produce high yields. Although desirable for modern agriculture, these traits come at a metabolic and physiological cost. The rapid growth of crop plants leaves little energy resources behind for stress responses when required. Previously, we showed that dehydrating shoots of the native Australian resurrection plant, *Tripogon loliiformis*, initiate autophagy and nutrient recycling as one strategy to reinstate homeostasis and suppress cell death. Here, we describe the affiliation between energy status, shoot and root source-sink relationships and the activation of cell death pathways in the desiccation tolerance strategies of *T.loliiformis*. We show that *T.loliiformis* engages an approach similar to the long-term drought responses of sensitive plants and continues to use the roots as a sink even during sustained stress. Dehydrating *T.loliiformis* roots contained more sucrose and trehalose-6-phosphate compared to shoots at an equivalent water content. The increased resources provide sufficient energy to cope with stress, and thus, autophagy is not required in the roots. These findings show how shoots and roots utilise different stress response strategies that result in starkly different cell death outcomes. We postulate that the accumulation of stress-associated metabolites in hydrated tissues is a "pseudo" short-term drought stress response that *T.loliiformis* uses to aid its survival. Unlike sensitive plants, mild to moderate dehydration of *T.loliifomis* triggers the early onset of a "long-term" drought response and the implementation of energy-efficient tolerance strategies. Sensitive plants have similar fundamental systems to resilient plants but not the regulatory finesse to utilise their energy resources as effectively. This information may have wide-spanning implications to modern agriculture by allowing the modulation of energy signalling to optimise a crop's capacity to grow, yield and tolerate stress.

Session 11: Biotic Interactions

Speaker 1: Carl Mesarich

Recent progress towards understanding the Venturia inaequalis–apple pathosystem: a focus on fungal cell surface modifications and effector biology

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Scab is the most economically important disease of apple worldwide, and is caused by the non-obligate biotrophic fungus, Venturia inaequalis. Following penetration of the host, V. inaequalis must undergo dramatic changes in cellular morphology that permit colonization of the extracellular environment located within the cuticle and above epidermal cells of apple leaves and fruit. These changes include the differentiation of subcuticular runner hyphae and stromata. The former are much wider in diameter than regular tubular hyphae found on the plant surface or in nutrient-rich agar, and are often fused lengthwise to form 'hyphal superhighways'. The latter are made up of single or multiple layers of pseudoparenchymatous cells, and are presumably required for nutrient acquisition and the delivery of effectors (molecules that promote host colonization). Subcuticular runner hyphae and stromata also give rise to asexual spores that further the pathogen's infection cycle. Strikingly, V. inaequalis is able to undergo similar dramatic changes in morphology in the absence of the host, inside cellophane membranes. We are particularly interested in understanding how V. inaequalis, through changes in its cell surface, is able to differentiate and maintain subcuticular runner hyphae and stromata during colonization of the host interior. We are also interested in determining whether cellophane membranes can act as an artificial host environment for studying these important changes. I will highlight our progress on this subject, and will give an overview of our research on V. inaequalis effector biology, with a specific focus on those effectors that activate the plant immune system (i.e. following recognition by a cognate host immune receptor). Ultimately, it is hoped that our research can be used to inform durable scab control programmes.

Session 11: Biotic Interactions

Speaker 2: Ilona Turek

News from the PUB: mechanisms of ubiquitination mediated by plant U-box E3 ubiquitin ligases and their impact on immune responses

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Ubiquitination is a prevalent post-translational modification involved in all aspects of cell physiology. It is mediated by enzymatic cascade and the E2 ubiquitin-conjugating enzymes (UBCs) lie at its heart. Even though E3 ubiquitin ligases determine the specificity of the reaction, E2s catalyze the attachment of ubiquitin and have emerged as key mediators of chain assembly. They are largely responsible for the type of linkage between ubiquitin moieties and thus, the fate endowed onto the modified substrate. However, in vivo E2-E3 pairing remains largely unexplored. We therefore interrogated the interaction selectivity between 37 Arabidopsis E2s and PUB22, a U-box type E3 ubiquitin ligase that is involved in the dampening of immune signaling. We show that whereas the U-box domain, which mediates E2 docking, is able to interact with 18 of 37 tested E2s, the substrate interacting armadillo (ARM) repeats impose a second layer of specificity, allowing the interaction with 11 E2s. In vitro activity assayed by autoubiquitination only partially recapitulated the in vivo selectivity. Moreover, in vivo pairing was modulated during the immune response; pairing with group VI UBC30 was inhibited, whereas interaction with the K63 chain-building UBC35 was increased. Functional analysis of ubc35 ubc36 mutants shows that they partially mimic pub22 pub23 pub24 enhanced activation of immune responses. Together, our work provides a framework to interrogate in vivo E2-E3 pairing and reveals a multi-tiered and dynamic E2-E3 network.

Session 11: Biotic Interactions

Speaker 3: Penelope Smith

Vacuolar iron transporter-like proteins import ferrous iron into the symbiosome in legumes

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Legumes form a symbiosis with rhizobia that convert atmospheric nitrogen (N_2) to ammonia, that is provided to the plant. A nodule is developed as part of the symbiosis and rhizobia are enclosed in the plant-derived symbiosome membrane (SM), to form the symbiosome. In exchange for fixed N, the plant provides many metabolites required by the rhizobia to fuel N fixation, among them malate and iron. As a consequence, iron is essential for an effective symbiosis. Despite the high demand for iron, the mechanism for iron transport into the symbiosome is not clear. To address this, we characterized GmVTL1 and GmVTL2, members of the vacuolar iron transporter (VIT) family, which includes *Arabidopsis* AtVIT1 and yeast CCC1 that transport Fe^{2+} into the vacuole. Since import into the symbiosome is similar to export from the cell and symbiosomes are considered to take on the role of the vacuole in cells infected with rhizobia, members of this family are excellent candidates for import of iron. Both GmVTL1 and GmVTL2 have high, nodule-enhanced expression and promoter-GUS fusions show VTL1 expression is concentrated in infected cells. Proteomic analysis identified both proteins on the SM and, when expressed in nodules, GFP-VTL1 localizes to the SM. GmVTL1, but not GmVTL2, can complement the yeast *Δccc1* mutant suggesting it functions as a Fe^{2+} transporter. The orthologue of *GmVTL1* in *Lotus japonicus* is *LjSEN1.* In *sen1* mutants symbiosome development is impaired and N fixation blocked. The function of the LjSEN1 protein has not been established but as GmVTL1 is able to replace it and restores nitrogen fixation, we suggest that both proteins are ferrous iron transporters. We conclude that VTL proteins are responsible for import of iron into the symbiosome in legumes to support symbiotic nitrogen fixation.

Session 11: Biotic Interactions

Speaker 4: Peter Ryan

Manipulating the root microbiome around wheat roots

Kawasaki, A.[1], Dennis, P.[2], Raghavandra, A.K.H[2], Forstner, C.[2], Delhaize, E.[1], Richardson, A.[1], Mathesius, U.[3], Gilliham, M.[4] and Watt, M.[5]

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The community of microorganisms around plant roots, the microbiome, is usually very differ from the bulk soil. Some microorganisms closely associated with root tissues (in the tissues or on the surface) can benefit plant growth by, for instance, preventing the proliferation of pathogens or improving mineral nutrition. These complex interactions could provide opportunities for improving the sustainability of crop productivity if the composition of the root microbiome can be manipulated in a beneficial way. To achieve this we first need to know how what a beneficial microbiome is and how to manipulate it in a predictable manner. We tested how the microbiome on wheat roots is affected by the release of simple organic anions. A transgenic rice line that releases malate from the roots and a pair of near-isogenic wheat lines that vary in citrate efflux (*Citrate-line* and *Null-line*) were grown in different soils and the bacterial and fungal communities at different positions along the length of the seminal and nodal roots were analysed. We found that the microbiome was affected by soil type (acidic or non-acidic), root type (seminal or nodal) and position on the root (tip or base). Malate and citrate efflux from the root tips also affected the microbiome composition but the effects were smaller than for the other variables. These results indicate that it is misleading to describe “the root microbiome” on a particular plant because we demonstrate that it varies with the environment, root type and with location on the root. We also demonstrate that the microbiome can be altered by the release of a simple organic compound. This information reveals further complexity of the system and helps us understand how the microbiome can be engineered in a reliable fashion.

Peter Goldacre Award

Speaker: Simon Williams

Activation and signaling by plant innate immunity receptors

Williams, S.[1]

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Dr Simon Williams utilises structural biology and protein biochemistry to study plant-microbe interactions. His research goal is to understand the structural-basis of pathogen recognition by plant immunity receptors. These large multi-domain proteins, known as NLRs (nucleotide-binding oligomerisation domain-like receptors), are capable of recognising virulence proteins from plant pathogens and activating defence pathways in the host that result in plant immunity. Simon's talk entitled "The structural-basis of plant innate immunity" will explore published works that have significantly advanced our understanding of plant immunity receptors structure and function and describe recent works originating from his recently established "plant structural immunology" laboratory at the ANU.

Speaker 1: Mao Long

Genomics improvement of wheat during polyploidization, domestication, and selection

Mao, L.
[1]

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Agriculture is an outcome of the interaction between the human and the nature with the ultimate goal to achieve the best gain in yield. Wheat as an allohexaploid evolves in a unique manner for yield increase in nature and, although, as a member of the monocotyledon family, it also follows general mechanisms during human selection. With the advance of sequencing technologies, the available of the wheat genomes, and various genomic tools, our understanding of wheat yield component is rapidly increasing although the current resolution is still quite low. In this talk, I will cover several developments in the genomics point of views that may contribute to wheat yield improvement, especially during the wheat genome polyploidization, domestication, and human selection.

Speaker 2: Allan Rattey

OMICS- Are they toys or tools for applied plant breeding?

Rattey, A., Godoy, J., Robinson, H., Moody, D., Mullan, D., Walmsley, T.

InterGrain is an Australian cereal breeding company established in 2007, with head office in Perth. It has two operational facilities (Perth, WA and Horsham, Vic) and conducts a large field testing program of over 200,000 yield plots annually. To improve the rate of genetic gain per unit investment, InterGrain is at various stages of testing, validating and upscaling technological tool kits for rapid deployment at targeted phases of barley, wheat and oat breeding. Direct InterGrain investment for improved genomic breeding will be applied via the XT bead chip, which has a significant price reduction compared to current platforms and explores sufficient genetic diversity with enhanced calling algorithms for improved imputation predictions. InterGrain are in advanced discussions regarding a framework for this genomic technology to be shared globally for greater linkage within the research community, and subsequent impact and path to market via applied breeding. Phenomics tools such as RGB drones with multispectral indexes are being validated across crops to determine if and when application is achievable and for what target traits that are valuable in applied breeding. Proteomics is also being tested in grain quality with PhD students. InterGrain is financed through End Point Royalty revenue and the business reinvests all profits back into the breeding programs and research targeting breeding improvements. We value working collaboratively with research partners and are interested in developing additional research relationships to continue the evaluation of 'omics to ensure they are more than toys and find their beneficial application in a breeding program.

Speaker 3: Tristan Coram

AGT - Advancing plant breeding through technology

Coram, T.

Australian Grain Technologies (AGT) is Australia's largest plant breeding company, with breeding programs for bread wheat, durum wheat, barley, canola and lupins. As a plant breeding research company, we get excited by new technology, innovation and scientific breakthroughs. Adopting new and novel scientific methods, or adapting new technologies to suit our objectives, helps us to continually improve and in turn, deliver greater value to Australian grain growers. Our talented and dedicated team use new mechanical solutions, robotics, computer science, GPS, tissue culture, and the latest biological and genetic theory on a daily basis. This presentation will focus on the development and application of innovative technologies in our breeding programs, such as genomic selection, high-throughput phenotyping and quality.

Speaker 4: Alex Johnson

Redesigning cereal grain for improved nutritional quality

Johnson, A. [1]

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Macronutrients and micronutrients localize to different regions of cereal grain, impacting not only on retention during milling but also their ability to be absorbed and utilized for physiological function by humans (i.e. bioavailability). Starch and protein are the main constituents of inner endosperm tissue and are readily digestible while micronutrient minerals such as iron (Fe) and zinc (Zn) accumulate primarily in the outer aleurone layer with phytic acid, polyphenols and other compounds that inhibit their bioavailability. As a result of the heterogeneous distribution of nutrients in cereal grain, milled products such as white rice and white flour are rich in dietary starch (and to a lesser extent protein) yet contain low amounts of bioavailable Fe and Zn.

We have used genetic engineering to increase biosynthesis of two endogenous metal chelators – nicotianamine and 2'-deoxymugineic acid – in bread wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.). The genetically engineered plants contain significantly increased Fe and Zn concentrations in milled grain, yield normally in multi-location field trials, and demonstrate improved Fe bioavailability in cell culture studies and animal feeding trials. We are now employing CRISPR-based genome editing to generate loss-of-function mutations in rice genes that may also increase grain Fe and Zn concentration and/or bioavailability. This presentation will describe our genetic engineering and genome editing strategies to produce Fe and Zn-enriched cereal grain.

Speaker 5: Chris Blanchard

How good are grains? – The potential health benefits of cereal and pulse consumption.

[1]*Blanchard, C.*

[1]*ARC ITTC for Functional Grains, Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, Australia.*

Grains have fallen out of favour with consumers in recent years. The poor public perception of grains has largely been driven by consumer who are concerned about consuming gluten-containing products and carbohydrates in general. The Functional Grains Centre (FGC) undertakes research projects that investigate potential health benefits of grain consumption. Some of the outcomes of FGC research includes the demonstration of a range of potential health benefits which are thought to be associated with grain-derived bioactive compounds. These potential benefits include anti-obesity, anti-cancer and anti-inflammatory properties. The FGC has studied the drivers of gluten avoidance and developed tools to breed new wheat varieties with reduced allergenicity. The effect of processing on potential health benefits of grains and grain products have also been investigated as well as new processing methods to develop healthy pulse-based snacks. FGC research has demonstrated that allergenicity of wheat has not changed or has potentially decreased over the past 150 years of wheat breeding in Australia. Also clinical studies conducted by the functional grains centre have demonstrated consumption of whole grains high in phenolic compounds may have significant health benefits for consumers. Based on this, and other similar research, it is hoped that the perception of grain consumption will change and that consumers will view grains as a healthy option.

Speaker 6: Angela Juhasz

The gene space of the 1RS/1BL translocation in wheat reveals new networks for drought stress resistance

[1]*Juhasz, A.*

The 1RS/1BL translocation is a well-established contributor to improved yield in wheat lines that carry the 1RS chromosome arm. Defining the high-resolution structure and gene complement of this chromosome 1RS.1BL has provided the basis for identifying target genes for a range of contributors to components of yield, biotic and abiotic stresses. Compared to the short arm of chromosome 1B 1RS chromosome region included significantly more gene models with expression in the grains and roots, however tissue specific enrichment in the 1RS chromosome arm was only detected in the grain tissues. Through transcriptome-based gene network analysis, multiple clusters were identified in the first 22 Mb region with genes primarily related to spike or root development and abiotic stress responses. Root-specific gene models related to root development and cell expansion encoding proteins with the domains associated with cysteine protease and cytosolic sulfotransferase were in a cluster with genes expressed more broadly. The interactions of these entities implied by co-expression include interactions on a local level within the 1RS region as well 3005 other genes (for the cysteine protease Pfam domain containing gene model) distributed across all the wheat chromosomes and covering a broad range of biological activities. Genes in a cluster that included the defensin and formin genes showed positive interactions with mitochondrial proteins expressed in the spike or during early grain development and included the 50S and 60S ribosomal proteins (TraesAK58CH1B01G010800, TraesAK58CH1B01G020200), and the mitochondrial inner membrane translocase protein TIM9 (TraesAK58CH1B01G022600). Genes with root-specific expression profiles were primarily related to specific aspects of plant growth and development as well as to abiotic and biotic stress responses. Also through transcriptome-based co-clustering with the secalin-protein coding genes, a set of transcription factors as well as other prolamin family members, histones and histone-like transcription factors directly affecting the storage protein accumulation were identified. The manual gene annotation captured some short (70-80 residue long proteins) not usually captured in automated gene annotation pipelines. The precise gene annotation of a 1RS region controlling yield and a range of abiotic stresses, including drought and heat, provides insights into genomic factors with the potential to contribute to these agronomic features of the plant.

Speaker 7: Lynne McIntyre

MAGIC and pre-breeding advances in wheat

[1]*McIntyre, L.*

Multi-parent advanced generation intercross (MAGIC) populations provide opportunities to dissect the complex genetic relationships between traits and the environment. At CSIRO we have developed several wheat MAGIC populations involving 4, 8 or 16 diverse parents. In conjunction with phenomic tools, these populations have provided significant insight into the genetic basis of traits, increased the frequency and robustness of QTL detection, increased the heritability of selection methods for major traits, provided a resource for the dissection of traits across the wheat genome, and assisted in the identification of candidate genes for traits of interest. These advances are now being used to develop elite germplasm more efficiently and effectively.

Speaker 8: Kate Howell

The wheat flour/yeast-bacteria microbiome interface in bread making to modify the aroma, crumb, nutritional and sensory properties of bread

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Yeast and bacterial communities inhabit a sourdough starter to make artisan-bread, and in this study the interactions of microorganism derived from Australian sourdough starters are shown to provide positive flavour, aroma, crumb and nutritional properties to bread. An investigation of Australian sourdough starters found that they contained *Saccharomyces cerevisiae* and *Kazachstania exigua* yeasts. When these yeasts were inoculated alone to ferment wheat flour in an extended fermentation, the bread had a heterogeneous crumb structure, a deeper colour and a distinctive chemical aroma profile than those made with commercial baker's yeast. When bread was made combining these yeasts individually and in combinations with lactic acid bacteria also isolated from these sourdough starters, including *Lactobacillus plantarum*, *L. brevis*, *L. rossiae*, *L. casei*, the bread aroma profiles and crumb structure were more distinctive, with compounds associated with *sour* aromas produced, altered crumb structures and preferred by sensory panels. The use of defined mixed cultures as the leaven in wheat bread-making, by exploiting the microbial diversity of artisan Australian starters, can produce bread with distinctive and attractive aromas, altered gluten structures and crumb properties. Diverse microbes interact with the macromolecules in dough in very specific ways to affect food quality and we argue that understanding the potential of microbes in the context of diversity in wheat flour from different wheat varieties is a new variable to be considered in achieving final product specifications.

Speaker 9: Michelle Colgrave

The role of mass spectrometry in a gluten-free-barley breeding program

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Gluten is a diverse class of proteins found in wheat, rye, barley and oats. Coeliac disease (CD) affects ~70 million people globally. When CD patients ingest gluten, it triggers an inappropriate auto-immune reaction resulting in intestinal inflammation and damage. The only current treatment is lifelong avoidance of dietary gluten; however, such diets are costly, and low in fibre, high in calories, which in themselves are health risks. The worldwide market for gluten-free products is predicted to grow by ~25% to over US$7 billion by 2022. Gluten-free foods are commonplace, however, current methodologies (ELISA) do not accurately measure gluten as the antibodies are non-specific and show cross-reactivity.

A novel ultra-low gluten (ULG) barley variety in which the hordein (gluten) content was reduced to below 5 ppm was achieved using traditional breeding strategies. Three recessive alleles, which act independently of each other, were combined to lower the hordein content in the parental varieties. By employing advanced proteomics analysis, it was possible to select the lines which showed the lowest gluten content and validate the low gluten content of the finished product.
Two LC-MS/MS approaches employing multiple reaction monitoring (MRM) and a data-independent acquisition strategy (SWATH) were used to quantify the complex protein mixtures present in nine barley varieties ranging from wild-type (gluten-containing) to ULG barley (gluten-free, < 20 ppm). The gluten peptide fragments were identified by high resolution LC-MS/MS with proteins identified from the *Poaceae* subset of proteins from Uniprot-KB supplemented by a database constructed from genomic and/or transcriptomic resources. An MRM-based approach was explored for specific protein quantification and the results were compared to those generated using variable window SWATH-MS.

The impact of using MS to support the plant breeding program was the acceleration of product development, halving the time (saving ~ 7 years) from bench to brewery.

Poster Session 1

#1: Abi S. Ghifari

Proline aminopeptidase completes the amino acid recovery in chloroplastic processing pathway

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The chloroplast requires the correct targeting and assembly of the majority of proteome from the cytosol using an amino-terminal targeting peptide. Upon import the targeting peptide is cleaved off and further degraded into single amino acids by a series of step-wise reactions catalysed by various peptidases. Recent studies in *Arabidopsis thaliana* have elucidated the essential peptidases required for targeting peptide cleavage, fragmentation, and recovery to single amino acids. Peptides undergo a multi-step degradation carried out by different enzymes, including various metallo-aminopeptidases that recover almost all amino acids. However, recovery of proline was not observed in these aminopeptidases, although observed in chloroplastic extract. Here, we report the characterisation of novel proline-specific aminopeptidases in *Arabidopsis thaliana* that are exclusively targeted to the chloroplast and has a specific activity to recover proline from short peptides. These results strongly suggest that proline aminopeptidase completes the targeting peptide processing in chloroplasts. Furthermore, to assess the biological roles of these proline aminopeptidases in plant growth and stress response, phenotyping studies of T-DNA insertional lines under normal and stress conditions will also be presented. Our results comprehend the current understanding on plant organellar peptidolytic network, especially the processing pathway and its roles in organellar homeostasis and normal plant growth.

Poster Session 1

#2: Cecilia Blomstedt

Investigation into the Molecular Regulation of Cyanogenesis in Forage Sorghum

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Sorghum bicolor produces the cyanogenic glucoside dhurrin, which breaks down to release hydrogen cyanide. Cyanogenic glucosides are traditionally thought to function primarily in defence, but recent research suggests additional roles in nitrogen storage and re-mobilisation, and mitigating oxidative stress. The synthesis of dhurrin involves two cytochrome P450s (CYP79A1, CYP71E1) and a UDP-glucosyltransferase (UGT85B1), with CYP79A1 considered rate limiting. These biosynthetic genes are known to be clustered in many, but not all cyanogenic species studied to date. In sorghum they are within 104kb on chromosome 1. Clustering of genes may have evolved to allow inheritance of these genes as a unit, avoiding toxic intermediates. It may also suggest co-regulation, possibly at the chromatin level. Dhurrin synthesis is developmentally and environmentally regulated, with potentially toxic levels present in young or stressed plants, but little is known about the molecular mechanisms involved. To investigate the molecular regulation of cyanogenesis in sorghum we have taken a number of approaches using a variety of genetic backgrounds: domesticated *Sorghum bicolor*, Australian endemic wild crop relatives, and EMS mutants. To investigate chromatin level regulation, the chemical 5-Azacytidine was used to assess *S. bicolor's* response to genome-wide demethylation. In addition, the methylation status of the region surrounding a CΔT mutation present in the *CYP79A1* promoter was determined in wild-type and mutant plants over two stages of development. Yeast-one-hybrid experiments have identified potential transcription factors regulating *CYP79A1*. Investigation of the biosynthetic genes of wild species suggest that cyanogenic glucosides are primarily used for storage of nitrogen in harsh environments, not defence. Our results indicate that the regulatory mechanisms involved in dhurrin synthesis differ between the shoot and the root. Understanding how cyanogenic glucosides are regulated is important to enable the prediction and control of potential toxicity in plant species important for human and animal consumption.

Poster Session 1

#3: Dayton Christopher Bird

Identify key miRNAs in the barley inflorescence development through small RNA sequencing

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MicroRNAs, small molecule RNAs, 18–24 nucleotides in length, are known to be fundamental regulators of gene expression at transcriptional and/or posttranscriptional levels that play key roles in plant reproductive development; including, inflorescence architecture, flower organ identity, flowering time, and the important yield components in crops. Even though a number of microRNAs have been identified that function during reproductive development in model plants such as *Arabidopsis thaliana* and rice (*Oryza sativa*), the genome-wide information and regulatory function of microRNAs in the inflorescence development of barley (*Hordeum vulgare*), one of the most important crops, remain unclear. To characterize the potential miRNAs involved in barley reproduction, three important stages of inflorescence development including spikelet initiation, floral organ differentiation and floral organ growth, samples at four different stages including seedling (vegetative control), double ridge, awn primordium, and green anther stages of inflorescence meristems in barely were collected for small RNA extraction and global sequencing. A total of 91,926,979 clean reads (18-44 nt) were obtained from four small RNA libraries, 43 known miRNAs and 153 novel miRNAs were detected. To investigate the function of known miRNAs differentially expressed during inflorescence development, GO analysis of predicted target genes by *in silico* analysis revealed that most of them are involved in cell division, anther development, brassinosteroid signalling pathway, and lignin biosynthesis process. Based on the differential expression pattern of miRNAs and predicted target genes in barley, *miR159* targeted *MYB* and *miR397* targeted *Laccase* gene, showed tissue- specificity pattern as their expression showed the highest peak in the awn primordium and green anther stage indicating that these miRNAs might play a key role in the inflorescence development and tissue-specificity including spike and spikelet formation in barley. Thus, our findings provide the insights of post-transcriptional regulatory mechanisms that might be exploited for fundamental understanding of inflorescence morphogenesis and crop improvement.

Poster Session 1

#4: Leilasadat Asadyar

Composition of Leaf Cuticular Wax from Banana Genotypes

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A decline in fossil fuel reserves and increases in carbon emissions are key drivers for bio-based fuels and chemicals. Plant cells produce cuticular waxes to protect against water loss, pathogens and insects. Due to their similarity to many petrochemical derivatives, demand for such bio-based waxes has increased in the past decade in applications such as lubricants and biofuels. However, the Brazilian palm tree (*Copernicia prunifera*) is one of only a few plants that produce economically viable amounts of wax (*i.e.,* carnauba wax). Bananas and plantains are the most consumed and traded fruits in the world with a worldwide production of over ~145 million tons per year. This results in ~300 million tons of plant waste and little consideration is given to utilising it efficiently. In the present study, we extracted and analysed wax from leaves of 16 different banana genotypes to investigate banana plant waste as a valuable resource. Using a range of analytical tools we report that banana leaf wax has physical properties comparable to carnauba wax. Furthermore, using chromatographic and mass spectrometric techniques, we identified and characterized long chain esters and saturated alkanes in banana leaf wax. Based on the similarities to commercially available waxes, we conclude that banana leaf wax has great potential in the production of biofuels and high value chemicals.

Poster Session 1

#6: Oliver Berkowitz

Cell-specific transcriptomic analysis of the stress responses to mitochondrial dysfunction using laser capture microdissection

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Plant growth and development is greatly affected by biotic and abiotic environmental stresses, which are responsible for major yield loss in agriculture. As the major energy generating organelle, mitochondria generate most of the cell's supply of ATP which is used as chemical energy to drive all of the energy-requiring processes including transport, synthesis and degradation of cellular components, signalling, defence, growth, and reproduction. Their energy metabolism has to be flexible and accommodate the changing requirements of the cell. Hence, mitochondria are in a prime position to sense cellular dysfunction when the plant is exposed to adverse growth conditions. The disturbance of mitochondrial function by stresses can induce specific signals which are transmitted to the nucleus and activate a transcriptional stress response. This mitochondria-to-nucleus communication is referred to as mitochondrial retrograde regulation. Identification of the underlying signalling pathways and their components is thus important to the understanding of a plant's ability to acclimate to stresses. Traditional molecular studies depended on the analysis on the whole plant or at organ levels. However, the cellular composition varies greatly between the different cell-types of plants, which will also impact mitochondrial functions.

We have performed laser capture microdissection on leaves of Arabidopsis wild type plants and mitochondrial signalling mutants treated with the inhibitor of mitochondrial electron transport antimycin A. Subsequent analysis by RNA-seq established cell-type specific differences in the transcriptomic response to mitochondrial dysfunction. This revealed key mitochondrial signalling–dependent processes on a spatially highly-resolved scale. Results contribute to our understanding of stress tolerance mechanisms and may help in improving the capacity of plant responses to environmental stresses. Knowledge obtained from this project will be beneficial to gaining a sustainable increase of crop yields in agricultural production.

Poster Session 1

#7: Thi Thanh Mai Nguyen

Correlation between key transcription factors controlling lignin biosynthesis and the lignification pattern within an elongating stem internode of Setaria.

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Lignocellulosic biomass from C_4 bioenergy crops, predominantly grasses, has emerged as a potential source of renewable energy. Foxtail millet (*Setaria italica*) and its wild ancestor, green foxtail (*Setaria viridis*), have been proposed as novel model species for undertaking functional genomics of C_4 grasses. Lignin, a principal component of plant biomass, poses a significant challenge to enzymatic digestibility during the process of biofuel production. The RNA-Seq investigation of the fifth elongating stem internode of *S. viridis* has revealed a cohort of regulatory genes likely to be involved in controlling lignin biosynthesis, with the most highly upregulated transcription factors (TFs) belonging to the MYB and NAC domain gene families. In particular, *MYB42*, *MYB59-like*, *NAC73* and *NAC63* were expressed most highly in the transitional (TZ) and maturation zones (MZ) of the elongating internode where lignin deposition occurs. Confirmation of the expression profile of the candidate TFs was sought in *S. italica*, with five accessions exhibiting a similar phenotype but predicted to possess differences in cell wall (CW) lignin content, selected for histological examination of stem anatomy and lignin content analysis. The correlation between TF expression profile measured by quantitative RT-PCR, lignification pattern within the stem CW and total lignin content will be reported.

Poster Session 1

#8: Aaron Elkins

Chemotyping of Medicinal Cannabis Phytocannabinoids

Elkins, A.C.[1], Rochfort, S.J.[1,2], Vincent, D.[1], Ezernieks, V.[1], Cogan, N.O.I.[1,2] and Spangenberg, G.C.[1,2]

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Cannabis is an herbaceous flowering plant of the Cannabis genus (Rosales) that has been used for its fibre and medicinal properties for thousands of years. In recent decades medicinal cannabis has become legal in several jurisdictions and the possibility of legalisation is being explored in many more. This legalisation has opened the field of medicinal cannabis research. The biochemistry of Cannabis is complex including phytocannabinoids, terpenes and phenolics. Each of these classes have biologically active compounds that contribute to the medical efficacy of Cannabis. The most abundant of these classes are the phytocannabinoids derived from the glandular trichomes of the flowering heads of the female plant. We have undertaken the targeted and untargeted chemotypic analysis of 70 diverse cannabis strains using liquid chromatography mass spectrometry (LCMS) to characterise the major and minor cannabinoids. Multivariate hierarchical clustering has been employed to assess similarities and differences between the strains. This provides an opportunity to target strains with specific chemotypic profiles to be used in the treatment of specific medical conditions.

Poster Session 1

#9: Bec Baillie

Cannabis Genomics for Diversity, Traceability and Regulatory Compliance

Baillie, R.C.[1], Braich, S.[1,2], Spangenberg, G.C.[1,2] and Cogan, N.O.I.[1,2]

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Cannabis produces a range of medicinal compounds that are beneficial for a variety of ailments. It is a flowering herb of the Cannabaceae and has a dioecious breeding system with female plants generating the highest floral bud and resin yield, although monoecious plants do exist. Since its legalisation in several jurisdictions, Cannabis research is one of the fastest moving fields in plant medical science, providing the capacity to supply and treat patients with approved medicinal cannabis products of reliable quality and known composition. Therefore, reliable traceability techniques, based on genomic tools for compliance requirements are paramount. In addition, to increase improvements in commercial strains, fast and reliable screening techniques for gender assignment and basic chemotypic profiling are essential for rapid plant selection. A PCR screen of immature seedlings can determine gender and initial chemotypic ratios of THC and CBD. This initial screen enables the immediate selection of desirable plants for the target breeding outcome. These PCR tools have also been evaluated for traceability requirements to support law enforcement agencies. Comprehensive genomic resources have been developed through extensive whole genome resequencing of over 600 diverse genotypes of Cannabis. The genotypes were selected to represent a broad range of chemotypes, morphology and origins. The genomic resources have been analysed for sequence variants and specific profiles for each plant genotype have been identified. These sequences are being used to develop genomic diagnostic tools to identify specific strains and fingerprint the proprietary strain collection.

Poster Session 1

#10: Christian Krill

Volatile Profiling of Medicinal Cannabis

Krill, C.[1], Ezernieks, V.[1], Elkins, A.C.[1], Vincent, D.[1], Rochfort, S.J.[1,2] and Spangenberg, G.C.[1,2]

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Cannabis is an herbaceous flowering plant of the *Cannabis* genus (Rosales) that has been used for its fibre and medicinal properties for thousands of years. In recent decades medicinal cannabis has become legal in several jurisdictions and the possibility of legalisation is being explored in many more. This legalisation has opened and stimulated the field of medicinal cannabis research. The biochemistry of cannabis is rich and varied including phytocannabinoids, terpenes and phenolics. Each of these metabolite classes contains individual compounds with biological activity. This chemical diversity and the interaction between molecules may underpin the 'entourage effect' that is believed to contribute to the medical efficacy of medicinal cannabis. Moreover, the volatiles produced by cannabis can directly impact patients based on their taste and smell. As part of a larger exploration into the biochemistry of medicinal cannabis, we have evaluated various extraction strategies and GC-MS analysis methodologies on milled dried biomass. Using optimised extraction techniques and quantitative GC-MS analysis, we profiled the volatolome of numerous plants from a variety of cannabis strains and achieved absolute quantification of significant volatiles. When coupled to genomics and proteomics data, these findings enable genomic selection strategies to breed for chemotypic specific strains. The data is also being used to annotate genes and gene pathways for the different molecular species.

Poster Session 1

#11: Delphine Vincent

Bottom-up, Middle-down and Top-down Proteomics of Medicinal Cannabis

Vincent, D.[1], Ezernieks, V.[1], Rochfort, S.J.[1,2] and Spangenberg, G.C.[1,2]

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Medicinal cannabis is used to relieve the symptoms of certain medical conditions, such as epilepsy. Cannabis is a controlled substance and until recently was illegal in many jurisdictions. Consequently, the study of this plant has been restricted. Proteomics studies on *Cannabis sativa* reported so far have been primarily based on plant organs and tissues other than buds, such as roots, hypocotyl, leaves, hemp seeds, and flour. As far as we know, no optimisation of protein extraction or enzymatic digestion from cannabis reproductive tissues has been attempted. Therefore, we set out to optimise methods for bottom-up, middle-down and top-down proteomics to recover, separate and identify the proteins of the reproductive organs of medicinal cannabis, apical buds and isolated trichomes. In this study, we first optimised protein extraction from medicinal cannabis apical buds and trichomes exploring six methods for top-down analyses by UPLC-MS. We then optimised protein digestion on the highest yielding protein extract by testing four endoproteases independently or combined for middle-down and bottom-up analysis by nLC-MS/MS. The most efficient methods will be applied to the comparison of different Cannabis strains.

Poster Session 1

#12: Doris Ram

Near InfraRed Spectroscopy For Cannabinoid Quantitation

Ram, D.[1], Rochfort, S.J.[1,2], Tran, J.[1], Elkins, A.C.[1] and Spangenberg, G.C.[1,2]

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Cannabis is usually a dioecious plant of the Cannabaceae family. It is an ancient plant utilised over millenia for medicinal, recreational, religious as well as for biofuel, food, paper and textile purposes. Cannabis has a rich and complex biochemistry. At least 113 different cannabinoids have been identified and isolated from various cannabis strains. Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two most researched and generally present at the highest levels compared to the other cannabinoids. Different cannabinoids have different therapeutic potential hence it is important to screen and select cannabis strains with optimum individual cannabinoid ratios, for targeted therapeutic applications. Commercially-sold cannabis products must be standardised for product quality assurance in terms of safety, consistency and potency for end users. Chromatography methods are generally viewed as a gold standard of analysis for major, minor and trace constituent cannabinoids. However, these methods are often time consuming and expensive, while non-destructive methods such as near-infrared spectroscopy (NIR) are rapid, cost effective, solvent-less and have the potential to be deployed beyond the laboratory. We have developed NIR technology for cannabis strain typing (high or low THC) and cannabinoid quantification. In this study, a Bruker MPAII spectrophotometer was used to collect NIR spectral data from ground bud material of 65 cannabis strains. Algorithms were developed and chemotypic clustering of the NIR data identified different cannabis strains and accurately predicted cannabinoid content for a range of cannabinoids including, for the first time, both the acid and neutral forms. A portable hand held, miniature NIR probe was also tested on the same material and new equations developed allowing the prediction of certain cannabinoids with good reliability. NIR has the potential for rapid chemotyping of large-scale breeding populations, in field/in glasshouse crop monitoring and as a rapid testing tool for regulatory authorities.

Poster Session 1

#13: Erez Naim-Feil

Medicinal Cannabis: Systems Biology Approaches to Cultivation and Precision Breeding

Naim-Feil, E.[1,2], Spangenberg, G.C.[1,2], Rochfort, S.J.[1,2] and Cogan, N.O.I.[1,2]

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Cannabis sativa L. (Cannabis), is a primaeval domesticated crop dispersed by humans across the globe and commonly cultivated for its fibre and oil, as hemp, as well as for its psychoactive chemicals. Drug-type strains were selected for their prolific resin productivity and contain up to 120 cannabinoids, that for millennia have been in use for their medicinal benefits. However, due to the drug-type classification and its inebriant effect, Cannabis was prohibited in most countries during the last century. This prohibition has led to cultivation and breeding initiatives being conducted under clandestine conditions, while scientific development of the crop ceased. Recently, the potential of medicinal cannabis has been reacknowledged and the generation of scientifically-characterised Cannabis strains for medicinal applications is under high demand. The application of a comprehensive systems biology approach to the improvement of medicinal cannabis is therefore critically needed. Targeted plant evaluation has been performed with over 500 genotypes being grown and extensively measured. This in-depth characterisation includes quantification of common secondary metabolites (cannabinoids and terpenoids) and evaluating the plants physiological (yield components, plant height, internodes, stem diameter, bud evaluation) and phenological (precocity) performance. In addition, each of the individual strain's genome sequence has been generated to enable the development of genomic selection predictions. The application of a rigorous scientific basis to accelerated precision breeding in medicinal cannabis will enable the generation and production of tailored strains for a range of applications.

Poster Session 1

#14: Jonathon Tran

CO_2 Supercritical Fluid Extraction of Medicinal Cannabis

Tran, J.[1], Rochfort, S.J.[1,2], Isbel, A.[3], Ezernieks, V.[1], Elkins, A.C.[1], Vincent, D.[1], Deseo, M.[1] and Spangenberg, G.C.[1,2].

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Medicinal cannabis is gaining popularity as a treatment option for many different conditions. Cannabis can be administered as a medicine by being smoked, vaped or consumed in the form of oils or other edibles. However, dose variability is a potential issue particularly with vaping and smoking due to the different methods of inhalation and plant variation making it difficult to control the amount of active compound administered. Cannabis oil extracts are a better alternative as the dose administered to the patient is quantifiable and consistent. CO_2 supercritical fluid extraction extracts the resin from cannabis biomass to provide a concentrated mix of cannabinoids suited for patient consumption. The resin is then diluted to specific concentrations in an oil for patient use. Alcohol extraction and hydrocarbon extraction to obtain the resin are possible, but are often associated with more unwanted by-products, inherent safety risks and additional purification steps to remove the extraction solvents from the resin. This makes the use of CO_2 as an extraction solvent the better alternative as there are no toxicity, flammability or environmental risks. We have fully developed and validated a method for the supercritical fluid extraction of medicinal cannabis ensuring a high-quality product for medicinal purposes. Factors such as extraction time, extraction pressure and CO_2 flow rate have been investigated as the major factors that influence yield and recovery of cannabinoids.

Poster Session 1

#15: Lennon Matchett-Oates

Informed sgRNA Design for Genome Editing in Medicinal Cannabis

Matchett-Oates, L.[1,2], Braich, S.[1,2], Rochfort, S.J.[1,2], Spangenberg, G.C.[1,2] and Cogan, N.O.I.[1,2]

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Cannabis has been used for industrial, recreational and medicinal use for in excess of 4,000 years. However, to date no successful attempts at editing genes involved in cannabinoid biosynthesis have been reported. The development of a best practices approach for the design and implementation of genome editing technologies in cannabis to target all genes involved in cannabinoid biosynthesis is needed. A large population of reference genomes were sequenced and mined to determine copy number variation and associated SNP variants for optimum target edit sites for genotype independent editing. Copy number variation and highly polymorphic gene sequences exist across specific genes in the genome making genome editing using CRISPR and Zinc Fingers more difficult. Evaluation of alleles and gene copy number variants was determined through nucleotide and amino acid sequence alignments with comparative analysis performed. From the determined gene copy number and the presence and location of SNPs, multiple CRISPR design tools were used to design sgRNA targeting every gene, accompanying allele and homologs throughout all involved pathways. The generated catalogue of constructs will enable a wide array of knockouts to be created for functional genomics studies. Universal sgRNA were designed for highly homologous sequences using MultiTargeter and visualised using Sequencher, creating unique sgRNA avoiding SNP and shared nucleotide locations targeting optimal edit sites. Using this framework, the approach established has wider applications to plant species with a range of ploidy level or highly homologous gene sequences.

Poster Session 1

#16: Meng-Han Lee

In planta discovery of novel cyclotide biosynthesis machinery

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Cyclotides are circular peptides produced naturally in plants as defence agents against plant pathogens. It is estimated that more than 150,000 unique cyclotides could exist across five major plant families; Rubiaceae, Violaceae, Fabaceae, Cucurbitaceae and Solanaceae. Given their diversity and structural stability, there is considerable interest in how cyclotides are produced, with potential applications in agriculture and therapeutic drug design. The stability of cyclotides is largely due to their unique cyclized backbone which is further strengthened by a knotted-like arrangement of three disulfide bonds. For maturation, cyclotides undergo post-translational processing, whereby the peptide is released from a larger precursor protein by the concerted action of resident proteases. In the final backbone cyclisation step, a special class of proteases termed asparaginyl endopeptidases have been implemented. However, gaps in knowledge remain on how and where this protease processing occurs, and on the importance of chaperone proteins on providing correct disulfide bond arrangements. In this work, we address these gaps by using the recently established BioID technology to proteomically map the *in vivo* protein-protein interactions of kalata B1 (kB1) in *Nicotiana benthamiana* (non-cyclotide producer) and *Petunia hybrida* (cyclotide producer). Early results have revealed that expressing BioID conjugated to the cyclotide precursor protein of kB1 still allows maturation and cyclisation of kB1. Future work will now focus on identifying the *in planta* interacting proteins with the ultimate goal of unravelling how cyclotides biosynthesis is controlled in both producer and non-native producer species. Anticipated results will allow further engineering of this fascinating biosynthetic pathway to develop plants as biofactories for therapeutic or agricultural relevant peptides.

#17: Noel Cogan

Generation of a Comprehensive Transcriptome Atlas and Transcriptome Dynamics in Medicinal Cannabis

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Cannabinoids are the main medicinal compounds of interest in the plant *Cannabis sativa*, that are primarily synthesised in the glandular trichomes; found on female floral buds. The content, composition and yield of secondary metabolites (cannabinoids and terpenoids) is influenced by the plant's genetics and environment. Some initial gene expression experiments have been performed from strains of this plant species that contrasted in cannabinoid production, however the present knowledge about detailed trichome transcriptomics in this species is limited. An extensive transcriptome atlas was generated by RNA sequencing using root, shoot, flower and trichome tissues from a female plant strain (Cannbio-2) and was enhanced with the addition of vegetative and reproductive tissues from a male Cannabis plant. Differential gene expression analysis identified genes preferentially expressed in different tissues. Detailed trichomics was performed from extractions specifically from glandular trichomes as well as female floral tissues at varying developmental stages, to identify stage-specific differentially expressed genes. Candidate genes involved in terpene and cannabinoid synthesis were identified and the majority were found to have an abundant expression in trichomes. The comprehensive transcriptome is a significant resource in Cannabis for further research of functional genomics to improve the yield of specialised metabolites with high pharmacological value.

Poster Session 1

#18: Shivi Braich

Reference Genome and Whole Genome Resequencing in Medicinal Cannabis for Genomic Selection and Genome Editing Enabling Accelerated Precision Breeding

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The legal use of medicinal cannabis has increased rapidly, however Cannabis is the only crop of significant dollar value that has largely evaded recent technology advances in agriculture in terms of accelerated precision breeding. Three *de novo* reference genome sequence assemblies of *Cannabis sativa* have been generated, using long-read sequencing technology. The genome sequences have been assembled and compared to other available genome data sets for the species. The structure and order of the genome sequences have been harmonised in order to represent and deliver an integrated structured resource. These genome sequences have also been targeted to cover a broad range of the diversity within the species as well as encompass both male and female plant types. The creation of these genomic resources are enabling detailed comparative genomics as well as permitting a pan-genome analysis, to identify structural variants within the genome. In addition, a collection of >600 individual genotypes of Cannabis have been whole genome resequenced at varying depths, enabling SNP identification across the genome. This work supports genomic selection and predictive breeding through the development of a reference population with ordered variants, that is complemented by detailed chemotypic data. The rapid implementation of genomic selection approaches as well as genome editing for the generation of novel designer strains of Cannabis will deliver significant benefit to the industry and enable tailored chemotypic profiles to be realised.

Poster Session 1

#19: Denise Fernando

Response to inundation of black box trees on two disjunct Murray River floodplains: a nutritional perspective

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Black box (*Eucalyptus largiflorens* (Myrtaceae)) trees are widespread across outer floodplains and inland lake systems of the Murray Darling Basin (MDB), Australia, where they are reliant on occasional flooding. This ecologically important species is endemic to the MDB, occurring on a range of soil types. Its unhealthy state in sections of the Basin is linked to ecological imbalances arising from and exacerbated by climate change and river water removal. Knowledge about black box nutrition relative to flooding in its varied ecosystems is virtually absent. This field study examined nutritional response to flooding by interrogating nutritional data for young and mature leaves gathered before, during and after major flood at each of two disjunct Murray River floodplains with differing soil types. Eight samplings were done during two separate one-year periods, i.e., at six sites at Chowilla, South Australia; and at three sites further upstream at Hattah, Victoria. Young and mature leaf data were compared before and after flooding, and within and between floodplains using standard multi-variate analyses to consider an array of twelve nutrients and aluminium (Al). Overall, nitrogen (N) and sulphur (S) varied least through time and between floodplains, whereas manganese (Mn) and Al varied most. Young and mature leaves differed significantly between floodplains, but not within floodplains, where differences were marginal for Chowilla and insignificant for Hattah. At Chowilla, flooding modified the nutritional difference between young and mature leaves at sites with differing flood histories, with calcium (Ca) contributing more to the difference post-flooding than it did pre-flooding. The variable effect of flooding on the nutritional dynamics of black box is tentatively attributed to differing flood histories interacting with differences in floodplain soils. Data analyses preliminarily suggest that the nutritional effects of flooding on black box trees varies with soil type.

Poster Session 1

#20: Krzysztof Piotrowski

Fluorescence of chlorophyll as a tool for assessing the vigor of Lemnaceae plants grown on digestate from biogas plant in Piaszczyna (Poland)

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High demand for energy, prospect of the exhaustion of conventional energy sources and growing ecological awareness of societies are important reasons for the dynamic development of alternative energy sources. Production of renewable energy from agricultural biogas allows Poland to comply with highly restrictive EU directives and the Climate and Energy Package assuming an increase in the share of renewable energy sources in final energy consumption to at least the level of 15% in 2020 and a further increase in this indicator in subsequent years (to 20% in 2030). The energy management model based on the development of renewable sources is implemented differently in EU Member States and in the world. Modern biogas plants are among the most promising renewable energy industries. The largest European biogas market is Germany, which has over 10,000 biogas plants and produces almost 50% of all biogas in the European Union. However, with the development of biogas technologies, problems have arisen with the management of waste resulting from biogas fermentation. Utilization of post-fermentation leachates in in vitro culture of Lemnaceae macrophytes is one of the solutions . The purpose of this work is to define a simple and economically justified method of monitoring the vigor of Lemnaceae plants grown on the leachate from biogas plants using the latest techniques for analyzing chlorophyll fluorescence.

The experiment was conducted in laboratory conditions on a model Lemnaceae plant grown in vitro at the Faculty of Biology and Environmental Protection of the University of Lodz. The plants were grown on a diversified culture medium including: standard "Z" medium, tap water and digestate from biogas plant in Piaszczyna. Plant cultivation was carried out under phytotron conditions at 24°C. After 10 days of cultivation, analyses of plant growth, as well as physico-chemical parameters, i.e. the index of chlorophyll content, gas exchange parameters, RNAse enzymatic activity and acid and alkaline phosphatase were performed and important physicochemical parameters of the medium were determined: COD, PO43- phosphates, NO3- nitrates, pH. On the last day of the experiment, chlorophyll fluorescence was determined in macrophyte shoot members using a specialized Handy PEA fluorimeter (Hansatech Instruments Ltd.), which recorded nearly 70 physiological parameters. The obtained data were analyzed by Pocket PEA Plus V1.10 software (from Hansatech Instruments Ltd.).

The conducted research revealed that a significant increase in the signal of maximum chlorophyll fluorescence (Fm/Fv) was associated with an increased load of nutrients in the medium supplemented with the digestate from the biogas plant in Piaszczyna (Poland). Increased maximum fluorescence values were observed in the variants characterized by optimal growth of Lemnaceae plants. The undertaken research direction is in line with modern trends in conducting research on the physicochemical state of macrophytes, including the evaluation of chlorophyll fluorescence. This research may contribute to significant simplification of vigor monitoring of Lemnaceae and other plants grown on digestate from biogas plants.

#21: Martin O'Brien

Investigating the Effects of CRISPR/Cas9-mediated Knockout of the Vacuolar Iron Transporter Genes in Rice

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Iron (Fe) is an essential co-factor in enzymatic reactions such as DNA replication, oxygen transport, and respiratory and photosynthetic electron transfer chains, yet its deficiency and/or excess is damaging to cells. Vacuolar iron transporters (VIT) help to maintain Fe homeostasis in plant cells by transporting cytoplasmic Fe into vacuoles. In rice, the OsVIT1 and OsVIT2 proteins play a major role in sequestering Fe into vacuoles of the flag leaf. In this study, we have used *Agrobacterium*-mediated transformation of rice cv. Nipponbare with CRISPR/Cas9 plasmids targeting the *OsVIT1* and *OsVIT2* genes to generate a collection of *osvit1* and *osvit2* mutants. Eleven and fifteen independent mutant alleles were generated for *OsVIT1* and *OsVIT2*, respectively. We present molecular and phenotypic analyses of two homozygous *osvit1* and three homozygous *osvit2* alleles, as well as their respective null segregants, and demonstrate loss-of-function of the *osvit1* and *osvit2* alleles using a yeast complementation assay. We also report on grain Fe concentration and agro-morphological performance of the mutant and null segregant plants grown in two different environments.

#22: Pornpun Yanaso

Genotypic variation in silicon accumulation capacity in paddy rice (Oryza sativa L.)

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Rice (*Oryza sativa* L.) genotypes have high capacity to accumulate silicon (Si), but the extent of genotypic variability in Si accumulation in paddy rice is unclear. This study aimed to characterize the capacity of paddy rice genotypes to accumulate Si, and to assess the effects of Si supply on Si accumulation and growth of paddy rice genotypes. A solution culture experiment in a completely randomized design with three replicates was conducted to characterize the capacity of 27 paddy rice genotypes to accumulate Si from the nutrient solution (1.50 mM Si) at 3 and 9 hours. The results indicated that the accumulation of Si from solution culture varied among the genotypes. Two high-Si accumulating genotypes; Somewake and Calrose, and two low-Si accumulation; Langi and YRL38 were selected and subsequently further studied under flooded soil culture supplemented with 0 and 0.48 g Si added kg^{-1} soil. The experiment was arranged in a completely randomized block design with three replicates. The Si application had a larger impact on the high-Si in comparison to low-Si accumulating genotypes, with a greater increase in growth and shoot Si accumulation in Calrose than YRL38, when treated with Si. Additionally, large root biomass and long root length influenced an increase in shoot Si accumulation in high-Si genotypes.

#23: Ricarda Jost

SPX4 - a phosphate sensor that integrates information on plant nutrient status with development

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Phosphorus and nitrogen are essential macro-nutrients that limit plant growth. The interplay between the two is intriguing, given that external nitrate supply promotes nitrogen uptake, while external phosphate supply suppresses phosphorus uptake by roots. This is one of the main causes of why crops only take up about 30 % of the phosphorus fertilizer applied by farmers. The SPX4 protein consists of a single SYG1/Pho81/XPR1 (SPX) domain, which can bind to inositol polyphosphates as a read-out of plant phosphorus status. Inositol polyphosphate-bound SPX4 interacts with transcription factors, such as the master regulator of the transcriptional phosphate starvation response, MYB transcription factor AtPHR1 / OsPHR2, or OsNLP3 which is involved in nitrate signal transduction. SPX4 binding prevents these transcription factors from entering the nucleus. It therefore acts as a negative regulator of downstream signalling. Phosphate limitation promotes ubiquitin-proteasome-dependent SPX4 turn-over, which releases PHR1 into the nucleus. The overall aim of this study is to alleviate SPX4's negative effect on phosphate uptake, without affecting overall nutrient homeostasis and growth.

Using the luciferase reporter we analyze *in vivo* SPX4 turnover. Using SPX4-RFP and PHR1-GFP fusion proteins in a custom-made nutrient-response Arabidopsis protoplast assay, we show cytosolic retention of PHR1 by SPX4 is both dose- and P-status dependent. In-depth physiological and transcriptome analyses of *spx4* and *phr1* T-DNA insertion mutants reveal that SPX4 is not only acting upstream of PHR1, but also upstream of transcription factors that are involved in plant development, such as ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN55 and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1. Furthermore, we can demonstrate that regulatory networks associated with SPX4 change during seedling development. Consequences of these findings for improving phosphorus efficiency in crops will be discussed.

Poster Session 1

#24: Ritushree Jain

Conserved and accession specific transcriptional responses of Arabidopsis thaliana under limited phosphorous availability

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Phosphorus (P) is an essential macronutrient for plant growth, development, and cellular responses. Limited availability of Pi (inorganic phosphate) in soil triggers phosphorus starvation response (PSR) in plants, characterised by morphological alterations in root structure architecture (RSA), transcriptional activation of phosphorus starvation induced (PSI) genes, and metabolic changes crucial for increased acquisition, translocation and remobilisation of Pi. Combined these responses optimise growth under stress, but still limited Pi results in a significant growth reduction in plants. Naturally occurring *Arabidopsis thaliana* accessions show varying degrees of PSR. We have identified eight accessions that exhibit tolerance and six that are sensitive towards Pi limitation based on contrasting shoot and root biomass ratios of seedlings in Pi-limited versus Pi-replete conditions. By conducting a meta-analysis of Phosphorus starvation studies we have defined core PSR genes in roots and shoots of *Arabidopsis thaliana* (Col-0). A component of the core PSR is conserved in all accessions irrespective of their sensitivity towards Pi limitation along with novel PSR genes which are specific to each accession. Transcriptomic profiling of these accession in Pi-limited and Pi-replete conditions accompanied with Weighted Gene Co-expression Network Analysis (WGCNA) identified presence of multiple molecular strategies of combating Pi limitation. PSR genes in tolerant accessions were overall less responsive to Pi depletion compared to sensitive accessions. Furthermore, the genes in two PSR-associated modules identified by WGCNA are primed for higher Pi starvation tolerance in tolerant accessions. The transcription factor analysis using WGCNA module hub genes identified known and novel transcription factors that are potentially involved in Pi homeostasis.

Poster Session 1

#25: Tannaz Zare

Tissue Specific Lipid Profiling of Two Commercial Chia Genotypes using High Performance Liquid Chromatography Techniques

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Chia (*Salvia hispanica* L.) is a superfood rich in omega-3 fatty acids that provide numerous human health benefits such as reduced risk of cardiovascular disease. Until now the Chia lipidome and composition of lipids in different Chia tissues has not been described. This study aimed to explore and quantify the lipid composition of various tissues of black and white commercial genotypes of Chia. The lipidomics analysis employed Triple Quadrupole Time of Flight mass spectrometer with high precision. A Liquid Chromatography Triple Quadrupole mass spectrometer along with multiple reaction monitoring was utilised to perform untargeted and targeted quantitative analysis, respectively. Here we present the first comparative analysis of tissue-specific lipid profiles of different Chia genotypes. In an initial experiment we detected more than 8,000 lipid species using an untargeted lipidomics approach. The analysis revealed significant differences between lipid profiles of both genotypes. Using principle component analysis, we determined that lipid species are present at different levels between the two genotypes in all tissues except seeds. The targeted analysis revealed the presence of two major lipid families of glycerophospholipids and glycerolipids in various tissues of both genotypes. The concentrations of phosphatidylcholines and phosphatidylserines in young leaves and upper stem were similar in both genotypes; whilst, in old leaves and lower stems they were significantly higher in the white Chia and phosphatidylcholines were highly present in the roots of the black Chia. Both genotypes contained a high amount of phosphatidylethanolamine in their leaves and stem tissues. The concentration of phosphatidylinositol in leaves and upper stems were higher in the white genotype. The response intensities of triacylglycerol in leaves and stems of both genotypes were similar; whilst in roots they were higher in the white genotype. In addition, the amount of diacylglycerol in young leaves and stems of the black Chia and in old leaves and roots of the white Chia were higher compared to other tissues.

#26: Zdzislawa Romanowska-Duda

Post-fermentation waste from biogas plants - a natural fertilizer in plant cultivation. Circular economy

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Utilization of intensively increasing amounts of plant biomass post-fermentation waste from biogas plants in sustainable agriculture and circular economy requires knowledge of their composition and development of ecological methods to maximize their fertilizing efficiency. The aim of research was to investigate the composition of biogas plant waste in Piaszczyna (Poland) and their application for plant growth.

The results show the novel information concerning a different composition of biogas plant waste and its positive impact on medium and soil properties and also on *Lemnaceae*, algae and sorghum development. The plant waste (1-20% in the medium and preferably 24-48 q ha^{-1}in soil), depending on the origin, increased the medium and soil fertility as well as development of *Lemnaceae*, algae and sorghum biomass. These improvements were proportionally to biogas plant waste doses and increased fluorescence and chlorophyll content in leaves, net photosynthesis, transpiration, stomatal conductivity, activity of alkaline and acid phosphatase, RNase and dehydrogenase and energy properties of biomass.

Supplementation of soil and production of media based on biogas plant waste increased growth and physiological properties of plants, as compared to chemical fertilizers and commercial media.
The used concentrations of waste caused a enhancements in sorghum, *Lemnaceae*, algae yields and improvement of their physiological properties and the studies indicated that the studied waste can be a new valuable fertilizer in the circular economy and sustainable agriculture.

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Poster Session 1

#27: Zeenat Rupawalla

Development of high-throughput process to optimise microbes for sustainable biofertiliser applications.

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The global economy is valued at US$127 Tn pa (2017 est.) and the agricultural sector contributes 6.4% of that at US$8.1 Tn pa (CIA, 2017). By 2050, 70% more food will be needed to support the population increase from 7.6 to 9.8 billion people (CIA, 2017). Therefore, enhancement of sustainable agricultural practices is increasingly important towards attaining a range of UN Sustainable Development Goals (eg. No Poverty, Zero Hunger, Clean Water and Sanitation). Several UN SDG's are aligned with the planetary boundaries (eg. Nitrogen and Phosphorus flows, Ocean Acidification, Land System Change, Climate change, biodiversity conservation and global fresh water consumption) (UN, 2018). The current problems associated with agricultural practices are, firstly, large-scale use of chemical fertilizer, which rely heavily on fossil fuels. The current IPCC report lists to limit Global Warming to 1.5°C, and decrease carbon dioxide (CO_2) levels by ~45% from 2010 to 2030, ultimately reaching the 'net zero' goal by 2050 (IPCC, 2018). Secondly, loss of microbiome biodiversity, thirdly, increase in run off materials (contains more nutrients since and causes eutrophication), thirdly, soil structure (lower water and nutrient holding capacity), fourthly, more susceptible to diseases due to decrease in biological biodiversity. Several different bio-fertilizer exist such as; azotobacter (non-symbiont free-living nitrogen fixers), *Rhizobium* spp. (symbiont), *Azospirillium*, Blue Green Algae (nitrogen fixers), and Azolla-Anabaena (symbiont). The advantages of using such systems are: 1) the production of bio-fertilizers is not energy intensive. 2) Increases soil biodiversity due to the interaction with other soil microbes forming symbiotic relationships. 3) Improved nutrient uptake (macro and micronutrients), better water retention, therefore, less run-off water containing nutrients. 4) Soil structure improvements by root architecture. 5) Provides protection to the plant through interaction with soil pathogens. Plants that have a good phosphorus status are far less susceptible to damage caused by pathogens. However, there are some constraints: 1) an appropriate (local) and efficient strain is not available. 2) The shelf life of most bio-fertilizers is short, and storage and transportation is another major concern, which adds to the cost of production. 3) No adequate awareness to the farmers about the usage. 4) Cost of production is high in comparison to the Haber-Bosh process. Microalgae fertilizer technology can offer some solutions to these constrains. The poster is focused on the production and use of microalgae as a bio-fertilizer and its advantages include: light and CO_2 uptake, production on non-arable land, can control inputs of heavy metals.

#28: Patrick J. Allen

MYB-WDR-bHLH regulates Arabidopsis seed coat development via a three-tiered transcriptional mechanism.

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MYB-WDR-bHLH transcription factor (TF) complexes (MWBs) regulate Arabidopsis seed development including seed coat mucilage and tannin biosynthesis. MYB5, MYB23 and TRANSPARENT TESTA2 (TT2) participate in the MWB complexes with the WD-repeat protein TRANSPARENT TESTA GLABRA1 (TTG1). These complexes regulate GLABRA2 (GL2) and TTG2 expression in developing seeds. Transcriptome analysis of *ttg1-1* and wild-type (Ler) developing seeds identified 246 TTG1-regulated genes which include all known metabolic genes of the tannin biosynthetic pathway. The first detailed TTG1-dependent metabolic pathways were constructed for the biosynthesis of mucilage, jasmonic acid (JA) and cuticle including wax ester in developing seeds. Many known genes and previously uncharacterized genes were assigned to these pathways. Several TTG1-regulated genes are required for the activation/inactivation of hormones, plant immunity and nutrient transport. The promoters of eight TTG1-dependent genes were active in many tissues including developing seeds. Expression levels and regulation of 23 genes were determined in developing seeds of the combinatorial mutants of MYB5, MYB23 and TT2, and the combinatorial mutants of GL2, HOMEODOMAIN GLABROUS2 (HDG2) and TTG2. These six TFs between them positively regulate mucilage biosynthetic genes while negatively regulating the wax biosynthetic genes examined. The six TFs also positively regulate four repressor genes which are likely required to repress wax biosynthesis. Additionally, these TFs function with partial redundancy to regulate trichome and root development. Chromatin immunoprecipitation analysis identified over 30 genes directly regulated by MYB5 including GL2, HDG2, TTG2, repressor genes, metabolic and cell wall biosynthesis genes. Protein Kinase CK2β3 subunit was shown to interact with MYB5 in a yeast two-hybrid system suggesting the MWB complex activity may be modified/activated through phosphorylation by protein kinase CK2. We propose a multifaceted, three-tiered regulatory mechanism by which MWBs regulate these metabolic pathways.

#29: Chaoqun Shen

Functional Analyses of SEPALLATA(SEP)-like Genes in Regulating Spikelet Morphogenesis in Barley

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SEPALLATA (*SEP*)-like genes, a subfamily of MADS-box transcription factor, play vital roles in determining floral organ and inflorescence meristem identity and maintenance. Barley (*Hordeum vulgare*) genome contains five *SEP*-like genes, while *HvMADS1*, *HvMADS5* and *HvMADS34* belong to the *LOFSEP* subgroup and *HvMADS7*, *HvMADS8* are homologs of Arabidopsis *SEP3* subfamily. The objective of this study is to investigate the function of three *LOFSEP*-like gene members in barley inflorescence and floral development and reveal their potential interaction with other MADS-box floral homeotic members. Phenotype analysis of all the *lofsep* single, double and triple mutants generated by CRISPR-Cas9 knockout system indicated that the three *LOFSEPs* have a redundant biological function in controlling barley floret development, especially in lemma development. Further expression patterns analysis of *LOFSEPs* and other floral homeotic genes in the ABCDE model showed that *LOFEPs* have potentially negative regulation with *AP1/FUL*-like genes *HvMADS14* and *HvMADS15*. The expression of the PI-like gene *HvMADS4* and the AG lineage gene *HvMADS3* were decreased in *lofsep* mutants, particularly in terms of their expression in lodicule and pistil. The *AGL6*-like genes HvMADS6 displayed a similar expression pattern with the *SEP3* subgroup genes *HvMADS7* and *HvMSDS8* in *lofsep* mutants, with their expression increase in the early inflorescence stage and palea, while in lodicule and pistil, decrease of expression were detected.

Overall, this study reveals that although the three *LOFSEP* members show similar functions in outside whorls of barley floret development, their biological functions or interaction patterns with other *ABCDE* genes in the inner three whorls seem to have a divergent role with other plants, which provides new insights of barley *LOFSEPs* in barley inflorescence development and crop breeding.

#30: Iain Searle

EXT is a conserved gene that controls anther orientation and seed production in plants.

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Angiosperms are the most diverse and dominant land plants and propagate by self or cross pollination. Out-crossing increases genetic diversity, avoids inbreeding depression and can increase reproductive fitness. Many plants develop adaptations to avoid self pollination and some examples include self-incompatibility, herkogamy, distyly and anther orientation. Within the Brassicaceae, anther orientation can be introrse to promote selfing or extrorse, twisted outwards, to promote out-crossing. Here we describe the underlying genetic control of anther orientation by *EXT* in *Brassica rapa*.

#31: Hoai Thi Thanh Phan

Roles for aquaporins in seed water relations.

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Seeds are the typical dispersal and propagation units of angiosperms and gymnosperms. Water movement into and out of seed plays a crucial role from the point of fertilization through to imbibition and seed germination. In agriculture, effective seed development and germination is critically important in determining yield and establishment of the crop. The large movement of water, and likely other solutes, within the seed appear to be assisted by membrane integrating channel proteins called aquaporins (AQPs). These highly diverse and abundant proteins have been linked to different processes important in the development, longevity, imbibition and germination of seed. However, it is not clear exactly how, when or which of the many aquaporins encoded in a plant's genome are involved. Here we review the literature, examining the evidence for AQP involvement in seed biology. We link this to an analysis of a series of Arabidopsis seed related transcriptomic data sets in an effort to highlight candidate AQPs important in seed water relations and possible additional roles of AQPs in seed biology.

Poster Session 1

#32: Joanne Ernest

Using omics tools to convince plants to stop having sex

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Apomixis is a naturally occurring phenomenon in which plants circumvent normal sexual reproduction to instead produce a seed with an embryo that is a genetic clone of the maternal parent. By harnessing this "switch" from sexual to asexual (apomictic) reproduction, we could enable faster and more efficient production of new crop varieties; furthermore, desirable (and complex) agronomic traits could be "locked in" from generation to generation. This technology thus not only represents an important contribution to global food security, but has the potential to disruptively revolutionise plant breeding and agriculture by making available more of the world's biodiversity for exploitation.

To understand the underlying genetic mechanisms of apomixis, our lab studies the genus *Boechera*, a wild relative of Arabidopsis with members which exhibit both sexual and apomictic reproduction. Boechera represents an apomictic model system in which we can apply molecular tools and knowledge from Arabidopsis, such as various comparative "omics" technologies, and forward and reverse genetics. Using these strategies, we identified *APOLLO*, a predicted DEDDh exonuclease with apomixis-specific polymorphisms conserved across all apomictic *Boechera*, whose expression is highly correlated with apomictic egg cell formation, suggesting that it is a key factor in regulating apomixis in this genus.

We are now using genome-wide proteomics approaches to investigate the structure, biochemical and cellular function of APOLLO in the reproductive tissues of apomictic and sexual *Boechera*. This work will aid our understanding of the ways in which APOLLO contributes to apomictic seed production, and how we can utilise this knowledge to engineer valuable asexual crop species.

Poster Session 1

#33: Shyama Chakma

Brassinosteroid influences root system architecture of *Arabidopsis* under salt and dehydration stress

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Root growth and development are intricately linked with phytohormones. Brassinosteroids (BRs), a relatively new class of plant steroidal hormone, play important roles in root growth and tolerance to environmental stresses. A unique aspect of BR is that it enhances stress tolerance without compromising plant growth. BRs are involved in many aspects of root development, such as root elongation, lateral root initiation, gravitropic response and nodule formation, but the role of BRs in modulating the root system architecture in response to salt and drought stresses has not been studied. *Arabidopsis* seedlings grown in the presence of 0.05 nM 24-epibrassinolide (EBR), a BR, had increased primary root length (PRL) and lateral root numbers (LRN) as compared to untreated seedlings. BR also significantly increased PRL and LRN under salt and dehydration stresses induced by 80 mM NaCl and 3% polyethylene glycol (PEG), respectively, but this effect was observed in the presence of 0.5 nM EBR. BR also increased PRL and LRN in the null *hkt1-1* mutant, which is hypersensitive to salt stress given that the *HKT1* gene is involved in unloading sodium from the transpiration stream. BR also significantly increased root apical meristem size and meristematic cortical cell number, and promoted reactive oxygen species (ROS) and calcium ion (Ca^{2+}) accumulation in root cells under salt stress. The latter act as signalling molecules at low concentrations and trigger systemic stress responses. The Salt Overly Sensitive genes, *SOS1, SOS2* and *SOS3,* play a key role in ion homeostasis and salt tolerance in plants. All three *SOS* genes were up-regulated by *BR* under salt stress. Together these results suggest that BR interacts with multiple regulatory pathways involved in stress response pathways.

#34: Calum Watt

Mapping of major grain size QTL is an important step to increasing yields and improving the economic value of barley (*Hordeum vulgare*)

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To meet the food requirements of nine billion people by 2050, it is proposed that agricultural production is going to have to increase upwards of 70% to meet demand. Barley is the fourth most widely grown cereal crop. It is important to improve its yield to meet future demand. Grain yield is a reflection of three key determinants; number of grain spikes, number of grains per spike and individual grain weight which is a reflection of grain size. Increasing grain size is a means by which to increase yield. Grain size is a quantitative trait controlled by many genes; genes that are little understood in barley and thus genetic mapping is an important first step to improving yields via this approach. Using a barley population derived from crossing two Australian varieties that have significantly different grain lengths (Vlamingh and Buloke) the authors have discovered many genetic regions that control grain length, width and thickness. A number of these regions are major contributors to grain size. Two of these regions in particular were able to explain a combined 45 % of the variation for grain length within the population. Genetic mapping approaches were used to narrow down these regions to intervals containing single candidate genes, one of which has previously been reported in other cereal crop species (wheat and rice) to control grain length. The identification of desirable alleles underlying grain size loci will enable the development of higher yielding and higher value barley varieties through marker assisted selection.

#35: Chunli Mao

The effect on mitochondrial biogenesis with overexpressing protein import components.

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Mitochondrial biogenesis requires the import of approximately 1000 different proteins through a complicated channel to reach the appropriate location. There are four major protein import pathways in plant mitochondria; (1) the general import pathway for protein directed to the matrix, via passage across TIM17:23, (2) the carrier protein pathway for assembly of carrier proteins into inner membrane, (3) the Mitochondrial Intermembrane Space Assembly (MIA) pathway for proteins carrying twin cysteine residue that are imported to inter-membrane space, (4) the Sorting and Assembly Machinery (SAM) pathway for assembly β -barrel proteins on the outer membrane. Tim17-2, Tim22 and B14.7 are the components of mitochondrial protein import apparatus, which belong to the Preprotein and amino acid transporters (PRAT) family. The effect of these components on mitochondrial biogenesis is still unclear, as knocking out any of these components resulted in lethal phenotypes. Thus, this study will characterize their effects on mitochondrial biogenesis with overexpressing Tim17-2, Tim22 and B14.7, including monitoring growth phenotype, analyzing the abundance of protein import and respiratory chain components, and detecting *in vitro* protein import rate.

Poster Session 1

#36: Cody Hall

Lysine Biosynthesis; an Uncharted Herbicide Target

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Invasive weed species have long been a significant contributor to decreased crop quality and yields, and pose a major threat to infrastructure, livestock, native flora and biodiversity. However, weeds have developed resistance to almost all herbicide modes of action, and yet, there has been a void in the herbicide discovery pipeline. This study explores the potential of targeting lysine biosynthesis in plants for the development of novel herbicide leads. Specifically, we focus on the enzyme responsible for the first committed step in lysine biosynthesis, namely dihydrodipicolinate synthase (DHDPS). By employing X-ray crystallography, rational inhibitor design and site-directed mutagenesis, we have identified the first plant DHDPS inhibitors within a novel binding pocket in DHDPS. Furthermore, we have shown efficacy of these novel inhibitors both *in vitro* and *in planta* by using a combination of enzyme kinetics and plant growth assays, respectively. Thus, this study provides for the first-time evidence that lysine biosynthesis represents a promising target for the development of herbicides with a novel mode of action to combat resistant weeds.

#37: Emily Mackie

Towards the development of herbicide leads with a novel mode of action.

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Herbicide resistant weeds pose a major threat to our ecosystem and agricultural industry. Weeds have now evolved resistance to 23 of the 26 known herbicide modes of action, yet there has been a void in the herbicide discovery pipeline. The inhibition of amino acid biosynthesis in plants has been employed as a successful strategy to develop herbicides, including glyphosate and ALS-inhibitors that target aromatic and branched chain amino acids, respectively. In order to revitalise the herbicide discovery pipeline, this project aims to validate the lysine biosynthesis pathway as a novel herbicide target. Specifically, this project focuses on using an innovative *in silico, in vitro* and *in planta* pipeline to identify inhibitors of an essential enzyme within lysine biosynthesis. The goal of this project is to identify herbicide leads with a novel mode of action to avert the rise in resistant weeds.

#38: Hee Sung (Fiona) Kang

Arabinan Plays a Critical in Bryophtye Development

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All plant cells are surrounded by a strong cell wall composed of a relatively small number of distinct chemical polymers that control the shape and growth of cells, as well as their utility for food, fibre and fuel. (1,5)-α-Arabinans are a type of pectic polysaccharide that play an important role in maintaining cell wall elasticity in plants. Arabinan is synthesised in the Golgi-apparatus by Arabinosyl transferases (AraTs) from Carbohydrate-Active Enzyme (CAZy) family 47, clade B. We found two AraT homologs in *Marchantia polymorpha,* orthologous to *Arabidopsis thaliana* ARABINAN DEFICIENT1 *At*ARAD1) and *Nicotiana alata* (*Na*ARAD-LIKE1). *M. polymorpha* is a member of the bryophytes, a lineage of plants that diverged from the land plant ancestor around 450 mya. It has a haploid dominant life cycle, a fully sequenced genome and is amenable to genetic manipulation, making it a suitable organism to study gene function. We report that *M. polymorpha* has high levels of arabinan (up to 15%) indicating that this polysaccharide plays a critical role in Marchantia plant growth. Furthermore, we describe gene expression and subcellular localisation protein patterns of *Mp*ARADL1 and *Mp*ARADL2 using C-terminally fused fluorescent proteins and GUS reporter constructs. In contrast to flowering plants, we found substantial growth defects when altering the expression of the two *Mp*AraTs in the haploid generation, which highlight the importance of arabinan in bryophyte growth. Detailed cell wall analyses, interaction analyses and activity assays are currently being undertaken to better understand the role of arabinan in plant growth.

#39: Kelsey Picard

An investigation into what makes plants stop flowering, and what it means for yield

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When a plant stops flowering, it diverts all its resources to its developing seeds to ensure they are strong and healthy, thus increasing its chance of passing on its genetic material. Although there has been substantial effort to understand what makes a plant start to flower, much less is known about what makes a plant stop flowering. However, manipulating this process to extend the reproductive phase is one potential strategy for increasing overall yield in some agricultural situations.

Field pea is an agronomically important crop grown predominantly in the southern and western grains regions of Australia, for human consumption, stock feed and fodder. As in many crop species globally, climate change is having unpredictable effects on phenology and performance of pea and other pulse crops in Australia, and in order to secure our future food supply we need a better understanding of how these species are responding to environmental cues and challenges.

We are using a genetic approach to explore the control of floral cessation in pea. We have generated several mutants affecting this process and are examining their roles and interactions in studies that include classical genetics, gene expression analyses, and an examination of physiological responses to daylength and temperature.

Determining the genetic interaction and control of these genes will offer possible gene targets for breeding programs to modify floral cessation independently of flowering onset to increase yield in legume crops.

#40: Meridy Price

To germinate or not to germinate? The potential impacts of climate change on germination capacity of three woodland forbs

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Australian native temperate grassy woodlands maintain high biodiversity, including many iconic plant species. These culturally and ecologically important communities are increasingly threatened by land-use and climate change and are the subject of many restoration efforts. Restoration of grassy woodland understorey species is challenging, especially in the face of a warming climate that is disrupting local seasonal patterns of temperature and rainfall. Indeed, it is unknown how such changes may impact a plant's regeneration capacity, e.g. via seed germination cues. We here investigate the temperature requirements for seed germination of three woodland forbs, *Arthropodium fimbriatum, Bulbine bulbosa and Microseris walteri*, to determine whether optimal germination temperatures are linked to home-site climate. Controlled condition cabinets were used to simulate temperatures expected under climate change at the home-sites of 15 provenances sampled from each species across their native ranges in New South Wales, Australia. Significant interactions between temperature and provenance were found, along with variation in 'transfer tolerance' range for each species. Optimum germination temperatures correlated with aridity of the home-site in all three species, such that seed collected from warmer, drier sites had higher proportion germinated at higher temperatures compared to seed collected from cooler, wetter sites, and vice-versa. Our results point to putative local adaptation of germination responses to home-site temperature, and we highlight the importance of such responses when considering climate-adjusted seed selection for restoration.

Poster Session 1

#41: Miing Tiem Yong

Mechanisms and adaptations of salinity tolerant Oryza species to prolonged salinity stress at reproductive stage in field

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Wild *Oryza* species are considered as an important gene pool for salinity tolerance improvement in rice (*Oryza sativa* L.). To date, only a few wild *Oryza species* were reported to have similar or better performance under saline condition compared to the well-known salt-tolerant landraces. Here, we evaluated the salinity tolerance (control and 100 mM NaCl) of 7 wild species with 3 *O. sativa* varieties at late tillering and reproductive stages over prolonged salinity stress in the field. We found that (1) Salinity tolerant wild rice species showed the least reduction in whole plant growth performance, gas exchange and chlorophyll content. Moreover, MIFE measurement showed that the net K^+, Na^+ and Cl^- efflux is smallest in the most tolerant group (*O.coarctata, O.latifolia, O.alta* and Pokkali), suggesting less toxic ion accumulation and higher K^+ retention in these Oryza species. Confocal imaging measurement of ROS and sodium accumulation in mesophyll demonstrated that tolerant group has lower salinity- induced Na^+ and ROS accumulation in mesophyll over prolonged salinity stress. qPCR experiments on the key transporter and ROS genes also confirmed the physiological results. Finally, correlation analysis indicated that most of the photosynthetic parameters (e.g. A, g_s, T_r, C_i, WUE) were significantly correlated to biomass, electrophysiological (net K^+ and Cl^- flux) and leaf mesophyll sodium accumulation. Our result suggested that low Na^+ accumulation and K^+ leakage in mesophyll is a key to maintain photosynthetic activities in tolerant *Oryza* group to confer long-term salinity stress.

Poster Session 1

#42: Mohammad Babla

High light induced photosynthetic performance and ion fluxes from tomato plants in greenhouse

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Light is not only a primary energy source for photosynthesis but also a vital regulator of numerous processes in plants. However, high light intensity induced responses of leaf mesophyll cell ion fluxes and nutrition homeostasis in plants is yet to be elucidated, particularly at different canopy levels of large horticultural crops in greenhouse. Microelectrode ion flux measurement (MIFE) and leaf gas exchange were investigated to test the effects of prolonged high light induced canopy wise photosynthetic performance and ion fluxes of tomato plants. Though top canopy of leaf canopy has better gas exchange rates, prolonged high light irradiance does not improve plant overall photosynthetic performance. However, most physiological parameters were significantly reduced upon high light treatment. Canopy wise K^+, Ca^{2+} fluxes from leaf mesophyll of tomato plants in long term high light differed significantly, but Cl^- flux was insignificant across the three canopies. Plants photosynthetic performance were highly correlated to K^+, Ca^{2+} and Cl^- efflux of leaf mesophylls as well as physiological traits of the plants in prolonged high light. In summary, canopy wise K^+ efflux, Cl^- efflux and Ca^{2+} efflux from leaf mesophylls are likely to be high light induced indicators in the acclimation of tomato plants grown in glasshouse. Our study will improve the understandings of the light induced signalling responses transduced from top canopy to bottom canopy leaf in light of the photosynthetic performance of tomato plants.

#43: Pawel Gluza

Understanding how plants control the delivery of substrates for cell wall biosynthesis

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The biosynthesis of the cell wall and glycan structures is exceptionally complex involving thousands of proteins and biosynthetic processes that are partitioned into distinct subcellular compartments. It is well established that the assembly of non-cellulosic cell wall polymers and glycan decorations found on proteins and lipids relies on the transport of nucleotide sugars from the cytosol into the Golgi lumen where the sugar moiety is subsequently incorporated into glycan structures by glycosyltransferases. We recently developed a nucleotide sugar transporter (NST) assay and its successful application to the Arabidopsis NST family revealed novel functions for 16 of its members.

Interestingly, the characterization of some of these NSTs identified an intriguing aspect of in planta regulation of nucleotide sugar transport. While some NSTs show broad substrate specificity in our in vitro biochemical transport assay; in planta they can have impact on only a specific cell wall polymer.

These results clearly imply yet unknown levels of control on nucleotide sugar delivery that are only present in planta. We propose that the targeted substrate delivery is mediated through either the formation of large metabolic complexes including nucleotide sugar transporters, glycosyltransferases and/or interconverting enzymes or through the spatially defined distribution of transporters within the secretory pathway. To better understand the mechanisms underpinning the sugar polymer-specific substrate delivery we investigate protein-protein interactions, localize the individual components to their specific sub-compartments within the cell and track changes in mutant plants where these processes are modulated. Here we present our recent findings on the bifunctional UDP-rhamnose/UDP-galactose transporter (URGT) family from Arabidopsis, discuss potential interactions with glycosyltransferases and provide insights into their sub-Golgi localization.

#44: Qi Guo

Understanding the role of membrane lipid remodelling and lipid metabolism in plant salt tolerance

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Salt is a natural part of the Australian landscape but has become one of the most serious environmental problems, threatening the productivity of agricultural lands. Salt tolerant crop breeding is imperative to help overcome this abiotic stress factor, however, more research into the molecular mechanisms underpinning plant salt tolerance is needed. Membranes that surround organelles or the cell itself, are an important line of defence against salinity stress, controlling the uptake and cellular distribution of the sodium and chloride ions that can be toxic to the cell. Changes in membrane fluidity and permeability have long been observed in plants under salt stress. These changes likely involve specific regulation of membrane lipids and proteins, and as such this remodelling would be specific to the cellular origin of the membrane.

The underlying cellular mechanisms involved in this regulation remain unclear due to the considerable obstacle of isolating well resolved subcellular membrane fractions. In the current study, innovative separation technology, Free Flow Electrophoresis (FFE), which separates membranes based on charge, was used for high-resolution fractionation of subcellular membranes from leaf tissue of untreated and salt-treated *Mesembryanthemum crystallinum*, a model halophytic plant. These fractions were used for proteomic and lipidomic analysis to identify the subcellular origin, and subsequently characterise salt-induced alterations in membrane lipid content and changes to proteins involved in lipid metabolism. In total 1040 proteins were identified across 28 samples corresponding to 56 FFE fractions from which marker proteins confirmed the distribution of multiple subcellular membranes. This information was used to identify fractions corresponding to tonoplast and plasma membrane for further analysis. This work will help to give a comprehensive understanding of the mechanisms of membrane lipid remodelling in plants under salt stress.

#45: Ruwanthi Nawarathna

Is it worth to redesign rice photosynthesis via screening for enhanced leaf vein density?

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The engineering of C_4 rice has been one of the major breeding foci in last decade. Enhanced leaf vein density (LVD) has identified as a prerequisite to establish C_4 biochemical pathways in rice. However, C_3 plants have potential to perform better than C_4 plants under elevated CO_2 levels and temperature with suppressed photorespiration. We screened sixteen rice cultivars of different regions of world to examine the variation in photosynthesis capacity and checked for association between photosynthesis and LVD. In our study, the mean photosynthesis was ranged from 31.48 $\mu molCO_2m^{-2}s^{-1}$ to 18.54 $\mu molCO_2m^{-2}s^{-1}$ where LVD vary from 5.3 veins/mm to 6.3 veins/mm in studied population. However, there was no relationship between photosynthesis and LVD (r=0.18631; p=0.4896). Furthermore, higher vein density candidates, Tetep and Ciwini showed photosynthesis rates of 24.55 and 21.14 $\mu molCO_2m^{-2}s^{-1}$ respectively where photosynthesis rates of 24.09 $\mu molCO_2m^{-2}s^{-1}$ recorded in Surjamuki, the lowest vein density candidate. These observations indicate that it will be a better approach to exploit photosynthesis potential in short term rather than for screening for high leaf vein density candidates to enhance the rice productivity. Identification of variation in term of LVD can be used as a breeding tool to underpin the genetic regulation of LVD to install Krantz anatomy to introduce efficient CO_2 pump and biochemical modification in rice to mitigate the impact of climate change and to feed rising population in Asia. Our findings highlight it is worth to study further and optimize selection of candidates to be used as genetic stocks to facilitate redesigning of C_4 rice.

Poster Session 1

#46: Sajal Zia

Melatonin and auxin – how similar are they?

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Melatonin (*N*-acetyl-5-methoxytryptamine) is a ubiquitous indolic molecule. It has been extensively studied for its biological roles in mammalian systems, mainly as a strong antioxidant and anti-stressor. Relatively, in plants, the knowledge about the effect of melatonin and its biological role is still in its infancy. It is a secondary metabolite with multi-functional roles in plants, such as enhancing growth and development, as well as mitigating a variety of abiotic (drought, salinity, extreme temperature, heavy metals, light) and biotic (pathogens) stresses. Hence, it is envisaged that melatonin has the potential for use in agronomic improvement strategies. In recent studies, melatonin has shown to be involved in crosstalk with plant hormones, such as salicylic acid, ethylene, and abscisic acid to regulate growth and alleviate stresses in plants. Of particular interest has been the relationship between melatonin and most well-studied plant hormone, auxin. Both have tryptophan as their biosynthetic precursor and an indole ring in their structure. Owing to this, it has been hypothesized that melatonin exhibits plant growth regulation similar to auxin. However, the current understanding of the relationship between melatonin and auxin remains controversial. In this study, we have used comparative promoter-activation and global transcriptome analyses to clarify whether melatonin and auxin regulate gene expression in a similar fashion in *Arabidopsis thaliana*. We have found that melatonin and auxin act quite differently on gene expression in Arabidopsis. The findings shed light on the distinct effect of melatonin in plants that warrants further investigation to harness its benefits for improving plant fitness.

#47: Xiaoyang Wei

Sucrose induces and regulates wall ingrowth deposition in phloem parenchyma transfer cells in *Arabidopsis thaliana*

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In *Arabidopsis*, the differentiation of phloem parenchyma (PP) transfer cells (TCs) in leaf minor veins is proposed to play a pivotal role in phloem loading. Sucrose (Suc) from mesophyll tissue is delivered symplasmically to PP TCs before being unloaded into the apoplasm prior to active uptake into the sieve element/companion cell (SE/CC) complex. We report that Suc is a key factor regulating wall ingrowth deposition, but the effects of endogenous versus exogenously-supplied Suc are different. Exogenous Suc significantly represses wall ingrowth deposition in both cotyledons and juvenile leaves in a dose-dependent manner. However, exogenously supplied glucose (Glc) or fructose (Fru) do not repress wall ingrowth deposition. Furthermore, exogenous Suc mimics the influence of cotyledons on wall ingrowth deposition in leaves 1 and 2, indicating that Suc as an endogenous signal can regulate PP TC development in *Arabidopsis* leaves. Endogenous Suc, on the other hand, is a signal inducing wall ingrowth initiation and positively regulates its deposition. Elevating endogenous Suc levels by high-light or constant light enhances wall ingrowth formation, whereas lowering endogenous Suc levels by dark treatment or genetically in the chlorophyll deficient mutant *ch-1* results in lower levels of deposition compared to controls. The repressive effect of exogenous Suc on wall ingrowth deposition appears to be achieved by inhibiting endogenous Suc accessibility to PP TCs by delaying the sink-source transition in juvenile leaves. The cell wall invertase mutant, *cwin1-1* is less sensitive to exogenous Suc repression compared to wild-type but shows a similar enhancement response to high-light treatment as wild-type. These results demonstrate that endogenous Suc induces wall ingrowth development in PP TCs, but this role can be disrupted by exogenously-supplied Suc.

Poster Session 1

#48: Yanqiao Zhu

Temporal proteomic and transcriptome profiling of mitochondrial biogenesis during *Hordeum vulgare* L. (Barley) germination

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Barley is an important agricultural crop. Previous study using shot-gun proteomic approach has identified proteins associated with various germination stages in the total protein background. However, the profile of mitochondrial biogenesis during barley germination has not been well characterized, while a number of studies have examined mitochondrial biogenesis in rice and Arabidopsis. As mitochondria play a critical role in seed viability and germination in plants, our study aims to provide a deeper insight into mitochondrial biogenesis during barley germination at both transcription and protein level over a 48 h time course. So far, the total oxygen consumption and the activity of mitochondrial respiratory complexes in barley embryos isolated at different germination stages have been measured. It revealed an increase in respiration via the cytochrome and alternative pathway. The increase of respiratory activity is likely due to the increasing abundance of the proteins in the respiratory pathway. In addition, transcriptome analyses using RNA-seq were applied to identify the genes critical for barley germination. Several clusters of genes with specific expression patterns were identified with some genes only showing a transient expression over the time course. These were then compared to orthologous genes in Arabidopsis and rice to reveal evolutionary conserved and species-specific co-expressed genes with importance for seed germination and the underlying processes. These results provide novel targets for optimization of malting barley breeding.

Poster Session 1

#49: Emma Gillingham

A characterization of phosphate dependent SPX4 degradation in *Arabidopsis thaliana*

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Phosphorus (P) deficiency results in a host of physiological responses in plants and is a primary agent of limitations in plant growth. In Arabidopsis thaliana SPX domain encoding proteins are essential for phosphate homeostasis, and with the exception of SPX4, are positively regulated in shoots and roots during the PSI response. SPX4 is suggested to act as a negative regulator, with proteins encoded by SPX4 being degraded in low P conditions. This study seeks to characterize the proteasomal degradation of SPX4 under P-limited conditions, and further understand the role of SPX4 as a transcriptional regulator of PSI responses. Upstream elements associated with the initiation of proteasomal degradation will be investigated by screening of EMS mutants generated from an SPX4:LUC reporter construct line. Candidates will be screened using a luciferase reporter assay, and those plants exhibiting sustained SPX4 protein accumulation in low Pi conditions will be used for further analysis. In order to establish whether the degradation of SPX4 proteins is associated with PHO2-mediated ubiquitination of target proteins in low P conditions, crosses are being generated and will be subject to further analysis. This investigation is currently in progress, and methods may be subject to revisions. It is the authors intention to further characterize the degradation of SPX4 proteins, improving the resolution of dynamic transcriptional changes occurring during PSR in Arabidopsis thaliana.

Poster Session 1

#50: James Lancaster

Identifying a paramutant locus in the 'Rogue' pea (*Pisum sativum)*

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The "Rogue" phenotype in *Pisum sativum* (Garden pea), characterized by narrow "rabbit-eared" stipules and leaflets, is an example of the epigenetic phenomena known as paramutation. Traditional Mendelian laws of inheritance do not apply to paramutation, as 'paramutant' alleles induce epigenetic alterations to 'paramutable' alleles resulting in drastically increased penetrance of a given trait/phenotype in progeny. Paramutation has been extensively studied in maize, where it was found RNA-directed DNA methylation and siRNA production are heavily involved. Paramutation has also been described and investigated in other eukaryotic organisms such as tomato, petunia and *drosophila*, though the paramutant rogue locus in *P. sativum* has yet to be identified and characterized at the molecular level. This project aims to identify the causal locus of the mutation, and to investigate its paramutation mechanism through combining molecular and phenomic analysis.

Poster Session 1

#51: Martino Schillaci

The effect of beneficial bacteria on the growth of Brachypodium distachyon under temperature stress and phosphorus deficiency

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Soil microorganisms can improve plant growth, especially in suboptimal environments. As a result, increasing our understanding of this interaction is a promising approach to enhance crop productivity, which is already threatened by climate change and decreasing availability of fertile lands. We investigated the effects of inoculation of the model plant *Brachypodium distachyon* with the plant growth promoting bacterium *Azospirillum brasilense*, focusing on root architecture and metabolome responses under temperature stress and phosphorus deficiency. Brachypodium makes an ideal model for temperate climate cereals due to its small size, short life cycle and small genome.

For phenotypic characterisation, inoculated and uninoculated plants were grown for three weeks in the GrowScreen-PaGe system, a soil-less setup specifically designed to allow high throughput and non-intrusive imaging of plant roots at various stages of their growth. The phenotypic analyses suggest that inoculation with *A. brasilense* improved plant growth during the later stage of seedling development, with an increase in root length and shoot biomass. Interestingly, the shoot nutrient content showed no significant difference between the treatments, suggesting that the positive role of *A. brasilense* is not a result of improved nutritional status in the plant.

To further investigate those mechanisms, root samples were collected to study their metabolome at various stages of development, focusing on both polar metabolites and lipids, using gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) techniques, respectively. Recent advances in GC- and LC-MS technology have enabled quantitative monitoring of the abundance of various biological molecules in a high-throughput manner. A preliminary analysis suggests a shift in the lipid profile between uninoculated and inoculated plants, which was enhanced with development. By integrating the results of the phenotypic and metabolomic approaches, we aim to increase our understanding on the interaction between plants and beneficial soil bacteria under environmental stress.

Poster Session 1

#52: Miguel Angel Ibeas

The diverse iron distribution in Eudicotyledoneae seeds: from Arabidopsis to Quinoa

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Iron is an essential micronutrient for all living organisms. Seeds accumulate iron during embryo maturation stages of embryogenesis. The role of iron in seed yields is an important agronomical trait because iron deficiency affects plant reproduction and limit crops yields. Using *Arabidopsis thaliana* it has been described that iron accumulates in the vacuoles of the endodermis during seed development. Using Perls/DAB staining we were able to identify this distribution pattern was conserved in different members from Brassicales. We extend this study to embryos belonging to species from different orders from Eudicotyledoneae. Our results suggest that iron pattern found in Arabidopsis is not extended to all Eudicotyledoneae. Noticeably, in *Chenopodium quinoa* embryos iron accumulates in several cell layers including cortex and endodermis cells. *Chenopodium quinoa* is a highly nutritious crop that is adapted to a wide range of ecosystems and has reach international attention because of the nutritional value of its seeds and demand for quinoa has soared in recent years in developed countries. We also found that iron loading during *Chenopodium quinoa* seed development is different than what we observed before in embryos belonging to the Brassicaceae family. So far, studies have been not be successful in to determine where iron accumulate in quinoa embryo. We detected high amount of phytoferritins in quinoa embryos. Ours results open new questions about the molecular mechanism controlling iron loading, distribution and accumulation in quinoa embryo seeds.

#53: Ahmad Mollazadeh Taghipour

Functional study of plastid-targeted protein 1 (PTP-1) associated with desiccation tolerance in the resurrection plant Craterostigma plantagineum

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Craterostigma plantagineum is one of the best studied resurrection plants that can adapt to extreme drought and survive under drought stress conditions. Plastid-targeted protein 1 (PTP -1) is an abscisic acid and dehydration-responsive gene from the PTP family. Our objective is to functionally characterize the cis-acting regulatory elements of the CpPTP-1 promoter. A promoter β-glucuronidase (GUS) reporter gene approach was used to analyze the activity of the CpPTP-1 wild-type promoter (CpPTP-1-wt). A series of deletion and site-directed mutagenesis constructs were prepared and analyzed. The promoter::GUS fragments transformed into Agrobacterium tumefaciens and used for stable and transient transformation in Arabidopsis thaliana and in C. plantagineum. Our results showed high activity of the CpPTP-1-wt promoter in stably transformed A. thaliana four week-old seedlings, leaves, flowers, siliques and seeds after dehydration and ABA treatment, as well in untreated samples. Transient transformation results showed that the CpPTP-1-wt promoter activity was higher under dehydration than ABA treatment and normal conditions in transiently transformed A. thaliana and C. plantagineum leaves. The relative promoter activity in ABA-treated leaves was lower than in untreated leaves in A. thaliana. The deletion construct 1 which only contains a DRE cis-acting element showed no promoter activity. However, after deleting the coupling element 3 in construct 2, we observed high promoter activity in C. plantagineum and A. thaliana under dehydration and ABA treatment in comparison to normal conditions. Site-directed mutagenesis of the MYC cis-acting element showed a dramatic decrease in the promoter activity in C. plantagineum and no significant change in A. thaliana under dehydration compared to CpPTP-1-wt promoter activity. Functional promoter analysis demonstrated that the CpPTP-1 promoter has high activity in a homologous and heterologous system. The MYC cis-acting element plays a critical role in the activity of the CpPTP-1 promoter.

Poster Session 2

#54: Alicia Quinn

The impact of drought on the nutritional quality of Cassava (Manihot esculenta Crantz)

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Cassava (Manihot esculenta), a perennial shrub of the Euphorbiaceae, is a major food source for over 500 million people. Cassava crops are grown primarily for their tubers which are the main source of carbohydrate for many people in tropical regions. Cassava produces the cyanogenic glucosides (CNGlc) linmarin and lotaustralin: compounds which release toxic hydrogen cyanide (HCN) when broken down. High levels of CNGlcs in cassava can lead to diseases such as Konzo, tropical neuropathy and goitre in vulnerable populations, including children and the undernourished. Cassava has been traditionally grown as a subsistence crop as it performs well under drought conditions and in marginal soils. These qualities have also made cassava an attractive crop for commercial production and efforts are in place to improve cassava yields. There have been some reports of an association between higher HCN concentrations and higher yield in cassava. It may be that selection for lines with improved growth and drought tolerance may inadvertently select for lines with higher concentrations of CNGlcs. In this greenhouse study we investigate the growth and toxicity of four cassava cultivars in response to drought and drought recovery. Cuttings of cassava cultivars available in Australia were established in pots and randomly allocated to one of three treatments: control, drought and drought followed by re-watering. Plants were grown for six weeks before harvesting. Growth and performance were measured with chlorophyll fluorescence, leaf relative water content, biomass and yield. Toxicity was determined for leaves and tubers by measuring evolved HCN spectrophotometrically. We found differences in growth, yield and toxicity between the four cultivars under drought conditions and during drought recovery. We discuss relationships between growth and CNGlc content and implications for cassava crop improvement efforts in an increasingly dry and unpredictable environment.

#55: Andreas Hartmann

Comparative analysis of transcriptomic responses involved in organellar stress in Arabidopsis, Barley and Rice

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As sessile organisms in a highly dynamic and ever-changing environment, plants need to respond and adapt to gradual and rapid changes, to balance the allocation of resources between productivity and stress tolerance mechanisms. Mitochondria represent the main organelles responsible for respiration and energy production and therefore play a crucial role in overall cellular metabolism. Accumulating evidence suggest that mitochondria are not only responding to impaired function, they moreover act as a sensor in the overall stress response. Specific signal transduction between the organelle and the nucleus is necessary to adjust nuclear gene expression in response to the physiological state of the organelle, as the vast majority of proteins is encoded in the nucleus. These signaling pathways and networks were mainly investigated in the model plant *Arabidopsis thaliana* (Arabidopsis). Relatively little is known about the mitochondrial retrograde response (MRR) or its contribution to stress tolerance in agriculturally important crops such as barley and rice. Recent advances in crop genomics and the identification of major species-specific differences in stress responses and corresponding signals, using alternative plant model systems, call for a re-evaluation of overly generalized tolerance models. To reveal the overall- and organelle specific responses between Arabidopsis and the agronomical relevant monocot species barley and rice, a comparative analysis of the genome-wide transcriptional response was carried out by RNA-seq. Plants were treated with different chemicals and UV radiation perturbing mitochondrial and chloroplast function. A core stress response across all species was defined representing a valuable resource for the selection of stress marker genes. Gene orthology-based co-expression clustering identified unique and conserved transcriptomic responses across mono- and dicots. Unique and overlapping transcriptional networks enable stress tolerance in each species and reflect the evolutionary divergence between them.

Poster Session 2

#56: Bo Eng Cheong

Phenotyping Reproductive Stage Chilling and Frost Tolerance in Wheat Using Targeted Metabolome and Lipidome Profiling

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Frost events lead to A$360 million of yield losses annually to the Australian wheat industry, thus improving chilling and frost tolerance is a vital trait for breeding. We established a controlled environment phenotyping method and used metabolomics and lipidomics to profile differences in cold acclimation over a four-day period for two Australian wheat cultivarss with contrasting chilling tolerance. Flag leaves of the sensitive cultivar Wyalkatchem showed a substantial reduction in amino acids after the first cold night, followed by an accumulation of sugars after prolonged treatment. Accumulation of osmolytes in Wyalkatchem is indicative of a water stress response and was not observed for the tolerant cultivar Young. The two wheat cultivars also displayed significant differences in lipid accumulation. Combined changes in two major lipid groups, one set consistently higher in Young and another set consistently higher in Wyalkatchem, resulted in a higher unsaturated to saturated lipid ratio in Young after four days. Young flag leaves may have a superior ability to maintain membrane fluidity following cold exposure, thereby avoiding membrane damage and water stress as observed for Wyalkatchem. Our study indicates that metabolite markers could be used to discriminate wheat cultivars with differences in cold acclimation.

Poster Session 2

#57: Celymar Angela Solis

Molecular cloning and characterization of salt-responsive membrane transporter genes in wild rice relative, Oryza coarctata

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Wild relatives of rice have long been known to have superior performance under salinity stress. Understanding the essential mechanism involved in differential salinity tolerance between wild and cultivated rice could highlight its potential use to provide breeders with novel resources in producing salt-tolerant rice varieties. Our previous experiments evaluated the adaptive mechanism of the known wild halophytic rice *Oryza coarctata* alongside cultivated salinity check lines Pokalli (tolerant) and IR29 (sensitive) upon exposure to high salinity stress in complementary Greenhouse and Field experiment. In both experiments, *O.coarctata* showed the least reduction on whole plant growth performance, gas exchange, biomass, and chlorophyll content compared with the cultivated lines. Moreover, one of our key findings demonstrates the cell-type-specific Na^+ accumulation in the leaf of *O.coarctata*. Na^+ distribution confocal imaging reveals apart from salt hairs, Na^+ is preferentially accumulated in bundle sheath cells avoiding Na^+ accumulation in photosynthetically active mesophyll cells. These initial findings paved way for us to clone known salt-responsive membrane transporter genes such as vacuolar ATPase (*OcVHA*) and Na^+/H^+ antiporter (*OcNHX*) which is significantly upregulated during salinity stress in *O.coarctata* compared to cultivated lines based on our qPCR results. Sequence homology of these genes was analyzed and drawn to characterize its genetic relatedness to other *Oryza* and halophilic species. To date, introgression of these genes to cultivated rice is underway to further characterize its role in salinity stress adaptation and provide a better understanding on breeding salinity tolerant rice using wild related taxa.

Poster Session 2

#58: Crystal Sweetman

Molecular responses of legumes to abiotic stress

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Legume plants are sensitive to environmental pressures such as drought and salinity, causing enormous production losses globally and annually. In this study we explore the relationship between environmental stress tolerance and the expression of genes from common stress-regulated pathways, including the mitochondrial alternative pathway of respiration. The alternative pathway consists of alternative NAD(P)H dehydrogenase (ND) and alternative oxidase (AOX) enzymes, which bypass complexes of the classical mitochondrial electron transport chain and can alter energy production, redox balance and metabolism in the plant cell. We use chickpea, *Cicer arietinum* as a model legume due to the increasing economic value of this crop in Australia and the need for improved tolerance to extreme growth conditions. We previously identified four alternative oxidase (AOX) genes in *C. arietinum* and one of these, *CaAOX1* was stress-responsive but otherwise expressed at a low level, while another, *CaAOX2a* was expressed at a high level in the absence of stress, especially in photosynthetic tissues. This suggested specialised functions of different AOX isoforms in legumes. For the present study, six cultivars of *C. arietinum* were selected. Based on plant yield and biomass measurements in response to salt exposure, these cultivars represented a wide range of salinity tolerance levels and provided good genetic material for exploration of gene expression relationships. In response to salt exposure, transcript levels of *CaAOX1* increased in leaves of most cultivars, while a putative external-facing alternative NAD(P)H dehydrogenase, *CaNDB2* followed a similar pattern of up-regulation. Therefore, like Arabidopsis, there may be coordinated expression of alternative pathway components in chickpea and these may play an important role in the response to environmental stresses.

Poster Session 2

#59: Felix Amuji

The effect of water stress combined with a heatwave on reproduction and yield of Roma-VF tomatoes

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Tomato (*Solanum lycopersicum*) is one of the wold's most common fruits, supplying considerable economic and nutritional benefits. However, its production can be substantially affected by extreme weather events, such as heat waves, flooding and drought. The objective of this study was to evaluate the combined impacts of heat and moisture stress on the reproductive traits and yield of the popular tomato variety, Roma-VF. Five weeks after sowing, sixty plants were divided into five treatments as well as a control. Heat-stressed plants were subjected to day/night temperatures of 35/23 degrees Celsius, compared to the control (28/23 degrees Celsius). Moisture stress was imposed by reducing the water received by plants in each 10-litre pot to 70% and 40% soil field capacity (moderate and severe stress, respectively). Treatments lasted for eight weeks, followed by five-week recovery period. Pollen morphology, number of flowers, fruits and aerial biomass were monitored. Our results showed that flowers from plants subjected to heat stress combined with either moderate or severe moisture stress did not produce any pollen during the treatment period. Further, by the end of the recovery period 27-38% fewer fruits had developed on plants subjected to either heat or moisture stress, while fruit production among plants subjected to both stresses simultaneously declined 90% relative to control. We conclude that Roma-VF tomato can be able to recover from heat stress provided plants are well irrigated. However, should heat and moisture stress co-occur even at slightest form, yield is likely to be greatly diminished as plants may not recover. This is the first report on yield response of Roma-VF tomatoes to the combined effect of heat and moisture. Key words: Climate change, food security, horticulture, plant growth, plant reproduction

Poster Session 2

#60: Hanmei Du

How sensitive is canola (Brassica napus L.) to acid soils?

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Soil acidity limits the yields of Australia's major food crops. Acid soils present many stresses to plants but chief among these is aluminium (Al) toxicity which inhibits root growth. Liming is the most effective management practice for reversing soil acidification but the use of Al-resistant crops enables production to continue while soil management proceeds. Wheat, barley and some pulses show a significant genotypic variation for acid soil-tolerance. Fewer studies have investigated the variation among cultivars of canola (*Brassica napus* L.) but the general consensus is that canola is sensitive to soil acidity. Breeding programs are not targeting this trait, in part, because the genotypic variation for Al resistance in canola has not been investigated in detail. Using hydroponics, we screened the Al resistance of several cultivars of open-pollinating and hybrid canola and compared them with Al-sensitive and tolerant wheat as well as to several related *Brassica* species. We demonstrate that canola is as sensitive to Al toxicity as Al-sensitive wheat and that some related species are promising sources of Al resistance. We conclude that any strategies that successfully increase the Al-resistance of canola cultivars should significantly improve canola production on acid soils.

#61: Bo Xu

γ-Aminobutyric acid (GABA), a new plant signalling molecule

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The non-protein amino acid γ-aminobutyric acid (GABA) has been proposed to be an ancient messenger for cellular communication conserved across kingdoms. GABA has well-defined signalling roles in animals; however, whilst GABA accumulates in plant tissues under stress, and can regulate plant tissue growth, it has not been determined if, how and when GABA acts as an endogenous plant signalling molecule. Here, we establish that endogenous GABA is a *bona fide* plant signal by demonstrating it antagonises stomatal movements in response to opening and closing stimuli, and modulates plant gas exchange through the direct inhibition of plasma membrane and tonoplast-localised anion transporters within stomatal guard cells. Furthermore, we show that GABA production within guard cells is necessary and sufficient to influence stomatal aperture by restoring drought tolerance to mutant plants with reduced GABA synthesis through stomatal specific genetic complementation, but not through mesophyll-specific complementation. We show that GABA control of stomatal movement is widely conserved across plant families including valuable dicot and monocot crops. Our findings demonstrate that GABA is a novel plant stress signalling molecule that acts via a mechanism not found in animals to fine tune plant gas exchange. Furthermore, we propose GABA acts as a memory of drought stress by resetting stomatal opening to a lower level the day following a reduction in soil water content. These discoveries open novel avenues for manipulating crop water use and tolerance to biotic and abiotic stress.

#62: Miaojing Qin

The role of mitochondrial heat shock proteins for thermomemory

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We have previously demonstrated a crucial role for a plastidial small heat shock protein (sHSP21) in the regulation of thermomemory (Sedaghatmehr et al., 2016). The main objective of our new project is to study the role of mitochondrial small heat shock proteins for thermomemory. Here we show that transcript and protein levels of Mit-HSP-1 and Mit-HSP-2 (two mitochondrial small heat shock proteins) are highly induced during the thermorecovery phase. We generated transgenic lines and characterized their thermomemory phenotype. We found that Mit-HSP-1/Mit-HSP-2 double knockout plants were impaired in thermomemory compared with wide-type plants, suggesting a role for mitochondrial sHSPs in the regulation of thermomemory. We measured seedling respiration levels to determine the role of mitochondrial small heat shock proteins in the protection of mitochondrial respiration under different heat shock conditions. We plan to assess chaperone activity of these HSPs and identify their target proteins under heat stress conditions. To this end, we will perform pull-down assays and proteomic analysis. Moreover, we attempted to identify transcriptional regulators of Mit-HSP-1 and Mit-HSP-2. We performed yeast one-hybrid assay using a library covering 1956 Arabidopsis transcription factors and identified 115 TFs binding to the Mit-HSP-1 promoter. We have selected two priming-induced TFs for further analysis. To understand how Mit-HSP-1 and Mit-HSP-2 regulate mitochondrial physiology in response to heat stress in Arabidopsis, we will measure the activity of selected mitochondrial matrix enzymes, i.e. fumarase and rhodanese in Mit-HSP-1 and Mit-HSP-2 transgenic lines as well as wild-type plants under different heat stress regimes.

Poster Session 2

#63: Moshin Tanveer

Tissue specific ion profiling reveals differential salinity tolerance mechanisms in halophyte and glycophyte

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Salinity affects over 800 million hectares of agricultural land, which impacts crop production across the globe. Salinity stress tolerance is a complex polygenic trait that is conferred by multiple physiological mechanisms. Various agronomical traits such as germination, survival and growth rates; the extent of leaf injury; and whole-plant physiological characteristics have been frequently advocated in terms of salinity stress tolerance. However, a considerable inter- and intraspecific variation in the overall growth and salinity sensitivity has been reported in different species. Previously traits related to sodium exclusion and sequestration in vacuoles and shoot have been considered as major factors determining salinity stress tolerance in plants. However, recent evidences have suggested that cytosolic K^+ retention also plays a critical role in plant performance under saline conditions. The current study aimed to evaluate the essentiality of this trait and examine the tissue specific regulation of ion homeostasis in halophyte (quinoa) and glycophytes (spinach) under saline conditions. Our results indicated that quinoa showed better biomass production and photosynthetic efficiency than spinach and this was positively correlated with ability of quinoa to retain K^+ in leaf mesophyll and root mature zone as compared with spinach. Moreover, data relating to the kinetics of xylem ion loading showed that quinoa uptakes more Na^+ than spinach, suggesting halophytes use Na^+ as a cheap osmoticum. Confocal imaging data showed that quinoa possesses better vacuole buffering K^+ capacity and use K^+ efflux as a signalling moiety to act as a 'metabolic switch' aimed to conserve energy for adaptation to saline conditions, while spinach (a glycophytes) lacks this mechanism.

Poster Session 2

#64: Nicholas Booth

Cellular regulation of key chickpea osmoprotectant systems under salinity stress

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Salinity stress is one of the major limitations to crop production globally. Not only do highly saline soils result in yield reductions, but also cause many areas to be agriculturally unviable. Chickpea (*Cicer arietinum*) is particularly sensitive to salinity stress, with salt concentrations as low as 40 mM shown to impair growth. An RNAseq dataset of the salt-sensitive cv. Rupali collected previously, identified a host of genes involved in the salinity stress response. More recently, it has been shown that cv. Rupali accumulated higher concentrations of Na^+ in the photosynthetically-active mesophyll cells compared to the salt-tolerant Genesis836 (Kotula et al., 2019). Therefore, this variety can be used as a model system to create a gene expression profile of the response to high accumulation of ions within plant tissues. We found that genes involved in the biosynthesis of osmolytes, required to help alleviate the immense osmotic shock produced by Na+ accumulation, were highly responsive to the treatment. These included members of the Galactinol Synthase (GolS), Late Embryogenesis Abundant (LEA) and delta (1)-Pyrroline-5-Carboxylate Synthetase (P5CS) families. The expression of GolS2L, LEA1, LEA2 and P5CS1 was validated by qRT-PCR and mirrored the results observed in the RNAseq data. We also observed a strong positive correlation between the expression of these genes and the level of oxidative damage, and identified a number of common motifs within their promoter regions, which include G-Box, MYB, MYC and a potential heat-stress-responsive element. This work helps to elucidate the responsive nature of osmoprotectant systems under salinity stress. Further characterisation by overexpression/knockdown mutants is required to fully understand their function. Kotula, L., Clode, PL., Jimenez, JDLC., and Colmer, TD. (2019) Salinity tolerance in chickpea is associated with the ability to 'exclude' Na from leaf mesophyll cells, *J Exp Bot*, erz241.

Poster Session 2

#65: Ronan Broad

CRISPR/Cas9-Targeted Mutagenesis of the GDP-L-Galactose Phosphorylase Upstream Open Reading Frame in Rice

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Ascorbate, also known as vitamin C, is the most abundant water-soluble antioxidant in plant cells and plays important roles in photosynthetic function and stress tolerance. The *GDP-L-galactose phosphorylase* (*GGP*) gene encodes the rate-limiting enzyme of the L-galactose pathway—the dominant ascorbate biosynthetic pathway in plants—and is a promising gene candidate for increasing ascorbate concentrations in crops. In addition to transcriptional regulation, GGP production is regulated at the translational level through an upstream open reading frame (uORF) in the 5'-untranslated region. Disruption of the *GGP* uORF in *Arabidopsis thaliana*, lettuce (*Lactuca sativa* L.), and tomato (*Solanum lycopersicum* L.) with CRISPR/Cas9-targeted mutagenesis has recently been shown to increase ascorbate concentrations and enhance oxidative stress tolerance. In this study, we have utilized CRISPR/Cas9-targeted mutagenesis in rice (*Oryza sativa* L.) to generate a collection of $uorf_{OsGGP}$ mutant alleles. The $uorf_{OsGGP}$ mutants have 1.3- to 1.6-fold more foliar ascorbate, relative to wild-type, under glasshouse conditions. Here we present findings from several analyses of the $uorf_{OsGGP}$ mutants including expression of the endogenous *OsGGP* gene, whole life-cycle phenomic characterization, and stress tolerance.

Poster Session 2

#66: Santosh Khanal

UVA stress and the expression of phenolic compounds by Eucalyptus camaldulensis ssp. camaldulensis: influence of genotype and leaf age

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The genus *Eucalyptus* is one of the most diverse groups of trees globally, comprising 700+ species endemic to diverse Australian habitats and some spanning a wide range of latitudes. Consequently, they are an excellent candidate for studying plant responses to stress. Plants synthesize phenolic compounds in response to biotic and abiotic stress and the broad distribution of compounds suggests they are important in a range of physiological and/or ecological roles. For example, eucalypts produce an array of polyphenolic compounds with the total composition of phenolic metabolites sometimes representing up to 40% of leaf dry weight and tannins alone representing up to 27% of DW. We used a metabolomics-based approach to study how UV_A affected the production of foliar metabolites in five genotypes of *Eucalyptus camaldulensis* subspecies *camaldulensis* (a species found across a wide latitude range) grown under glasshouse (two constant levels of UV exposure) and ambient conditions (natural UV exposure). These genotypes encompass the native range of the subspecies (spanning 5 degrees of latitude) and have presumably therefore evolved under different levels of exposure to solar radiation. Since the composition of polyphenolic compounds changes as leaves age, we stratified our sampling to include leaf age. Analyses of young leaves (up to 3 months old) exposed to high UV_A ("summer" conditions) revealed high abundances of various hydrolyzable tannins, in particular gallotannins and ellagitannins. In contrast, older leaves (> 6 months) had high abundances of condensed tannins and flavonoids. The influence of leaf age was more apparent than that of genotype. Nevertheless, genotypes from lower latitudes (higher ambient UV_A) had higher abundances of phenolic glycosides and gallic acid than genotypes from higher latitudes (lower UV_A). Our findings are consistent with photoprotection of young leaves by hydrolysable tannins and protection from herbivory of old leaves by condensed tannins.

Poster Session 2

#67: Sapna Badhan

Understanding the drought tolerance mechanism and designing strategies to mitigate abiotic stress in chickpea

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RNA sequencing of leaf tissues from two contrasting chickpea genotypes reveals mechanisms for drought tolerance Sapna Badhan, Pravas Kole, Andrew Ball, Nitin Mantri∗ Our current study is focused on understanding the drought mechanism and pathways which are responsible for drought tolerance in two contrasting chickpea genotypes. Chickpea (Cicer arietinum L.) is the second most important winter crop which is consumed globally due to its high nutritional value. Chickpea as one of the leguminous crop is important in crop rotation with cereal crops like wheat and barley. The main constraints for chickpea production are abiotic stresses such as drought, salinity, and heat. Among these, drought is a major cause of the decline in chickpea production in worldwide. Studies conducted so far have provided a limited insight into different genetic pathways associated with drought tolerance/response. In this study, the leaf tissue from shoots apical meristem stage of drought tolerant (ICC8261) and drought sensitive (ICC283) genotypes were analysed using RNA sequencing to identify genes/pathways associated with drought tolerance/sensitivity in both genotypes. It was observed that genes related to ethylene response, MYB-related protein, xyloglucan endotransglycosylase, alkane hydroxylase MAH-like, BON-1 associated, peroxidase 3, cysteine-rich and transmembrane domain, vignain and mitochondrial uncoupling were specifically up-regulated in the tolerant genotype whereas, same genes were down-regulated in sensitive genotype. The crosstalk between the different hormones and transcriptional factors involved in drought tolerance and sensitivity in both genotypes make them great candidates for future research. We are also trying to look into the role of epigenetic factors such as DNA methylation in the regulation of gene expression and further validation of selected genes using CRISPR/Cas 9 gene editing. Badhan, S., Kole, P., Ball, A., and Mantri, N. (2018) RNA sequencing of leaf tissues from two contrasting chickpea genotypes reveals mechanisms for drought tolerance.

Poster Session 2

#68: Sneha V. K. Gupta

Investigation of lipids in salt stressed barley roots inoculated with *Trichoderma harzianum*

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In the past few decades, an alternative strategy to improve salt tolerance of plants has been the use of salt tolerant microbes that enhance crop growth under salinity conditions [1-3]. Their interactions with the plants induce several local and systemic responses which improve metabolic capacity of the plants to fight against abiotic stresses [4]. Metabolomics is increasingly being used for generating deep insights into abiotic stress responses. Recently, focus on exploring and discovering compounds that stimulate plant growth inoculated with beneficial microorganisms, to alleviate stress is gaining popularity. We investigated the effects of inoculation of the cereal crop barley with the salt tolerating beneficial fungus Trichoderma harzianum, focusing on biomass and lipid changes under salinity stress. Barley is chosen for this study as it is Australia's second most important cereal crop and is the most salt tolerant of all cereal crops. For confirmation of fungal association with roots, brightfield microscopy was performed on inoculated roots after sixteen days (9DPI). For biomass study, seedlings were grown on agar plates with barley agar growth media and in PVC pipes with sandy soil. The phenotypic analyses suggest that inoculation with T. harzianum improved plant growth at the time of harvest, with an increase in root length and shoot biomass. To further investigate the lipidome affecting those interactions, root samples were collected to study the changes in lipids using liquid chromatography mass spectrometry (LC-MS) technique. Further, Mass Spectrometry Imaging (MSI) was performed on roots harvested from agar plates, to reveal subcellular co-localisation of lipids within root tissues. Our analyses using both techniques suggest a shift in the lipid profile between inoculated and uninoculated plants. Phospholipids was the major class of lipids detected using LC-MS and MSI. With the integration of results obtained from phenotypic and metabolomic approaches, we aim to understand the biochemical basis of these interactions so that a broader picture of how plants interact with belowground life to tolerate abiotic stress, particularly salinity, can be created.

Poster Session 2

#69: Tetsuya Ishikawa

Revealing physiological basis of differential salinity stress tolerance between cultivated rice (Oryza sativa) and its halophytic relative (Oryza coarctata)

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Soil salinity is one of the major constraints for the global agricultural production that results in massive (over US$27 billion per year) economic losses. Rice is the second most popular cereal crop in the world, but this plant is highly sensitive to salinity. Understanding the mechanisms of salinity tolerance is essential to breed salt tolerant rice cultivars that will contribute to the global food security. *Oryza coarctata* is the only halophytic relative in the genus of rice cultivars (*Oryza* sp.) which grows in the coastal area of South-East Asian regions and is tolerant to high salinity levels lethal to normal rice cultivars (*Oryza sativa*). A series of electrophysiological assays was conducted on root epidermis of *O. coarctata* and *O. sativa* (cv. Koshihikari) by using microelectrode ion flux measurement (MIFE^{TM}) technique, to reveal the key mechanisms of ion transport differentiating their salinity tolerance. First, *O. coarctata* demonstrated the ability to exclude Na^+ from mature root zone by efficient operation of Na^+/H^+ exchanger while in cultivated *O. sativa* that exclusion was confined only to the root apex. Second, *O. coarctata* effectively retained root K^+ in response to salt treatment that is essential to maintain normal plant growth and metabolisms. *O. coarctata* was also more energy-efficient and did not activate the plasma membrane H^+-ATPase upon salinity exposure, thus being able to redirect more ATP towards cell metabolism and growth. Finally, oxidative stress (10 mM H_2O_2)-induced Ca^{2+} flux signatures were different between two species. Taken together, *O. coarctata* possesses superior abilities of controlling ion transport under salinity stress, however its detailed mechanisms of signalling pathways and transcriptional regulations are still highly unknown. Further investigations are required to apply the key traits of ion transport into the future breeding programs to produce salt tolerant rice.

Poster Session 2

#70: Waseem Ashfaq

Application of Infrared thermal imaging technique and physiological indices for wheat genotypes screening against drought and heat stress

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Wheat (*Triticum aestivum* L.) is one of Australia's major cereal crop, which often experience various environmental stresses during critical growth stages. Drought and heat are one of the primary causes of yield reduction due to climatic change. The purpose of this study was to identify the drought and heat stress tolerant wheat genotypes from a set of 46 wheat genotypes which were procured from Australian Grains GeneBank, Horsham, VIC Australia. Two separate pot experiments were conducted in controlled growth chambers using augmented randomized complete block design with four biological replicates. Previously identified genotypes RAC875, Excalibur, Drysdale, Axe (tolerant) and Kukri (susceptible) were being used as checks in drought and heat stress experiments. 10 days after anthesis, drought stress was induced by complete withholding of water for 14 days, while for heat stress study, diurnal temperature in growth chamber was maintained to 36/22 °C for three consecutive days. Stress tolerance and susceptibility of genotypes were determined based on a set of physiological traits including rapid, non-destructive measures of plant canopy temperature (Tc) and crop water stress index (CWSI) using infrared thermal imaging (IRTI). Genotypes with higher RWC, Photosynthetic activity, quantum yield and grain yield with lower values of excised leaf water loss under drought stress were classified as tolerant. Similarly, genotypes with higher values of quantum yield, chlorophyll content and grain yield with lower values of Tc and CWSI under heat stress were identified as tolerant genotypes. Based on this preliminary screening, ECH-957, RAC875 and Excalibur were identified as promising entries while Kukri, RAC704, CM59443 were classified as susceptible genotypes under both drought and heat stress treatments. After these results, it can be concluded that IRTI along with other physiological parameters proved to be high-throughput screening tools against drought and heat stress to estimate the stress tolerant variability in wheat genotypes.

Poster Session 2

#71: Yan Wang

Mitochondrial retrograde regulation at post-transcriptional level

Wang, Y.[1], Lyu, W.[1], Meng, X.[1], Mao, C.[1], Zhu, Y. and Whelan, J.[1]

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Under adverse growth conditions, mitochondria generate signals to alter nuclear genes expression, generally referred to as retrograde signaling pathways. These organelle-signaling pathways are important for optimal growth and development, as chloroplasts and mitochondria provide the energy and many of the metabolite building blocks that are required for cell growth and division. The induction of Alternative Oxidase 1A (AOX1A), a cyanide insensitive terminal oxidase, is widely used as a marker of mitochondrial signaling, as it is induced when mitochondrial function is perturbed. A number of studies using forward and reverse genetic approaches have identified some components involved in mitochondrial retrograde signaling in plants. The first component identified as a regulator of *AOX1a* in Arabidopsis was the transcription factor ABSCISIC ACID INSENSITIVE4 (ABI4) through identification of a conserved binding site for ABI4 in the promoter of *AOX1a* using promoter studies and mutagenesis experiments. Likewise, a number of WRKY transcription factors have been identified as positive and negative regulators of *AOX1a*. A family of endoplasmic reticulum (ER) bound NAC transcription factors have been identified, that are released from the ER after proteolytic cleavage upon mitochondrial dysfunction to activate gene expression. In contrast, MYB DOMAIN PROTEIN 29 (MYB29) was identified as a negative regulator as the mutation led to a longer lasting mitochondrial retrograde response. A number of kinases, *i.e*. CDKE1 (cyclin-dependent kinase E1), a subunit of the mediator complex, and KIN10 (SnRK1 - Sucrose non-fermenting Related protein Kinase 1), and several components involved in auxin signaling, have also been characterized as regulators of the induction of *AOX1a* in Arabidopsis. So far, most of the identified components regulate *AOX1a* at transcript level. Here, my study shows two novel components, Retarded Root Growth Like Protein (RRL) and Regulator of AOX1a 9 (RAO9), are requested to complete retrograde response at post-transcriptional level. RRL is required for the protein stability of AOX1A through interacting with the component of Ubiquitin Proteasome System. RAO9 is proposed to regulate AOX1A protein expression through alternative spicing of *AOX1a* or the translation/protein degradation machinery that is requested for the efficient translation and/or protein stability of AOX1A.

Poster Session 2

#72: Ying Meng

The Role of GABA under salt and hypoxia stress

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Gamma-aminobutyric acid (GABA) is a nonproteinogenic amino acid which is mainly synthesized from alpha-decarboxylation of glutamate (Glu) catalysed by glutamate decarboxylase (GAD) (Yin et al., RSC Advances 2018). GABA accumulates under a variety of stress conditions of which salinity and flooding are among the most intractable abiotic stresses that plant face (Bown and Shelp, Trends Plant Sci 2016). GABA accumulation occurs to the greatest extent during hypoxia and anoxia (Kinnersley and Turano, Critical Reviews in Plant Sci 2000), however, its exact role is still to be elucidated. Here we study the role of GABA during submergence stress in *Arabidopsis* thaliana. The materials used include Arabidopsis wildtype and mutants (Col-0): GABA-depleted lines (*gad1, gad2 and gad1/2/4/5*), GABA-accumulating lines by either GABA-T deficiency (*pop2*) or *GAD2*-overexpression (*B33* and *B97*). In the salt experiment, *pop2* total root length is significantly larger than WT after 9 days on 1/2 MS, mainly because their lateral roots developed faster, and this is brought down by 50 mM NaCl treatment to WT level. Interestingly, *gad1/2/4/5* and *pop2* have a greater primary root Relative Elongation Rate (RER) than WT on 1/2MS but not under salt, while these were not seen in *B33* or *B97*. In the hypoxia experiment, preliminary results of Relative Water Content (RWC), photosynthesis system Ⅱ (Fv/Fm) and phenotyping showed that *pop2* and *gad2* was the most and least sensitive respectively during a 6-day submergence. Other mutants were not affected compared to wildtype and were able to recover after 3-day de-submerge, while *pop2* couldn't. RNAseq and metabolites data analysis currently being performed will allow us to have a clearer and deeper understanding of whether GABA accumulates only as a result of metabolic perturbation under stress, or if GABA has a critical function for flooding tolerance.

#73: Chatchawan Jantasuriyarat

Genetic diversity of avirulent gene, AVR-Pik of rice blast fungus and its molecular interaction with blast resistant gene, Pik, in rice

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Rice blast disease, caused by the ascomycete Magnaporthe oryzae, is one of the most destructive rice diseases worldwide. The resistant rice varieties usually become susceptible to the disease within a few years of release because the instability of avirulence gene in blast fungus. We analyzed fungal characteristics, spore morphology, spore germination rate and the genetic variation of effector gene, AVR-Pik in 58 rice blast isolates from Thailand. Inter simple sequence repeat (ISSR) and Sequence-related amplified polymorphism (SRAP) markers were used to analyse the genetic diversity, 14 ISSR primers and 17 pairs of SRAP primers that produced clear and polymorphic banding profiles were selected for assessing genetic diversity. AVR-Pik gene was analysed for the sequence variation. We identified new allele and studied the interaction between AVR-Pik and resistant gene Pik in rice. The pathogenicity assay showed that the novel allele of AVR-Pik is extremely aggressive. No natural occurring allele of Pik gene can recognise this novel allele. Genetic and genomic tools were used to characterise their binding interaction. The results will be presented and the possible approaches to protect the infection process will be discussed, which the information obtained here will lead to the understanding in the mechanism and the evolution of rice blast fungus to overcome the resistance in rice.

Poster Session 2

#74: Courtney Winning

Characterisation of the nodule-specific UmamiT proteins in Medicago truncatula

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Root nodulation results from a multi-stepped symbiosis between rhizobia and legumes. Rhizobia housed in mature nodules fix atmospheric nitrogen (N) into ammonia and are responsible for supplying a substantial proportion of the biologically-available N in global agroecosystems. N-fixation is, however, energetically and metabolically costly. The cost/benefit ratio is favourable when legumes grow under low-N; here rhizobia can supply the host's entire N needs. Rhizobia that infect the plant prior to the N-fixation stage do not fix N. During this pre-N-fixation stage of the symbiosis rhizobia are encased in plant membranes called infection threads. Therefore, an unresolved question is what nutrients are supplied to rhizobia in the pre-symbiotic stages of infection. During these initial steps rhizobia require external N, which is likely supplied as host-derived amino acids. Unfortunately, an obvious candidate plant transporter for the supply of amino acids to rhizobia has been lacking. Recently, a family of plant-specific amino acid transporters, called the 'Usually Multiple Amino acids Move In and Out Transporters' (or UmamiTs), were characterised in *Arabidopsis* as possible amino acid exporters. Interestingly, a subset of these UmamiTs show a nodule-specific expression pattern in the pasture legume, *Medicago truncatula.* Since UmamiTs evolved early in the viridiplantae lineage (prior to nodulation), it is likely that these nodule-specific UmamiTs have been repurposed to have a symbiotic context from UmamiTs with prior functions. We have investigated whether these nodule specific UmamiTs mediate amino acid transport. We aim to identify the specific amino acids that are likely to be provided to rhizobia during the pre-symbiotic stages of nodulation, and the importance of this supply to the development of the infection. This will lead to a greater understanding of the evolution of legume-rhizobial symbioses and provide insights into the mechanisms controlling the pre-symbiotic relationship of rhizobia with legumes

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Poster Session 2

#75: Peter McGuiness

Brassinosteroids regulate early nodulation events in concert with ethylene

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Nodulation is a globally important symbiosis between plants and nitrogen fixing bacteria. It is formed by a variety of mainly leguminous plants, including the model legume species pea (*Pisum sativum*), and symbiotic bacteria that convert atmospheric nitrogen into ammonia to support plant growth. In return the plant supplies the bacteria with energy. Plants have evolved complex regulatory systems to control this symbiosis involving many plant hormones. This study focused on the brassinosteroid plant hormones. Prior studies suggested that brassinosteroid signalling might increase nodule numbers (Ferguson et al., 2005; Foo et al., 2016). However, little is known about the mechanisms by which brassinosteroids are involved in nodulation. This study used brassinosteroid biosynthesis and receptor mutants in pea to investigate various stages of nodule development: root hair curling, infection thread formation, nodule initiation, nodule growth and nitrogen fixation. Brassinosteroids were found to repress infection thread formation but promote nodule initiation. Prior studies demonstrated that ethylene signalling was epistatic to brassinosteroid signalling for visible nodule formation (Foo et al., 2016). This study used a brassinosteroid deficient ethylene insensitive double mutant to determine if this was the case for infection thread formation. This study found that ethylene and brassinosteroids inhibit infection thread formation in an additive manner, suggesting that they utilise independent pathways to regulate the number of infection threads formed. Additionally, we used acetylene reduction assays and metabolomics to investigate the role of brassinosteroids in nodule function. The former technique measured the rate of nodule nitrogen fixation, while the latter examined key metabolites such as asparagine, glutamate, homoserine, and sucrose. Both approaches did not show an effect of brassinosteroids on nodule function, suggesting that brassinosteroid mutants produce fully functional nodules. Overall, this study suggests that brassinosteroids inhibit infection thread formation but promote nodule organogenesis and do not alter the function of developed nodules.

Poster Session 2

#77: Rebecca Vandegeer

Double trouble: Can silicon improve plant resistance to drought and herbivory?

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Silicon (Si) is abundant in soils, where it is taken up by plant roots, transported within the plant, and deposited into organs as silica bodies (SiO_2). Si has been widely reported to improve plant resistance to biotic (e.g. herbivory) and abiotic (e.g. drought) stress. However, few studies have explicitly tested for the underlying physiological mechanisms generating this putative drought tolerance, or the implications of Si-induced drought tolerance on insect performance. We grew tall fescue (*Festuca arundinacea*) in a hydroponic system with or without Si. After 8 weeks, half the plants from each Si treatment were treated with a 20% polyethylene glycol treatment (PEG; osmotic stress), to simulate drought conditions. After 14 days, half the plants were harvested for plant physiological parameters (photosynthesis, stomatal conductance, water potential, biomass, Si content, specific leaf mass). The other half of the plants were each exposed to an individual 4th instar cotton bollworm larva (*Helicoverpa armigera*) for an additional 7 days to determine herbivore relative growth rate (RGR). Contrary to previous studies, we found little evidence that Si alleviates the negative effects of osmotic stress on plant physiological performance. In fact, Si further reduced leaf water potential and photosynthesis of plants exposed to osmotic stress. In contrast, both Si supplementation and osmotic stress reduced herbivore RGR. Our findings show that Si plays a role in the resistance of grasses to herbivory during osmotic stress, but suggest that the role of Si during drought may be limited.

#78: Suree Nanasombat

Control of postharvest fungal decay on tomato by organic acid and salt solutions

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In this study, 10 samples of fresh tomato were evaluated for fungal contamination by dilution plating method. These tomato samples were highly contaminated by molds and yeasts ($1.0x10^5$ - $1.4x10^8$ CFU/g). Among all fungal strains isolated including *Geotrichum*, *Penicillium*, *Rhizopus*, *Candida*, *Cryptococcus* and *Rhodotorula*, the highest proportion of molds found in tomato were *Penicillium* and *Rhizopus*. Then, the effect of organic acid (ascorbic acid, lactic acid, acetic acid and tartaric acid) and salt (potassium sorbate, sodium benzoate, potassium acetate, potassium metabisulfite and ammonium carbonate) solutions on controlling of molds and yeasts were studied by determining the minimum inhibitory concentration (MIC). Salt solutions showed more inhibitory effect to fungal growth than organic acid solutions. *Rhizopus stolonifer* isolated from tomato was sentitive to potassium sorbate and sodium benzoate (0.01-0.1% MIC), while *Alternaria alternata* and *Penicillium citrinum* was sentitive to potassium metabisulfite and ammonium carbonate (0.5-1.0% MIC). Ammonium carbonate and potassium metabisulfite effectively inhibited the growth of *Candica guilliermondii* and *Rhodotolula mucilaginosa* isolated from tomato (0.25-1.0% MIC). Furthermore, lactic acid was found to be more effective to inhibit most of mold and yeast strains tested as compared to other acids. Application of organic acid and salt solutions to control the growth of *R. stolonifer* on tomatoes was evaluated. Salt solutions were more effective to inhibit the growth of this mold on tomatoes, compared to acid solutions. Among all salt and acid solutions tested, potassium metabisulfite completely inhibited the growth of *R. stolonifer* on tomatoes at 1% concentration. However, use of potassium sorbate to inhibit this mold on tomatoes was effective at 3% concentration.

Poster Session 2

#79: Jose Barrero

Molecular analysis of the impact of heat during grain filling on wheat dormancy

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Short episodes of extreme high temperatures during early-mid grain filling can eliminate dormancy, and also reduce grain size and alter composition. This problem is expected to increase its frequency under the climate change scenarios with forecasted increasing temperatures in many wheat growing areas of the world. Because of that, a comprehensive genetic analysis of the resistance to extreme temperatures is needed. To fill this gap and provide the necessary information for isolating wheat cultivars with heat-resistant dormancy we have analysed a panel of Australian and Chinese wheat commercial lines and landraces. To genetically dissect the effect of heat during mid-grain filling, plants were grown under control conditions in a glasshouse and tagged at flowering. At a set date early in grain filling, some plants from each genotype were moved to a second glasshouse to be exposed to a heat treatment for one week, and then returned to control conditions. Grains were harvested at maturity and their dormancy, anatomy and composition was analysed. Although the majority of the genotypes were impacted by heat, we found some lines that were able to maintain their grain characteristics. This has allowed us to identify genotypes with “temperature-sensitive dormancy” or with “temperature-insensitive dormancy”. Samples were also collected for the expression analysis of major genes involved in the regulation of dormancy in order to understand if there is a link between any of these major genes and the resistance to heat.

Poster Session 2

#80: Chris Brown

The effect of photon flux density on cell wall components in different genotypes of Cynodon.

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Limiting the photosynthetic capacity of a plant by reducing photon flux density (PFD) restricts the availability of components required to synthesise the cell wall. Plants grown under low light conditions exhibit etiolation, characterised by decreased biomass and elongated internodes with altered cellulose microfibril and lignin content. *Cynodon dactylon* and hybrids with other *Cynodon* species are commonly referred to as Bermuda grasses. Widely used as forage and turf grasses they exhibit elevated drought tolerance. To determine the impact of reducing PFD on *Cynodon* grasses a selection of eight genotypes of C. dactylon and *C.dactylon* x *C. transvaalensis* hybrids were grown in 2.4 x 1.2m plots before being subjected to one of four shade treatments 0%, 30%, 50% and 70%. Plots were clipped to a height of 20mm twice weekly over 12 weeks at Redlands Research Station (QDAF) in Queensland. Clippings were analysed using partial least squares (PLS) regression models based on Fourier transform infrared (FTIR) spectra to predict cell wall composition and ultimately digestibility, in terms of animal forage. PLS regression models were calibrated using a two-stage sulphuric acid hydrolysis protocol to determine the composition of the cell wall. Using this approach, it is possible to identify genotypes that tolerated reduced PFD without detrimentally impacting the composition of the cell wall and thereby digestibility.

Poster Session 2

#81: Diego Lozano-Claros

Developmental time adjustment for high throughput plant phenotyping (HTPP) systems

Lozano-Claros, D[1], Meng, X[1], Custovic, E[1], Deng, D[2], Berkowitz, O[2], Whelan, J[1] and Lewsey, M.[1]

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The time of sowing is typically considered as the temporal reference for plant experiments in HTPP systems. However, this reference can result in misleading outcomes because it relies on the assumption that germination and seedling establishment are uniform across the population. On the contrary, seedlings show a different time response for both bioprocesses even within a species under similar environmental conditions. It will lead to increased variance in quantitative phenotyping approaches. We developed a method called “Digital Adjustment of Plant Development” (DAPD). It combines time-series trait measurement with normalisation by developmental stage in an automated manner. It synchronises the start-point of timelines for all plants in the population by their number of leaves. To achieve this, we also present a new leaf counting algorithm that outperforms existing algorithms. We apply it to evaluate the relative growth rate in Arabidopsis thaliana and demonstrate that it improves uniformity in measurements, permitting a more informative comparison between individuals. The algorithm allows users to select and define the starting leaf number relevant to their experiment.

#82: Georgia Guild

The use of EDXRF for rapid screening of micronutrient concentrations in biofortification breeding programs

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Energy Dispersive X-Ray Fluorescence (EDXRF) analysis is a new tool for analysing iron (Fe) and zinc (Zn) concentration in staple food crops such as wheat, rice and maize. The benefits of this method include little to no sample preparation, minimal consumable costs, rapid analysis and its ease of use by operators. We have developed crop specific calibrations for HarvestPlus target crops to ensure the most accurate results. The quantification range with EDXRF is crop dependent but can range from 3 – 140 mg kg-1 for Fe and 7 – 110 mg kg-1 for Zn, making this technique suitable for the range of micronutrient found in the target crops. Validation of these calibrations showed that results from EDXRF are, on average, within 4.5 mg kg-1 of the results from reference ICP analysis. When compared to conventional micronutrient analysis (ie ICP-MS, ICP-OES and AAS) the total analysis time is significantly reduced (1 minute per sample). Samples are analysed either as whole grain or flour without the need for acid digestion. This further reduces analysis time and cost per sample compared to conventional techniques. The high throughput and low cost (~$1 AUD) make this technology ideal for rapid screening of grains and to date, 22 plant breeding labs worldwide are using this technology.

Poster Session 2

#83: William Salter

An Affordable 3D Scanner to Phenotype the Complex Architecture of Chickpea Plants

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Phenotyping of plant structural traits is important to understanding underlying plant physiology and in determining plant productivity, however, until recently we have been limited to fairly crude and often destructive measurement techniques. 3D imaging has been proven to work well as a non-destructive tool for acquisition of structural data from monocot cereal species such as wheat, rice and maize. Plants with more complex architecture, such as chickpea, remain elusive due to their small leaves and highly branching nature. We have developed a novel, low-cost 3D scanner and a data processing pipeline to accurately reconstruct 3D models of individual chickpea plants. These models can be used to acquire detailed data of plant architecture. The scanner platform consists of a motorised turntable, LED lighting and three DSLR cameras, all automatically controlled with a user-programmable microcontroller. The software used to reconstruct the 3D models is open source, semi-automated and can be run on a Windows PC. In total, the scanner platform costs less than AUD $1500, considerably less than commercially available alternatives. Our aims are to use the scanner to phenotype traits such as plant surface area, plant height, projected plant area index, branching angles and number of leaves, flowers and pods (amongst others). In early testing of the system we have been able to predict plant height to within 1 cm of the true height using our 3D models and are now ground truthing for plant and leaf surface area. We hope that this technique will help to inform future breeding decisions and open up the possibility of 3D imaging to more plant scientists.

Poster Session 2

#84: Alban Mariette

Molecular Insights on Arabinosylation Pathway in *Arabidopsis thaliana*

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The cell wall is one of the main energy sinks in plants primarily composed of polysaccharides and proteins. The synthesis of most of the polymers and the glycosylation of the proteins occur in the Golgi Apparatus (GA). Nucleotide sugars, the building blocks of the cell wall, are transported from the cytosol to the Golgi by the nucleotide sugar transporters (NSTs). The glycosyltransferases (GTs) then facilitate the polymerization of these imported nucleotide sugars as well as glycosylation of cell wall proteins. Of the different components in the cell wall, arabinose constitutes 10% of the cell wall monosaccharides in leaves and is present in pectins forming the side-chains of Rhamnogalacturonan-I and Rhamnogalacturonan-II. Arabinose also extensively decorates Extensins and Arabinogalactan-proteins. The arabinose residues of the cell wall exist in the furanose ring conformation. Cytosolic UDP-arabinomutase is involved in the isomerization of UDP-arabinopyranose into UDP-arabinofuranose which is then transported into the Golgi via the UDP-arabinofuranose transporters (UAfTs). Several GTs putatively involved in arabinofuranosylation have also been identified for pectin synthesis and proteins arabinosylation. These findings suggest the presence of protein complexes channelling UDP-arabinofuranose to the GTs. The UAfTs are therefore a central player of the arabinofuranosylation pathway yet a detailed understanding of the pathway is lacking. We aim to decipher the existence of protein complexes and the regulation of the arabinosylation pathway in the GA. We will do so by means of molecular characterization of higher order mutant, gene expression patterns of putative genes involved in arabinosylation and undertake protein interaction studies.

Poster Session 2

#85: Cheka Kehelpannala

Investigating the Tissue-Specific Lipidomes of *Arabidopsis thaliana* Across Development

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A comprehensive analysis of lipids from the model plant Arabidopsis has been challenging due to the complexity of lipid species and difficulties in identifying lipids. To tackle this problem we developed an analytical workflow for the identification and quantification of lipids from Arabidopsis using both targeted and untargeted approaches. This workflow will allow us to build a plant lipid-specific database to be mined by the scientific community. Furthermore, we aim to develop an open-source *in-silico* lipid map of Arabidopsis tissues across development, which will improve our understanding of lipid biosynthetic pathways and the roles of specific lipids in plant growth and development. We compared multiple lipid extraction techniques and mass spectrometric methods to develop a robust, high-throughput extraction and detection workflow. Lipids were extracted from different tissues and organs of plants grown under standard conditions. An Agilent QTOF 6545 coupled to a 1290 HPLC was employed to detect lipids which were subsequently identified using the LipidMatch software. We then applied this optimized workflow to investigate the tissue-specific lipid profiles of Arabidopsis during development. Currently, the most complete study identified 140 apparent polar lipids from six different tissues of Arabidopsis. By contrast, the untargeted lipidomic analysis workflow that we developed was able to putatively identify more than 600 lipid species belonging to 14 different lipid classes from seven different tissue types. In consistence with published data, monogalactosyldiacylglycerols and triacylglycerols were the most abundant lipid classes found in all tissues. Leaves were found to contain more monogalactosyldiacylglycerols and digalactosyldiacylglycerols compared to other tissues, while seeds contained more triacylglycerols. Significant differences were observed in lipid species and content of each tissue. The lipid composition of leaves was significantly different from all other tissues, while it was similar between stems and seedlings and between flowers and siliques.

Poster Session 2

#86: Christina Offler

Enzymes Contributing to the Hydrogen Peroxide Signal Dynamics that Regulate Wall Labyrinth Formation in Transfer Cells

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Transfer cells (TCs), specialized for resource transport, are characterized by an amplified plasma membrane area supported on a wall labyrinth comprised of a uniform wall layer (UWL) upon which wall ingrowth (WI) papillae are deposited. Adaxial epidermal cells of developing *Vicia faba*cotyledons, when placed in culture, undergo a rapid (h) *trans*-differentiation to a functional epidermal TC (ETC) phenotype under control of a signalling cascade comprising auxin, ethylene, apoplasmic reactive oxygen species ($_{apo}$ROS) and cytosolic Ca^{2+}. Hydrogen peroxide (H_2O_2) was confirmed as the active $_{apo}$ROS species regulating UWL and WI papillae formation. Informed by an ETC-specific transcriptome database, a pharmacological approach identified a temporally changing set of H_2O_2biosynthetic enzymes comprising a respiratory burst oxidase homolog, polyamine oxidase, copper amine oxidase and a suite of class III peroxidases that generated two consecutive bursts in $_{apo}H_2O_2$production. Spatial organization of biosynthetic/catalytic enzymes was deduced from responses to pharmacologically blocking their activities on cellular and sub-cellular $_{apo}H_2O_2$ distribution. The findings were consistent with catalase activity constraining the $_{apo}H_2O_2$signal to the outer periclinal wall of the ETCs while strategic positioning of class III peroxidases in this outer domain shaped sub-cellular $_{apo}H_2O_2$signatures that differed during assembly of the UWL and WI papillae.

Poster Session 2

#87: Cunman He

ALTERNATIVE OXIDASE 1c has a Crucial Function for Plant Growth and Development

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The cyanide-insensitive alternative oxidase (AOX) terminates the alternative pathway of mitochondrial electron transport and uncouples oxidation of substrates from mitochondrial ATP production, leading to improved plant performance under adverse growth conditions. Under standard growth condition the activity of AOX is considered wasteful, as the electron flow is non-phosphorylating and the energy dissipated as heat. *Arabidopsis thaliana* possesses five AOX isoforms (AOX1A-D and AOX2). *AOX1a* and *AOX1c* genes are expressed in a variety of tissues while the other three *AOX* genes display organ- or development-specific expression. While AOX1a is the major isoform responsive to stresses impacting mitochondrial function, little is known about the specific function of the other AOX isoforms. Out of the five AOX isoforms, *AOX1C* is expressed at the lowest level when compared to *AOX1a*. *AOX1c* might play a housekeeping role as it is unresponsive to most stress treatments as shown by expression analysis (Clifton et al., 2006). We show that an *aox1c* knockout line displays growth retardation, pale leaves and altered development compared to wild type plants even when grown under normal growth light conditions. Complemented mutant lines are similar to the wild type, but interestingly – and somewhat counterintuitively due to its 'energetically wasteful' nature – *AOX1c* overexpressor lines accumulate more biomass than the wild type as witnessed by bigger rosette size. RNA-seq analysis identified differences in the transcriptomes of these genotypes and possible underlying mechanisms for AOX1c function. Further analysis of these genotypes will reveal the role of *AOX1c* in plant development.

Poster Session 2

#88: David Collings

X-Ray Microtomography Investigations into the Origin of Grain Patterns in Wood

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X-ray microtomography (uCT) uses X-ray images taken at different angles to reconstruct 3D organisation. Compared to traditional observation methods, uCT can observe much larger samples at near cellular resolution, and provides data sets that can be recut in different orientations. uCT and image analysis in ImageJ were used to investigate wood grain in commercially important timbers to understand the development of grain patterns, a process that remains poorly characterised at a cellular and molecular level. The tropical angiosperm Khaya (African mahogany) shows interlocked grain where grain oscillates by up to 20^{o} or more from vertical, with these grain variations being the origin of the optical properties of mahogany veneers (figure or lustre). Imaging confirmed that wood fibre orientation slowly rotated in these bands, with distinct inflexion points where the direction of grain change rapidly reversed. Xylem vessels showed similar periodicities, but with different amplitudes and phases: vessel inflexion sites lay slightly inside the wood fibre inflexion points, and vessels ran at steeper angles than wood fibres at these inflexion sites. These pattern differences are interpreted to be the result of a mobile signal, suggested to be auxin, that acts in multiple locations and roles inside the developing vascular cambium. By contrast, the gymnosperm *Pinus radiata* (radiata pine) develops spiral grain patterns in which grain become left-handed during the first several years of growth, and then slowly reverts towards vertical. Such spiral grain significantly devalues this corewood timber. uCT imaging of young trees showed that these grain changes were relatively smooth, and that they were unaffected by rocking the trees. However, the induction of compression wood, a reaction wood formed on the lower side of trees following tilting, inhibited this development of increasingly left-handed grain. In summary, uCT approaches will provide significant benefits for understanding complex plant development problems.

Poster Session 2

#89: Francois Barbier

The Role of Sugars in Shaping Plant Architecture

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Shoot branching and plant architecture are the result of interactions among different endogenous and environmental factors. Of these factors, sugars and hormones play an important role in the control of shoot branching, notably through the regulation of apical dominance. The continuous flow of auxin produced by the growing shoot apex inhibits the outgrowth of axillary buds, in part, by upregulating the synthesis of strigolactones, a class of hormones inhibiting this process. Additionally, the strong sink strength of the growing shoot apex deprives axillary buds from sugars that are required to promote bud outgrowth. In diverse species including pea, rice, rose and Arabidopsis, enhanced sugar availability, as in the case of decapitation or increased photosynthesis activity, appears to alleviate the inhibitory effect of strigolactone. To achieve this, sugars inhibit the perception of strigolactone by inhibiting the expression of MAX2/D3/RMS4, an F-box involved in the signal perception complex. The sugar-inhibition of MAX2 expression is at least partly mediated through the transcription factor bZIP11. These findings support a signalling role for sugars during shoot branching. Indeed, sugar signalling pathways such as the trehalose 6-phosphate and hexokinase pathways have been implicated in the regulation of shoot branching. Altogether, our results provide support in the signalling role of sugars during shoot branching and their interaction with a hormone controlling this process.

Poster Session 2

#90: Haoyu Lou

Cellulose synthase-like (*Csl*) Genes Control Barley Root Growth and Differentiation

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Plant cell walls are composed of polymers that confer distinct chemical and mechanical properties. Cell wall enzymes are encoded by large gene families that represent attractive targets to modify cell wall by creating new crop varieties for novel applications in agronomy and food processing. However, we currently lack information about the functions of many genes. The barley *CslF* gene family includes 9 members. Characterization of the barley *ClsF* genes has focused mainly on the grain, to date, only *CslF6* has been shown to play a significant *in planta* role in the primary cell wall polysaccharide synthesis, (1,3;1,4)-β-glucan. Gene expression analysis reveals that *CslF3* and *CslF9* accumulate in the barley root tip, suggesting a potential role during root development. In order to further understand the roles of *CslF3* and *CslF9*, genetic, molecular, microscopic, and computed mathematical approaches were used. GOIs peak in the elongation and young differentiation regions of root, and levels are notably higher than *CslF6*, which was the most highly expressed *CslF* gene in other studied tissues. In *CslF3* and *CslF9* RNAi knock-down lines, roots exhibited slower growth rates due to the reduced elongation length and decreased cortical cell layers in mutants. Shorter seminal, lateral roots and less root hairs are observed in soil and agar environments result in significant reduction of root system and surface area. In consistence, we observed negative effects on leaf area, shoot height and biomass in transgenic lines. By expressing *CslF3* in Arabidopsis, the growth of root hair was enhanced, and change of epidermis cell proliferation was observed in transgenic Arabidopsis, which confirmed a role of CslF3 in controlling root hair development. Currently, we are examining CRISPR/Cas9 knockout lines to determining the effects of these genes at a cellular level. In summary, we are developing a better understanding of the genes encoding CSL members.

#91: Joanna Kaptur

Elucidating the role of LEUNIG and LEUNIG_HOMOLOG in regulation of early embryo patterning in *Arabidopsis thaliana*

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Formation of apical-basal (A-B) axis is one of the crucial steps of early embryogenesis and leads to the establishment of shoot and root apical meristems at opposite ends. Despite the fact that several regulators involved in A-B patterning have been identified, our understanding of how the action of these factors is orchestrated remains elusive. Our work focuses on transcriptional co-regulators LEUNIG (LUG) and LEUNIG-HOMOLOG (LUH) and their role in the early embryo patterning. Here we show that lug luh double mutant embryos display aberrant cell divisions along A-B axis, which correlates with defects in auxin distribution as well as altered expression of transcription factors promoting shoot and root identities. As LUG and LUH cannot bind DNA directly, they form higher order complexes with adaptor proteins such as SEUSS (SEU) and SEUSS-LIKEs (SLKs) that facilitate binding to specific transcription factors. Based on yeast and in planta assays we report that LUG/LUH and SEU/SLKs physically associate with variety of WUSCHEL-RELATED HOMEOBOX (WOX) transcription factors. In addition, extensive genetic studies of LUG/LUH and WOX provide further support for observed interactions. Finally, we present evidence that a LUG-WOX complex regulates shoot patterning by promoting expression of the HOMEODOMAIN LEUCINE ZIPPER class III (HD-ZIP III) transcription factors. Together these observations provide new insight into transcriptional programs involved in formation of the basic body plan of the plant.

Poster Session 2

#92: John Patrick

Enzymes contributing to the hydrogen peroxide signal dynamics that regulate wall labyrinth formation in transfer cells

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"Transfer cells (TCs) are specialized for resource transport in plants conferred by an enlarged plasma membrane area, enriched in nutrient transporters, supported on a wall labyrinth (WL) comprised of an uniform wall layer (UWL) from which wall ingrowth (WI) papillae arise. Employing adaxial epidermal cells of cultured *Vicia faba* cotyledons as an experimental system, previous studies have revealed that an auxin-ethylene-apoplasmic-H_2O_2-cytosolic Ca^{2+} signalling cascade orchestrates formation of their WL (Andriunas et al., 2013; Zhang et al., 2017b). Transcriptomic analyses of the epidermal cells *trans*-differentiating to a TC phenotype identified additional signalling cascade candidates (Zhang et al., 2017a): gibberellin (GA), abscisic acid (ABA), brassinosteroid (BR) and jasmonic acid (JA). We tested whether these candidates regulated WL formation and, if so, how they interact with other participants of the sigalling cascade. Our findings showed that GA and ABA initiate the signalling cascade by elevating TC auxin levels that elicit a burst in ethylene biosynthesis. In turn ethylene, in conjunction with BR and JA, activates an apoplasmic-H_2O_2-cytosolic Ca^{2+} signalling hub that directs assembly of the WL.

Poster Session 2

#93: Lingling Yin

The Transcriptional Regulatory Network Controlling Responses to Hormones in Arabidopsis thaliana Etiolated Seedlings

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Seed germination is an essential step of the plant life cycle and is critical to agricultural production. *Arabidopsis* seedlings undergo etiolated development in the absence of light. The germinating seedlings establish an elongated hypocotyl and form an apical hook structure which enable them to push and emerge through the soil while minimizing mechanical damage. The process of etiolated seedling development is tightly regulated by crosstalk between multiple hormones. However, there have been few systematic studies of hormone-responsive transcription factor (TF) networks and the molecular mechanisms of regulation during *Arabidopsis thaliana* etiolated seedling development. We have generated time series transcriptomes (RNA-seq) from etiolated seedlings treated with the hormones brassinosteroid (BR), jasmonate (JA), salicylic acid (SA) and strigolactone (SL). We also obtained published time series RNA-seq datasets for abscisic acid (ABA) and ethylene (ETH) treatment. The phytohormone treatments cause dynamic changes in the transcriptome over time, which we can use to characterize hormone response gene regulatory networks. We have determined the target genes of 14 major hormone-related TFs using chromatin immunoprecipitation sequencing following hormone treatment. These data, along with literature-obtained transcription factor-target gene interactions from the AGRIS (Arabidopsis Gene Regulatory Information Server) and DAP-seq databases, provided a framework to construct a TF network and to predict significant regulators important to hormone responses in the *Arabidopsis* etiolated seedling. We applied the Signaling and Dynamic Regulatory Events Miner (SDREM) approach to build the gene regulatory networks, integrating time-series gene expression data with transcription factor-target gene interaction data. This yielded a comprehensive regulatory network in *Arabidopsis* etiolated seedling responding to six major phytohormones. Key regulators with potential roles in hormone crosstalk were defined, enlightening the relationship between different gene expression patterns in response to hormones and a complex phytohormone regulatory network.

Poster Session 2

#94: Maheshika Herath

The role of LEUNIG_HOMOLOG and MADS-box transcription factors in seed mucilage extrusion in *Arabidopsis*

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Upon hydration, the outer layer of the *Arabidopsis* seed coat releases polysaccharides that form a protective halo around the seed. This gel-like substance, termed mucilage, is primarily composed of the pectin rhamnogalacturonan I (RG-I) with smaller contributions of the pectin homogalacturonan, the hemicellulose galactoglucomannan and cellulose. Due to the availability of naturally occurring and induced mutations affecting the biochemical and biophysical properties of mucilage, together with the ease of visualizing mucilage using the stain ruthenium red (RR), seed mucilage has become an ideal system to investigate the regulation, synthesis and secretion of cell wall polysaccharides in plants. It has been shown that mutations in the transcriptional regulator LEUNIG_HOMOLOG (LUH) lead to a mucilage release phenotype that arises from a change in the hydration properties of RG-I. As this defect is associated with decreased expression of *MUM2*, a gene encoding a secreted b-galactosidase that removes galactose residues from RG-I, it has been proposed that LUH is a *MUM2* activator. Subsequent studies identified a series of MADS-box transcription factor binding sites in the large first intron (*I1*) of *MUM2*, which when mutated result in reduced activity of a promoter/intron-reporter in the seed coat. MADS-box transcription factors SHATTERPROOF1 (SHP1), SHATTERPROOF2 (SHP2), SEPALLATA3 (SEP3) bind to *MUM2 I1* where they are thought to form a higher order complex with LUH. Here we present further evidence to support the proposed model of *MUM2* regulation using phenotypic and genetic experiments. A newly established quantitative RR assay revealed a significant reduction in mucilage extrusion from *shp1 shp2 sep3* triple mutants compared to *shp1 shp2* double, *shp1*, *shp2*, *sep3* single mutants and wildtype seeds. Furthermore, examining the morphology of mucilage secreting cells (MSCs) in the seed coat of these mutants detected changes in cell area and structure but not overall morphology. Current work is focusing on placing the MADS-box transcription factors in a well-established gene regulatory network controlling mucilage biosynthesis, secretion and modification.

Poster Session 2

#95: MitchellRey Toleco

Investigations into the seemingly promiscuous nature of the plant mitochondrial carrier family: Focus on the Dicarboxylate/Tricarboxylate Carrier

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The Dicarboxylate/Tricarboxylate Carrier (DTC; AT5G19760) is one of the 58 members of the Arabidopsis mitochondrial carrier family (MCF). DTC has been reported to facilitate an obligatory electroneutral exchange of tricarboxylates such as citrate, and dicarboxylates such as malate or 2-oxoglutarate. However, the *in vitro* transport data seemed to show that DTC is highly promiscuous, allowing a plethora of substrates to cross the inner mitochondrial membrane (IMM). Given the level of control that we expect at the IMM, we set forth to scrutinize the substrate promiscuity of DTC *in planta*. Initial analysis by laser scanning confocal microscopy of DTC-GFP fusion lines showed that DTC seemed to localize in the mitochondria. Metabolite profiling of overexpression lines by gas chromatography-mass spectrometry indicated metabolic perturbation especially the accumulation of citrate, fumarate and malate relative to the wild-type control. When DTC is knocked-out using CRISPR-Cas9 system, the gene-edited plants exhibited severely stunted growth, early-flowering, and low biomass and seed yield, indicating that DTC plays a vital role in normal growth and development. Mega-phylogenetic analysis of more than 10,000 putative MCF proteins across Domain Eukarya revealed that DTC does not cluster with known citrate transporters from either yeast or humans, but with a class of MCFs known as M2OM which facilitates malate/2-oxoglutarate exchange. Work is underway to untangle the *in planta* function of DTC and to re-analyze transport function using a novel mass spectrometry-based transport assay system.

Poster Session 2

#96: Shuchao Dong

The HD-Zip class I transcription factor JUB2 modulates growth and development by regulating JUNGBRUNNEN1 in *Arabidopsis*

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The *Arabidopsis thaliana* NAC transcription factor (TF) JUB1 is a central regulator of development and the interplay between growth and stress responses. Employing a yeast-one-hybrid (Y1H) assay and transcript analysis of transgenic lines, we identified an HD-Zip TF as a potential upstream regulator of *JUB1*, and we named it JUB2. Here, we characterize the biological functions of JUB2 and unravel its gene regulatory networks. We found that overexpression of *JUB2* results in a GA (gibberellic acid)-deficient phenotype with a compact rosette, shorter stem, impaired filament elongation, fewer siliques and delayed senescence, compared to wild type. These phenotypes are similar to *JUB1* overexpressors. Furthermore, overexpression of *JUB2* in the *jub1* mutant background largely rescued growth and developmental defects, suggesting that JUB2 acts upstream of *JUB1*. We also found that overexpression of *JUB2* or *JUB1* significantly enhanced drought tolerance. By contrast, double mutant lines overexpressing *JUB2* in the *jub1* mutant did not perform better than wild type in response to drought stress, suggesting that the JUB2-*JUB1* cascade is also involved in the drought response. To identify other target genes of JUB2, we performed gene expression profiling by RNA-seq and found *GA2OX* genes to be upregulated by JUB2. GA2OXs are GA 2-oxidases that convert bioactive GAs to inactive isoforms. Indeed, we found that more bio-inactive GAs and less bioactive GAs accumulate in *JUB2OX* plants compared to wild type. Using ChIP-qPCR and gene expression analysis by qPCR, we confirmed that JUB2 directly and positively regulates *GA2OXs*. Taken together, our results suggest that JUB2 controls a complex regulatory system underlying growth and development programs.

#97: Stephanie Kerr

Strigolactone regulation of shoot branching in garden pea

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"Strigolactone (SL) is a key inhibitory hormone of shoot branching in plants. DWARF53 (D53) in rice and members of the SUPPRESSOR of MAX2-LIKE (SMXL) protein family in Arabidopsis have been identified as targets of the SL receptor complex. These targets are proposed to suppress SL-response genes in the absence of SL. In order to identify transcriptional targets of the SL signaling complex for the regulation of shoot branching in pea, we used RNA-Seq techniques to identify genes that are transcriptionally regulated by the synthetic SL, rac-GR24, in Pisum sativum buds over a six hour time-frame. More than 20 genes were identified as SL-responsive and were validated using qPCR. BRANCHED1 (BRC1), a transcription factor that acts downstream of SL in the regulation of shoot branching, was required for the regulation of nine SL-regulated genes, confirming its role as a mediator of SL transcriptional responses in axillary buds.

The plant hormone cytokinin (CK) acts antagonistically to SL to promote shoot branching. The transcription factor BRC1 also responds to SL and CK in an antagonistic manner. We tested response of the SL-responsive genes we identified to CK and found many of these genes were also antagonistically regulated by CK, further supporting a shared signaling pathway between the two hormones in the regulation of shoot branching. Similar to CK, decapitation of the growing shoot tip also leads to increased shoot branching and decreased expression of BRC1. Further examination of our SL-responsive genes found that many of these genes are also regulated by decapitation, implying that decapitation may also signal, at least partially, through a common pathway to both SL and CK."

Poster Session 2

#98: Sukanya Varape

Investigations on the role of vernalization pathway genes during soybean flowering

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Soybean (*Glycine max*) is one of the major oilseed legume crops. It is a rich source of proteins and other nutrients for humans and animals. Success of flowering determines the yield in legume crops such as soybean. Despite the importance of flowering in defining yield in legumes, studies regarding the molecular pathways that control flowering in soybean are limited. Floral initiation is governed by the cross talk between many internal and external cues. Temperature is one of the important factors that control flowering. The promotion of flowering by cold treatment called 'vernalization. This process is regulated by vernalization pathway genes and has been extensively studied in *Arabidopsis thaliana*. Soybean does not have quantitative/obligate vernalization requirement however, during the duplication event, it retained large number of vernalization genes. According to RNA sequencing study on soybean shoot apical meristem (SAM) undergoing floral transition [Wong et al.2013, PLOS ONE 8(6), e65319], many of these temperature responsive vernalization genes are differentially regulated. Using genomics approach, we have identified thirteen different vernalization pathway genes. These genes showed considerably high peptide sequence homology with their respective Arabidopsis homologues. Based on phylogenetic relationships, these genes were grouped into three different subfamilies namely *VRN1* (eight paralogues), *VRN2* and *VRN5* (four paralogues). A fundamental gap in our knowledge remains in elucidating the function of vernalization pathway genes in soybean. Therefore, to study the role of soybean vernalization genes, we performed integrated genomics and molecular characterization. Expression analysis showed that vernalization genes are photoperiod responsive in soybean. Overexpression of soybean vernalization genes in *Arabidopsis* and *Nicotiana tabacum* showed both conserved and diverged functions.

Poster Session 2

#101: Lim Chee Liew

Temporal cell-specific regulation of transcriptomes during barley (Hordeum vulgare) seed germination

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The development of a complex multicellular organism is governed by distinct cell types which have differential transcriptional profiles during growth and differentiation. The spatial and temporal gene expression profiles of these individual cell types are critical to identify transcriptional regulatory networks that govern developmental processes. The germinating seed is an excellent model to study genome regulation between cell-types since the majority of the transcriptome is differentially expressed in a short period, beginning from a uniform, metabolically-inactive state. In this study, we applied laser-capture microdissection RNA sequencing to small numbers of cells from barley seed embryonic organs (plumule, radicle tip and scutellum) of germinating barley seeds every 8 h, over a 48 h time course. Laser capture microdissection is a microscopic technique for isolating single cells or areas of tissue in a contamination-free and precise manner. We determined that plumule, radicle and scutellum accumulate and store transcripts differentially during seed development and embryo maturation. During barley seed germination, organ-specific gene expression was notably common in plumule, radicle tip and scutellum. Co-expression of genes had strong spatiotemporal structure, with most co-expression occurring within one organ and at a subset of specific timepoints during germination. Overlapping and distinct enrichment of functional categories were observed in the cell-specific profiles. We identified cis-regulatory motifs enriched in the promoters of genes expressed in each organ as well as candidate transcription factors amongst these that may be regulators of spatiotemporal gene expression programs. Our findings contribute to the broader goal of generating an integrative model that describes the structure and function of individual cells within seeds during germination.

Poster Session 2

#102: Yanfei Zhou

Increased salt tolerance by betacyanin production in transgenic Nicotiana tabacum

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Salinity tolerance is an important trait for food producing plants, given that the arable land area affected by high salinity is increasing in response to climate change, irrigation practices, and other factors. Identifying the different mechanisms responsible for salt tolerance in plants is therefore useful. Our previous studies have shown that foliar betacyanin pigments are associated with salinity tolerance in *Disphyma australe*, a halophyte capable of growing under high-salt conditions. It is unknown if salinity tolerance conferred from betalain pigment production can be transferred to other plants. To address this we generated betalain producing *Nicotiana tabacum* (*N. tabacum*) plants using a transgenic approach, and assessed their salt tolerance. We found that betalain over expressing *N. tabacum* lines predominantly produced betacyanins: betanin, isobetanin and betanidin. The presence of betacyanins delayed *N. tabacum* leaf senescence, conferred photo protection, and enhanced seedling survival under severe salt stress. Our results confirmed the association of betacyanins with salinity tolerance in a second plant system. The output from this study provides new insights on salinity tolerance mechanisms in plants, and could inform the development of novel biotechnological approaches to improving agricultural productivity in salinity-affected areas.