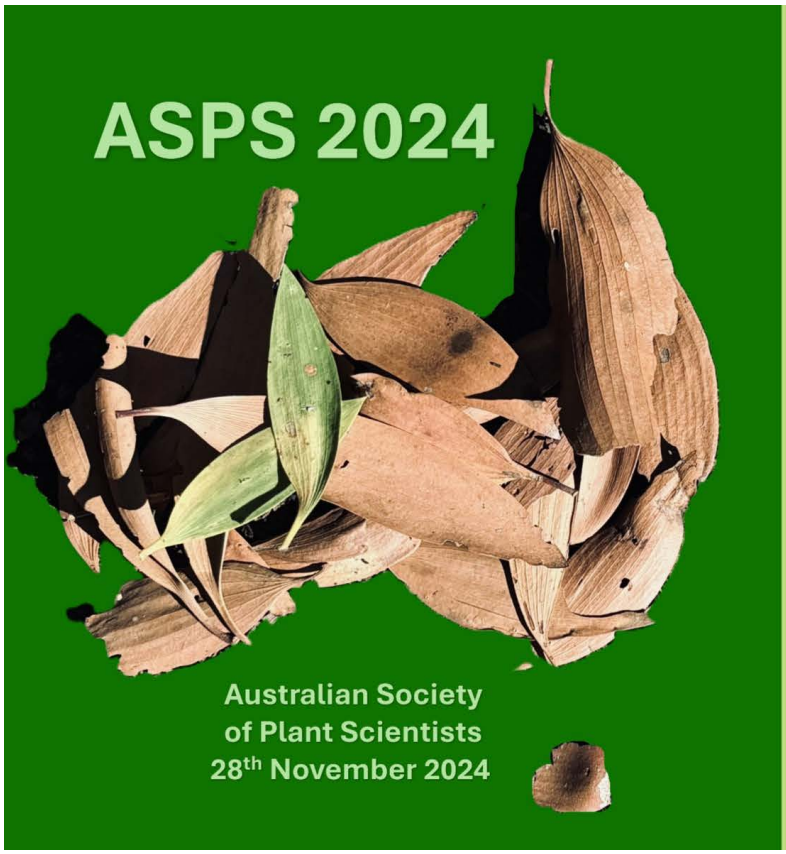
ASPS2024 28th Nov

Adelaide Program and Abstracts

ASPS2024 Adelaide

Level 14, Festival Tower





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ASPS2024 28th Nov

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Welcome to Flinders University City Campus!

Reminder: ASPS AGM 29th Nov. @ 11:30am
https://www.asps.org.au/about/agm

8:00 am	**Registration, poster installation (Tea and coffee available on arrival)**
8:30 am	**Conference Open, Welcome**
9:00 – 10:30 am	***Session 1 (Chairs Dr Scott Boden and Zane Marks)***
	Prof. David Day - Carbon-nitrogen exchange in nitrogen-fixing soybean nodules
	Dr Alison Gill - Autonomous agriculture for space exploration
	Dr Yue Qu - Spatial transcriptomic study of early developing wheat inflorescence using STOmics Stereo-seq
	Dr Haoyu Lou - High-throughput AI-assisted 3D trait extraction of cereal spikes using non-destructive X-ray CT imaging differentiation
	Dr Nick Booth - GABA as a marker for heat stress tolerance
10:30 – 11:00 am	**Morning tea sponsored by Waite Research Institute**
11:00 – 12:00 pm	**Session 2** ***(Chairs Dr Sunita Ramesh and Lauren Philp-Dutton)***
	Saber Sohrabi - In-planta transient transformation of Hemp (*Cannabis sativa*) using the RUBY reporter
	Sreshtha Malik - Repurposing failed antibiotics in the pursuit of novel herbicides
	Yiting Xie - Multi-modal approach to predict flowering time of individual wheat plants
	Zhale Hekmati - Exploring the role of TIPs in transporting water and ions in plants
12:00 – 1:00 pm	**Lunch sponsored by Flinders University**

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1:00 – 3:45 pm ***Session 3: Award Lectures (Chair Prof. Martha Ludwig, A/Prof. Lynette Brownfield)***

NZSPB Roger Slack Award: Dr David Chagne
Genomics for restoring a critically threatened tree species in the rohe of Rangitāne o Manawatū

ASPS Goldacre Award: Dr Peter Crisp
The DNA hypomethylome: unlocking crop epigenomics to uncover and engineer hidden diversity

ASPS Education and Outreach: Dr Ashley Jones & A/Prof. Benjamin Schwessinger
Genomics for all: a cross-sector effort to make cutting-edge genomics a cornerstone of education, industry, government, and public health

Break (15 mins)

NZSPB Elected Fellow to the NZ Royal Society: Dr Andy Allan and Dr Kevin Davies
Fast flowering as a tool for gene discovery in woody perennials

ASPS Jan Anderson Award: A/Prof. Jenny Mortimer
Sweet green tales: efforts to unravel the complexities of plant polysaccharides

RN Robertson Lecture: Prof. Sergey Shabala
Cell-based phenotyping for breeding crops for future climates

3.45 – 4:15 pm **Afternoon tea sponsored by the ARC Centre of Excellence in Plants for Space**

4:15 – 5:25 pm ***Session 4 (Chairs Dr Megan Shelden and Dr Yue (Julian) Qu)***

Dr Thi Thanh Hoai Phan - FPB Best Paper Award: Characterisation of overexpression Arabidopsis UBI:nGFP::PIP2;1 seed during germination and under saline condition

Dr Sunita Ramesh - The effect of water stress on root exudation and microbial diversity in wheat

Dr Steven Hussey - Functional investigation of five R2R3-MYB transcription factors associated with wood development in Eucalyptus using DAP-seq-ML

Dr Beth Loveys - Can learning how to correctly use laboratory equipment be improved by use of virtual resources?

5:30 – 6:00 pm **Prize Announcements (prizes sponsored by WRI and APPN), Conference Close**

6:30 – 10:00 pm **Dinner: Mrs Q 1/128 Gouger St**

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Oral Presentations

Talk 1-1

Carbon-nitrogen exchange in nitrogen-fixing soybean nodules

Nick Booth[1], Penelope Smith[2], Sunita Ramesh[1], Kathleen Soole[1], Steve Tyerman[3], David Day[1]

[1] College of Science & Engineering, Flinders University, Adelaide, SA
[2] School of Life Sciences, La Trobe University, Bundoora, VIC
[3] ARC Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, SA

The basic metabolic exchange between the plant and bacteroids during symbiotic nitrogen fixation in legumes is carbon from the plant, in the form of malate, for fixed nitrogen from the rhizobial bacteroids, in the form of ammonia/ammonium and possibly amino acids. During the symbiosis, legumes develop highly specialised organs on their roots, called nodules, which are infected by the rhizobia. Inside the infected nodule cells, the rhizobia differentiate into nitrogen-fixing bacteroids that are excluded from the plant cytosol in organelle-like structures termed symbiosomes. The symbiosome membrane acts to regulate metabolite exchange between the symbionts through transport proteins synthesised by the plant. A number of transport processes have been biochemically characterised in isolated symbiosomes, but the molecular identity of many of the transporters involved remains unknown. We have recently characterised candidates for these transporters using genomic analyses and heterologous expression in Xenopus oocytes and yeast.

We suggest that the soybean aquaporin GmNOD26 is a multifunctional channel that facilitates both ammonia and ammonium transport across the symbiosome membrane and that malate transport is catalysed by a member of the NPF transporter family.

Talk 1-2

Autonomous agriculture for space exploration

Alison Gill[1,2], Jenny Mortimer[1,2], Matthew Gilliham[1,2]

[1] University of Adelaide, Urrbrae, Australia
[2] ARC CoE in Plants for Space, Urrbrae, Australia

As humans prepare to travel further from Earth and enter a new era of Space exploration, food production *in situ* will be vital. Plants can provide renewable fresh food, in the form of necessary calories, foods to counteract menu fatigue, vital nutrition and psychological benefits. The full food requirements of astronauts cannot be taken as payload on longer-term missions due to mass and nutrient degradation, and astronauts time is extremely expensive, so they are unable to act as gardeners and are not trained plant scientists. Space exploration will thus be limited by the ability to provide fresh food and constrained by solving these challenges, necessitating the development of robust, resource-efficient autonomous plant growing systems. The aim of this United Kingdom Space Agency and Australian Space Agency-funded project is to deliver state-of-the-art autonomous controlled environment agriculture facilities to grow fresh, varied, and appealing plant food to support space missions. In collaboration with partners at Vertical Future, Axiom Space, the University of Cambridge, the University of Western Australia, the University of Southern Queensland, and Saber Astronautics, we are developing imaging methods to capture and analyse leaf movement as an indicator of plant health. Results from optimised parallel experiments across nodes have allowed large collection of data, standardisation of stress detection, and synchronisation tests. Our research contributes necessary knowledge for autonomous food production in space, reducing risks of failure, and is one step forward in sustaining human health over long-term space missions. Additionally, this work generates outcomes for on-Earth controlled environment agriculture and sustainability, autonomous growth facilities for remote or changeable environments, robust communication systems, and early-stress detection methods.

Talk 1-3

Spatial transcriptomic study of early developing wheat inflorescence using STOmics Stereo-seq

Yue (Julian) Qu[1], Scott Boden[1]

[1] School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA, 5064, Australia

Cereal grains are pivotal to the global diet, with wheat alone providing 20% of the world's calorie and protein intake. The double ridge (DR) and lemma primordia (LP) stages are crucial phases in wheat development that significantly influence yield potential, particularly in terms of final spikelet and floret numbers. This study employs spatial transcriptomics to create a comprehensive map of gene expression in wheat inflorescence during these early developmental stages. DNA libraries of wheat inflorescence samples at the DR and LP stages were generated and QC'd by Qubit for quantity and Tapestation for size distribution. Pooled libraries were sequenced on the MGI DNBSEQ G400 which resulted in 1.76 billion Passing Filter (PF) reads. The sequencing results were processed using the SAW (Stereo-seq Analysis Workflow) pipeline and aligned to the Chinese Spring v1.1 genome. Unsupervised clustering based on 20-bin spots revealed 14 distinct clusters in DR samples and 16 in LP samples, providing insights into the spatial organization of gene expression during these critical developmental phases. Each cluster underwent functional analysis through Gene Ontology (GO) term analysis, allowing us to elucidate the biological processes associated with the identified gene sets. We also investigated the expression patterns of well-studied genes related to wheat inflorescence development, such as *PPD1, PDB1, ALOG1, VRN1, APO1, FT2, VRT2, SVP1, DUO1* and *etc*. The spatial expression patterns observed in our study were found to be consistent with previously published mRNA *in-situ* hybridization results, validating the reliability of our spatial transcriptomic approach. This study aims to provide a high-resolution spatial transcriptomic atlas of early wheat inflorescence development, offering insights into the molecular mechanisms underlying spikelet and floret formation.

Talk 1-4

High-throughput AI-assisted 3D trait extraction of cereal spikes using non-destructive X-ray CT imaging

Haoyu Lou[1], Fouzia Syeda[1], Bettina Berger[1]

[1] Australian Plant Phenomics Network, The Plant Accelerator, University of Adelaide, Urrbrae, South Australia, 5064, Australia

Wheat and barley are major crops with substantial economic impact, where spike architecture and grain structure critically influence yield and quality. To enhance grain production and resilience, breeders seek to understand the dynamic relationship of genotype, environment, and management that shapes phenotype expression. Fast, high-throughput, and automated methods for collecting spike, spikelet, and grain traits are of particular interests to advancing breeding programs. However, traditional phenotyping often requires manual input, resulting in qualitative, time-consuming, and destructive processes that cannot easily quantify complex traits like grain volume, surface area, and spatial arrangement.

Utilizing the X-ray Computed Tomography (CT) technology at The Plant Accelerator, Australian Plant Phenomics Network, we have developed a non-invasive 3D imaging system that quantifies essential wheat and barley grain traits – including count, dimension, volume, surface area, 3D position, and density – with high accuracy and automation. This method provides new insights into the 3D structure of cereal spikes, facilitating advanced analyses of grain and spikelet arrangement, sterility detection, and spike architecture, which could guide breeding programs more precisely.

To further increase efficiency, machine learning models are being trained to automate the extraction of morphological traits such as grain arrangement, spikelet detection, and nodal positioning. By examining these traits in relation to genetic and environmental variables, we aim to deliver a more comprehensive understanding that can guide trait selection for breeding programs, directly addressing the demand for scalable, quantitative, and non-destructive phenotyping in cereal research.

Talk 1-5

GABA as a marker for heat stress tolerance

Nicholas Booth[1], Apriadi Situmorang[2], Scott Boden[2], Sunita Ramesh[1]

[1] Transport Physiology and Signaling Group, Collège of Science and Engineering, Bedford Park, Flinders University, SA 5042
[2] School of Agriculture, Food and Wine University of Adelaide Plant Research Centre, Waite Campus, Glen Osmond, SA 5064

γ-aminobutyric acid (GABA) is a non-protein amino acid found in plants, animals, and microorganisms, having diverse physiological functions (Bouche and Fromm. 2004). In plant tissues GABA accumulates in response to both biotic and abiotic stress, having important roles during stress adaption including regulation of cytosolic pH, osmo-protection, altering carbon and nitrogen metabolism, limiting ROS production, and suppling TCA intermediates via the GABA shunt pathway (Shelp, et al. 1999; Bouche and Fromm. 2004; Ludewig et al. 2008; Li et al. 2021). The natural variability in endogenous GABA content amongst wheat *Triticum aestivum* cultivars remains largely unstudied, and the responsiveness of the GABA shunt pathway to increasing temperature warrants further investigation.

We have screened the GABA content of 67 wheat cultivars of diverse breeding backgrounds and observed 10-fold variability in the natural GABA concentrations under control conditions. Amino acid and transcript analysis was performed on a subset of lines exposed to temperatures of 17°C, 22°C or 27°C. The GABA content varied amongst the cultivars under control conditions (17°C) from 0.1195 µmoles/gFW in SONORA 64 to 1.034 µmoles/gFW in EDPIE_56, with an average GABA ~0.4 µmoles/gFW observed across all cultivars. Numerous trends arose amongst the cultivars; in some cultivars GABA was not responsive to temperature treatment, while in others a moderate increase was observed at 22°C but then followed by a decreased at 27°C. In SONORA 64, GABA positively increased with temperature, but an opposing effect was observed for EDPIE_56. Transcripts of the GABA shunt pathway varied with cultivar, with a strong negative correlation between SSADH expression and temperature observed. This data highlights the genetic variability in the responsiveness and endogenous concentration of GABA amongst wheat cultivars and warrants further investigation. We believe that the natural diversity in GABA content could be used as a biomarker to screen for heat tolerance.

Talk 2-1

In-planta transient transformation of Hemp (Cannabis sativa) using the RUBY reporter

Saber Sohrabi[1], Sara Jalali[1], Philip Brewer[2], Rachel Burton[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae, SA 5064, Australia
[2] Institute for Future Farming Systems, Central Queensland University, Rockhampton 4701, Australia

Hemp (*Cannabis sativa*) is recognized as a recalcitrant plant species, presenting a significant challenge for genetic transformation and gene editing studies due to the absence of a reliable regeneration protocol. Despite numerous studies aimed at developing regeneration and transformation methods, published protocols often exhibit low efficiency and reproducibility. Here, we present a rapid and highly efficient *Agrobacterium*-mediated transient transformation method that circumvents the need for a regeneration phase. We used excised apical meristems from 10-day-old germinated hemp seeds as explants and cultured them on MS media. After one week of culture, the apical meristems were inoculated with *Agrobacterium* carrying the *RUBY* construct, which comprises three genes under the control of the *CaMV* 35S promoter enabling the conversion of tyrosine to the red betalain pigment. Our results demonstrate a transient expression of *RUBY*, evident through the distinct red coloration of explant leaves and meristems after two weeks. However, observations four weeks post-inoculation revealed a reversion to the typical green coloration of leaves. This reversion could be due to insufficient tyrosine presence as a precursor, partial or non-insertion of the genes involved in the betalain synthesis pathway, or gene silencing post-insertion into the hemp chromosome. Nevertheless, this approach shows promise for hemp transformation and other recalcitrant plant species where regeneration poses a challenge in transformation studies.

Talk 2-2

Repurposing failed antibiotics in the pursuit of novel herbicides

Sreshtha Malik[1], Andrew Barrow[1], Tatiana Soares da Costa[1]

[1] The University of Adelaide, Adelaide, Australia

Weeds are a major threat to our agricultural industry, resulting in lower crop yield and higher harvesting costs [1]. Herbicides remain the most cost-effective tool for the management of weeds [2]. However, the lack of new herbicides introduced to the market in the past 40 years combined with the rise in herbicide resistant weeds means that the identification of novel herbicides is of the highest priority [3]. Our work focuses on a novel herbicide target, shikimate kinase (SK) – an enzyme involved in the biosynthesis of aromatic amino acids in plants, bacteria and fungi. Previous studies have identified *in vitro* inhibitors of bacterial SK that had no antibacterial activity and therefore such compounds could not progress through the antibiotic discovery pipeline. Given the high degree of structural similarities between plant and bacterial SK, we hypothesised that these failed antibiotics could be repurposed as new herbicide candidates. To test this, SK from plant *Arabidopsis thaliana* (At) was cloned, expressed and purified to >95% homogeneity. The bacterial SK inhibitors were docked into AtSK and their binding affinity values were examined. The top 10 most promising compounds were synthesised in-house and assessed for *in vitro* activity against recombinant AtSK, as well as *in planta* activity against *A. thaliana*. We identified the compound with the most promising activity, and subsequently synthesised a series of analogues that had higher potency than the parent compound. This study reports the first plant SK inhibitor with herbicidal activity. Moreover, our work provides evidence that a repurposing approach combined with structure-guided synthesis can generate highly specific and potent herbicide candidates, which can significantly expedite the herbicide discovery process.

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Talk 2-3

Multi-modal approach to predict flowering time of individual wheat plants

Yiting Xie[1,2,3,4], Stuart Roy[1,3], Rhiannon Schilling[1,4,5], Huajian Liu[1,2]

[1] University of Adelaide, Adalaide, Australia
[2] Australian Plant Phenomics Network, Urrbrae, Adelaide
[3] ARC Training Centre for Future Crops Development, Canberra, Australia
[4] South Australian Research & Development Institute, Urrbrae, Australia
[5] Flinders University, Bedford Park, Australia

Accurate prediction of flowering time in individual wheat plants is critical for advancing wheat breeding and ensuring biosafety compliance in genetically modified (GM) plant breeding. Hybrid wheat breeding, which requires forecasting individual flowering times approximately ten days in advance, depends on these predictions to schedule cross-pollination. In Australia, regulatory requirements mandate forecasting of initial flowering at least 14 days in advance for GM field trials [1]. Existing models, such as APSIM [2], achieve 72% accuracy at the population level and are unsuitable for precise, individual predictions necessary for field trials. Recognizing the substantial influence of environmental factors on individual wheat flowering times, this study introduces a novel multi-modal machine learning model that integrated daily imaging and environmental data to forecast the anthesis of individual wheat plants efficiently. Environmental variables data—including daily maximum and minimum temperatures, sunlight duration, and rainfall—were recorded. The workflow began with object detection, identifying the swollen boot or head on individual wheat plants using a fine-tuned *You Only Look Once* v8 model, achieving a mean Average Precision of 0.88 [3]. Detected objects were cropped and combined with environmental variables to form an image-weather combo. Image features were then extracted via the imaging model, capturing plant growth status details, while weather data was processed using a recurrent neural network for feature extraction [4]. Both feature sets were merged and fed into a final classifier to predict whether a plant would flower before or after a set number of days. Integrating environmental data notably enhanced prediction performance, with accuracy increasing from 74% in the model without weather data to 85% in the model with weather data, marking an 11% improvement. This approach reduces the need for manual field assessments and sets a foundation for broader applications in anthesis forecasting, providing an efficient and economical tool for wheat breeding.

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Talk 2-4

Exploring the role of TIPs in transporting water and ions in plants

Zhale Hekmati[1], Jiaen Qiu[1], Steve Tyerman[1], Megan Shelden[1]

[1] The University of Adelaide, Urrbrae, Australia

Tonoplast Intrinsic Proteins (TIPs) are aquaporins (water channels) that are essential for plant water regulation in response to abiotic stresses, such as salinity and drought. They facilitate the bidirectional movement of water and small neutral molecules between the cytoplasm and vacuole, thereby maintaining cellular water balance and osmotic adjustment. Under salt stress, TIPs assist in ion compartmentalization within the vacuole and regulate water flux to mitigate osmotic imbalances. My research aims to investigate the role of TIPs in regulating water and ion movement across the tonoplast and their contribution to abiotic stress tolerance in barley (*Hordeum vulgare L*.) and Arabidopsis. We focus on barley HvTIP2;2 from Sloop and Sloop SA, which differ by a single amino acid in the third transmembrane helix (Leu117 and Phe117, respectively), as well as Arabidopsis TIPs, AtTIP2;1, AtTIP2;2, and AtTIP2;3. Water permeability and ion conductance were assessed by expressing TIPs in *Xenopus oocytes*, and using NaCl, KCl, NH_4Cl, $MeNH_4Cl$, and TEA solutions to explore how external cations affect water transport through TIP aquaporins. Results indicated that $MeNH_4Cl$ (10 mM) inhibited water permeability of AtTIP2;2, while TEA (10 mM) inhibited water transport of AtTIP2;1 and AtTIP2;2. The water permeability of HvTIP2;2 Sloop and HvTIP2;2 Sloop SA significantly decreased with NH4Cl (5 mM) at pH 6. Two-electrode voltage clamp (TEVC) results suggested that AtTIP2;1, AtTIP2;2, and AtTIP2;3 may not transport Na^+or NH_4^+. Phenotyping of Sloop and Sloop SA roots after one week growth in a paper roll-supported hydroponic solution with 150 mM NaCl treatment indicated that total root length, root volume, and surface area significantly decreased in HvTIP2;2 Sloop after salt treatment, while no significant difference was observed for Sloop SA. Future work will involve knocking out TIP2;2 in barley and phenotyping the mutant plants.

Talk 3-1

Genomics for restoring a critically threatened tree species in the rohe of Rangitāne o Manawatū

Colan Balkwill[1], Keith Funnell[2], Emily Koot[2], Julie Deslippe[1], Alana Nuku[3], Paul Horton[3], Wayne Blissett[3], David Chagné[2,4]

1 Victoria University of Wellington, Wellington

2 Plant & Food Research, Papaioea

3 Rangitāne o Manawatu, Papaioea

4 Genomics Aotearoa

Swamp maire (*Syzygium maire*; maire tawake) is an endemic tree species of Aotearoa's swamp forests that is currently listed as nationally critical due to habitat loss and, most recently, infection by myrtle rust. With fewer than twenty mature trees of swamp maire remaining within the Rangitāne o Manawatū rohe, including a remnant population under threat from the construction of Te Ahu a Turanga Manawatū Tararua, a Mana Whenua-led project was set up for conserving the species in the rohe, in accordance with Rangitānenuiarawa (Rangitāne o Manawatū tikanga). Genome sequencing of naturally occurring trees and seedlings from within the rohe was performed to generate knowledge of genetic diversity. A high-quality reference genome was assembled for the species, becoming the first genome sequence to be named by an indigenous group (Ngā Hua o te Ia Whenua). This genomics-based mahi focused on understanding the past and current population structure, how much inbreeding has occurred and how related trees are to each other and to other populations in Aotearoa. This research contributed to developing a restoration plan integrating Mātauranga Māori, genetic diversity and habitat suitability for replanting.

Talk 3-2

The DNA hypomethylome: unlocking crop epigenomics to uncover and engineer hidden diversity.

Peter Crisp[1]

[1] School of Agriculture and Food Sustainability, The University of Queensland, Brisbane, Qld, 4072, Australia

Keywords: Epigenetics, DNA methylation, genomics, gene editing, cis-regulatory elements.

Decoding the information stored in nucleic acids has been transformative to our understanding of life and inheritance. However, beyond the sequence of genes, it has been more challenging to understand the rules of the DNA regulatory code in the non-coding portion of plant genomes, particularly in the vast genomes of many crop species. In addition to the DNA bases A, T, G and C, heritable information can also be stored using modified bases, such as 5-methylcytosine, commonly known as DNA methylation. Profiling the patterns of DNA methylation now enables us to rapidly distil a genome down to the relatively small fraction of regions that are functionally most valuable for trait variation. Genomic regions that lack DNA methylation, named Unmethylated Regions (UMRs), provide very useful information for decoding a plant genome because they can predict loci enriched for cis-regulatory elements. We have developed approaches that use DNA methylation profiling of a single tissue (e.g. a leaf) to discover and characterise plant UMRs, which collectively comprise the ‘hypomethylome’ of a species. We are using this approach in multiple plant species, in particular cereals such as sorghum, wheat, barley and maize to annotate the regulatory portion of these genomes and to investigate natural epigenetic variation. Using the new hypomethylome annotations we have also selected novel gene regulatory regions as targets for gene editing to engineer gene expression variation for trait improvement.

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Talk 3-3

Genomics for all: a cross-sector effort to make cutting-edge genomics a cornerstone of education, industry, government, and public health.

Ashley Jones[1] and Benjamin Schwessinger[1]

[1] Australian National University, Canberra, ACT, 2601, Australia

Keywords: Genomics, Education, Community, Outreach

Rapid advancements in long-read DNA sequencing technologies offer unprecedented potential to transform genomic research. However, the complexity of these technologies can hinder widespread adoption. Over the past seven years, we have been at the forefront of integrating long-read sequencing into various sectors, including academia, industry, government, and public health. Through hands-on workshops, open-source resources, and collaborative networks, we have facilitated knowledge sharing, skill development, and the application of genomic technologies across diverse fields. Our efforts have led to significant advancements in student education, empowering them with practical skills, leading to improved engagement and learning outcomes. In the community, we have applied genomics to develop valuable genome resources for Australia's native flora, enhance biodiversity conservation and improve agricultural genotyping. Our collaboration with government agencies has enhanced biosecurity practices to rapidly detect invasive pathogens, while partnerships with public health organisations have contributed to addressing pressing issues like the COVID-19 pandemic. We believe our collaborative approach to education and outreach fosters a scientifically skilled workforce that empowers individuals to utilise the full potential of genomics for innovative advancements.

Talk 3-4

Fast flowering as a tool for gene discovery in woody perennials

Allan, A.C.[1,2]

[1]Plant & Food Research, Mt Albert, Auckland, New Zealand
[2]School of Biological Sciences, University of Auckland, Auckland, New Zealand

Plants should be considered as a third of the solution to the climate crisis, as they fix CO2 and make all our food (directly or indirectly). Moving to a more plant-based economy requires both new crops and enhanced climate-resistance of existing crops.

New Zealand's horticultural sector is based on temperate perennials. Breeding woody perennials requires a very long-term program. However, can genetic gain be quick enough in crops which have long generation cycles (seed-plant-seed)? New Breeding Technologies (NBTs) use molecular methods that quickly provide step changes in traits. We are using NBTs to make novel crosses with plants that are more floral.

The question remains of how NZ will respond to such plants, which have no additional DNA and harbour only new variants of genes which are identical to "natural" variants already in the environment. In most countries (but not NZ) these resulting plants are not regulated. NZ must quickly decide if NBTs will play a part in our response to a changing climate.

Talk 3-5

Sweet green tales: efforts to unravel the complexities of plant polysaccharides

Jenny C. Mortimer[1,2,3,4]

[1] School of Agriculture, Food and Wine, & Waite Research Institute, University of Adelaide, 5064, Australia
[2] ARC Centre of Excellence in Plants for Space, University of Adelaide, 5064, Australia
[3] ARC Training Centre in Future Crops Development, University of Adelaide, 5064, Australia
[4] Joint BioEnergy Institute & Lawrence Berkeley National Laboratory, Berkeley, CA 94608, USA

Keywords: Cell wall, biofuels, sphingolipids, synthetic biology, space

Plant glycosylation is a highly complex and essential biological process, yet it is still poorly understood. From glycan modifications of proteins and metabolites, to storage polysaccharides such as starch, to the structural complexities of cell wall polysaccharides, these shape how plants grow and respond to the environment. My research has focused on understanding how these complex glycans are formed, how their structure relates to their function, and how we can harness them to support a transition away from fossil-fuel based technologies.

Here, I will highlight our work in identifying key enzymes involved in cell wall and sphingolipid biosynthesis, and our progress in linking glycan structure to function. I will also explore how we are engineering these glycans to enhance biomass traits for biorefinery applications, extending their utility beyond serving as a carbon source for microbial conversion to a broader range of sustainable uses. Finally, I will discuss how understanding the regulation of plant cell wall biosynthesis is important when growing plants in a controlled environment, whether in a vertical farm on earth, or on the lunar surface.

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Talk 3-6

Cell-based phenotyping for breeding crops for future climates

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Keywords: abiotic stress; salinity; drought; flooding; climate change

Agriculture is vulnerable to climate change, and sustainable agricultural food production will be not achievable by the current agronomical and breeding practices, due to impact of climate changes and associated abiotic stresses on crop performance. At the same time, tolerance to key abiotic stresses (such as drought; heat; salinity; flooding) is conferred by multiple mechanisms. Each of them operates in a specific tissue/cell type and is regulated by multiple genes. In this context, the (empirical) whole-plant phenotyping (regardless of whether it is hyperspectral imaging, or ionomics, or any other whole-plant based trait) will be always critical for a final validation of genetic material (e.g., in field trails) but is unlikely reveal the role of a specific mechanism/gene, amongst others. To be more effective, breeding targets can be directed towards specific mechanisms. In this talk, I will argue for a need for a paradigm shift from whole-plant to cell-based phenotyping approach and discuss its current prospects and limitations. Using salinity stress as an example I will show the pitfalls of the whole-plant phenotyping approach for crop breeding, and then illustrate how using cell-based phenotyping platforms allow to overcome this problem. I will then demonstrate how combining novel electrophysiological and imaging techniques can be used for discovery of the candidate genes and/or QTLs conferring not only salinity but also tolerance to other abiotic and biotic stresses. I will also argue for a need for a broader use of wild relatives, to regain abiotic stress tolerance that was lost during domestication process.

Talk 4-1

Characterisation of overexpression Arabidopsis UBI:nGFP::PIP2;1 seed during germination and under saline condition

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In Arabidopsis GPF labelled AtPIP2;1 overexpression lines UBI:nGFP::PIP2;1 could be used as a tool to explore how AtPIP2;1 overexpression influences the phenotype of Arabidopsis plants where the localisation of AtPIP2;1 can also be reviewed by imaging GFP fluorescence. Here the expression of UBI:nGFP::PIP2;1 driven by the Ubiquitin-10 gene promotor (PUBQ10) was checked in putative transgenic lines using BASTA spray, laser scanning confocal microscopy and polymerase chain reaction (PCR). Homozygous plants were used to localize the AtPIP2;1 protein in germinating seed by laser scanning confocal microscopy. In imbibing seed UBI:nGFP::PIP2;1 expression was clearly observable from 30 hours after sowing in root tips, root stele, developing stems, and developing guard cells, and was especially abundant in root cell membranes. Seeds of lines overexpressing UBI:nGFP::PIP2;1 had delayed germination rate relative to wild type (WT, Col-0) lines, and had longer T_{50} in saline conditions (75 mM NaCl) compared to WT. Criteria related to AtPIP2;1 contribution to water, ion and H_2O_2 transport in transgenic seeds during imbibition and germination were consider in this study, along with the potential influence of the fusion of the nGFP.

Talk 4-2

The effect of water stress on root exudation and microbial diversity in wheat

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The United Nations and the World Health Organisation recommend intensifying global food production by ~50% by 2050 to meet the increased demand for food due to increasing global population. Increasing frequency of adverse weather conditions combined with decreasing agricultural land is making this harder to achieve. We need robust strategies that improve crop stress resilience and increase productivity in a sustainable and practical way. Root associated microbiomes contribute in several ways to plant growth, development and health. The roots provide unique niches for diverse microbial colonisation driven by root exudates which contain both high molecular (carbohydrates and protein) and low molecular weight compounds (e.g., organic acids such as malate; sugars, amino acids and gamma aminobutyric acid – GABA). These play a pivotal role in in improving plant resource-use by facilitating communication between roots and microbes, supporting plant growth, development, and influence interactions with various physicochemical and biological factors in the rhizosphere. Little information is available on the interactions between root exudates and microbes in mitigating stresses such as heat or drought in wheat.

Thus, the overall aim of this project is to investigate the interactions between root exudates and soil microbes in mitigating water stress. In this study two cultivars of wheat – Scepter and EDPIE_56 were grown to flag leaf stage and water stressed to 40% field capacity. Biomass, root exudates and soil samples were collected when plants reached 40% field capacity (T1) and four days after water stress (T2). Changes in root and shoot weights were observed between the cultivars and between treatments. Analysis of root exudates revealed changes in concentrations of sugars, organic acids and amino acids between treatments. The 16S sequencing of microbial DNA extracted from rhizosphere soil samples showed significant changes in the diversity of bacterial species between treatments and between cultivars. Future research will involve investigating fungal diversity in these samples and functional attributes of microbes that may confer an advantage under stress.

Talk 4-3

Functional investigation of five R2R3-MYB transcription factors associated with wood development in Eucalyptus using DAP-seq-ML

Stephen Hussey[3], Lazarus Takawira[1], Ines Bachir[2], Raphael Ployet[1], Jade Tullock[1], Helene Clemente[2] Nanette Christie[1], Eshchar Mizrachi[1], Alexander Myburg[1], Fabien Mounet[2]

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A multi-tiered transcriptional network regulates xylem differentiation and secondary cell wall (SCW) formation in plants, with evidence of both conserved and lineage-specific SCW network architecture. We aimed to elucidate the roles of selected R2R3-MYB transcription factors (TFs) linked to *Eucalyptus* wood formation by identifying genome-wide TF binding sites and direct target genes through an improved DAP-seq protocol combined with machine learning for target gene assignment (DAP-seq-ML). We applied this to five TFs including a well-studied SCW master regulator (EgrMYB2; homolog of AtMYB83), a repressor of lignification (EgrMYB1; homolog of AtMYB4), a TF affecting SCW thickness and vessel density (EgrMYB137; homolog of PtrMYB074) and two TFs with unclear roles in SCW regulation (EgrMYB135 and EgrMYB122). Each DAP-seq TF peak set (average 12,613 peaks) was enriched for canonical R2R3-MYB binding motifs. To improve the reliability of target gene assignment to peaks, a random forest classifier was developed from *Arabidopsis* DAP-seq, RNA-seq, chromatin, and conserved noncoding sequence data which demonstrated significantly higher precision and recall to the baseline method of assigning genes to proximal peaks. EgrMYB1, EgrMYB2 and EgrMYB137 predicted targets showed clear enrichment for SCW-related biological processes. As validation, *EgrMYB137* overexpression in transgenic *Eucalyptus* hairy roots increased xylem lignification, while its dominant repression in transgenic *Arabidopsis* and *Populus* reduced xylem lignification, stunted growth, and caused downregulation of SCW genes. EgrMYB137 targets overlapped significantly with those of EgrMYB2, suggesting partial functional redundancy. Our results show that DAP-seq-ML identified biologically relevant R2R3-MYB targets supported by the finding that EgrMYB137 promotes SCW lignification *in planta*.

Talk 4-4

Can learning how to correctly use laboratory equipment be improved by use of virtual resources?

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Junior science students often struggle with correct use of laboratory equipment (pipettes), yet these skills, once mastered, can become second nature. Our cohort of budding scientists is increasingly diverse, and supporting all students to foster their success can be challenging.

Pipettes are a key piece of equipment used in all research laboratories. In our experience, students can struggle to learn how to use a pipette correctly, which in turn impacts on the quality of the data generated in a laboratory session. We have designed a simple self-paced virtual tool to help students become confident in the use of these essential laboratory tools. Students enrolled in multiple level II courses offered across several STEM degree programs at the University of South Australia and The University of Adelaide were invited to be part of study examining the impact of an interactive virtual tool that gives students the opportunity to practice setting and reading a pipette correctly and converting between common volume units.

We will report data that demonstrate that after use of the virtual resources students make few errors is reading and setting volumes on pipettes, make few mistakes in unit conversion and feel more confident in their use of pipettes in the laboratory. Students commented that the virtual tool was very easy to use and reduced their worry about damaging equipment.

Posters

Poster 1

Two routes from the Fertile Crescent led to the introduction of common vetch in Europe

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Vicia sativa (common vetch, n=6) is an annual leguminous plant with high drought tolerance and high grain protein content. In the agricultural industry, vetch is used for biocontrol of weeds, carbon amelioration, nitrogen fixation, and feeding grazing livestock. In this study, we whole genome sequenced 308 *Vicia* accessions from a wide geographic range covering western Eurasia and North Africa to construct a comprehensive nucleotide variation map. Population structure analyses indicate that the Middle Eastern region was the likely centre of origin of common vetch, which then entered Europe in two distinct waves. One wave propagated through Turkey into the Carpathian Basin before spreading throughout Europe via “Danubian” and “Mediterranean” routes. We could not resolve the route for the other wave, which followed admixture between two common vetch groups in the Middle East. Demographic revealed a significant bottleneck for all common vetch groups initiated during the Last Glacial Maximum, followed by an . We also identified selection sweeps for the floral time regulator *SOC1* and *FTb2* in common vetch population at high northern latitudes. Overall, our dataset provides valuable genomic resources for conservation and breeding programs of common vetch.

Poster 2

Characterisation of developmentally critical cell-wall biosynthesis genes in Arabidopsis

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Plant biomass is a promising sustainable bioenergy feedstock, but for its efficient use we must have a comprehensive understanding of plant cell wall synthesis, structure and function. Cell wall polysaccharides are synthesised through a sequential process involving different glycosyltransferase enzymes (GTs). Despite significant efforts, the vast majority of GTs remain uncharacterised. This is in part due to the essential nature of many of these genes, where a loss of their function results in plant death or sterility. However, we previously observed that several cell wall GT mutants with severe growth phenotypes could be maintained easily as calli in tissue culture. We since developed a CRISPR-mediated gene editing method to knockout gene function and generate transgenic *Arabidopsis* callus lines via *Agrobacterium*-mediated transformation [1]. This provided us with stably transformed, mutant homozygous tissue to biochemically characterise, for genes where null mutants were previously reported as lethal due to critical defects in plant development. We will present data on some of these mutants, one which we identify as required for the addition of CMP-β-Kdo in rhamnogalacturonan II (RG-II) biosynthesis [1].

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Poster 3

Growing astronaut food: Physiological responses of duckweeds under LED lighting

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Duckweed has the potential to support astronauts on lunar and deep space missions as a sustainable, fresh and fortifiable food source. As part of a cyclical system, duckweed would be harvested and consumed by crew members providing essential macro- and micro-nutrients. Astronaut waste would then be recycled to support continuous growth of fresh food. Exhaled CO2 would be concentrated in plant facilities, boosting food production and supporting oxygen conversion. Additionally, studies have found that astrofarming supports crew mental health- due to enrichment in daily tasks as well as in combatting dietary fatigue. As part of the optimization process required for astrofarming, different light environments are being investigated for use in duckweed food production. The aim of the presented work is to investigate how lighting can be manipulated to alter growth rates, pigment composition and volatile compounds reflecting changes in overall biomass production, nutritional quality and flavor. To do this three distinct three lighting strategies were used to grow *Spirodela polyrhiza* (9509), *Lemna minor* (8389) and *Wolffia australiana* (8730); (i) determining the essential wavelengths required to optimally grow nutritious duckweed by removing key wavelengths from the growth spectrum, (ii) increasing the ratio of wavelengths in the growth spectrum to determine combinations which optimally grow nutritious duckweed, and (iii) manipulating duckweed development through end of day monochromatic lighting corresponding to plant photoreceptors. By using patterns of spectra and light intensities this work attempts to 'program' duckweed growth- providing insight into how growth rate and pigment content can be manipulated by LED lighting. In addition, chlorophyll fluorescence was used for the non-invasive assessment of photosynthetic parameters. These parameters were used as indicators of plant performance. Across each of the light experiments, *Wolffia australiana* had the highest growth rates and greatest pigment production – supported by higher operating efficiencies of photosystem II and lower nonphotochemical quenching at the grow light intensity. Findings have supported conceptualization of scaled up *Wolffia australiana* growth, with identified light recipes of interest used to manipulate nutritional quality. Further work includes characterization of duckweed stomata, and novel preliminary studies into measuring duckweed whole plant respiration.

Poster 4

Development of a non-GMO biological control bacterium GTI-5813 for control of grapevine crown gall disease.

Xingyu Wu[1], Iain Searle[1]

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Crown gall disease of grapevines is a major issue that impacts the production and propagation of grapevines in several large regions worldwide, including North America (especially California), Australia, Europe and China, and is estimated to cost the wine and table grape sectors over $2 billion/year. 90% of crown gall disease is caused by *Allorhizobium vitis*, and the remaining 10% is caused by *Agrobacterium spp*. Current biological control agents, such as *Rhizobium rhizogenes* K84 and its improved derivative K1026, are not effective in controlling grape crown gall disease. Previously, *A. vitis* strain F2/5 was demonstrated to effectively control *A. vitis*-induced grape crown gall disease however, this strain also causes unwanted necrosis on grapevine tissue. Interestingly, inactivation of *F-avi5813* by using a homology directed mutagenesis approach abolished necrosis but maintained the grape crown **g**all **t**umour **i**nhibition phenotype (GTI +, Zheng and Burr MPMI, 2013, patent number US 9,717,252 B2) however market pull for this GMO is low. Unconfirmed reports of another *F-avi5813* mutation abolished both the necrosis and GTI phenotypes and hence was not useful to control pathogenic *A. vitis* strains.

We used CRISPR/Cas9 genome editing of *A. vitis* F2/5 to produce a mutational series of *F-avi5813* and the mutants resulted in a range of necrosis and GTI phenotypes using the *in vitro* model carrot taproot disks (Wu *et al.,* 2024), *Nicotiana benthamiana* leaves and on grapevines. Our results showed that mutant *F-avi5813-5* had the same level of crown gall inhibition (GTI+) as the wild type F2/5 strain against pathogenic *A. vitis* strains K377 and K306 in carrot disks and grapevine stem assays. We then showed that *F-avi5813-5* induces less necrosis on *N. benthamiana* leaves and *in vitro* on grape stems. We renamed *F-avi5813-5* to GTI-5813 for our field trial and preliminary results showed more callus formation at GTI-5813 treated grafting unions than F2/5 presumably due to less tissue necrosis. Further field testing over multiple environments and years is required before releasing GTI-5813 to the viticulture market to control global grapevine crown gall disease.

Poster 5

A re-purposing strategy for the accelerated identification of herbicide scaffolds with new molecular targets

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The utility of current herbicides is becoming increasingly threatened due to the rapid evolution of herbicide-resistant weeds [1]. As the use of herbicides has underpinned the sustainability of weed management practices, the development of new herbicides is urgently needed to ensure global food security. By capitalising on the evolutionary similarities found between bacterial and plant kingdoms, we propose that *in vitro* inhibitors of bacterial enzymes that lack antibacterial activity could be re-purposed as herbicidal scaffolds to fast-track the identification of herbicides with new molecular targets. Specifically, we explored the herbicidal efficacy of an aromatic amino acid biosynthesis inhibitor that targets dehydroquinate synthase (DHQS) in bacteria. Using *Arabidopsis thaliana* DHQS, we confirmed with structural and kinetic studies that plant and bacterial DHQS enzymes possess a high degree of structural conservation, and the re-purposed inhibitor retains micromolar potency against plant DHQS *in vitro*. Then, upon subsequent discovery that the re-purposed inhibitor exhibits herbicidal efficacy in-soil, we improved the herbicidal potency of the inhibitor through development of the structure-activity relationship series. Our lead analogue displays potent post-emergent activity against one of Australia's most problematic weed species, wild radish. Thus, this study demonstrates that the re-purposing of inhibitors that target the aromatic amino acid biosynthesis pathway is a viable approach to identify herbicide scaffolds with new molecular targets to combat rising herbicide resistance in weeds.

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Poster 6

Biosynthesis of Omega-3 in Duckweeds

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Lemnoideae, commonly known as duckweeds are monocot aquatic plants and are the smallest flowering plants that can be found all over the world. They have been proposed as a novel crop for both global food shortage and space foods due to their fast growth rate and rich nutrient profile. For example, they could provide all the essential amino acids. However, some key dietary components are still missing. Omega-3 fatty acids play an important role in inflammatory reactions, brain and visual development, carcinogenesis, and thrombosis. Omega-3s can be divided into marine sources that provide eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and plant sources that provide alpha-linolenic acid (ALA). This project aimed to further enrich the nutrient value of duckweeds by introducing genes mainly from algae into duckweeds. These genes encode proteins responsible for further modification of ALA that already exist in duckweeds into DHA and EPA. Lipid profiles of different life stages of duckweed, including seeds, fronds and turions, will also be explored in this project. Preliminary lipid profiling on *Lemna minuta* and *Wolffia australiana* showed that the latter has much lower ALA than the former. We are interested in whether this holds true for other strains of *W. australiana* and how will this affect DHA and EPA biosynthesis. DHA and EPA producing duckweeds as space foods can eliminate the limited shelf life of omega-3s supplements and help astronauts fight against radiation damage and bone loss. They also could be an alternative source of omega-3s for the global shift into a plant-based diet and reduce the dependence on decreasing fish stocks.

Poster 7

Identifying the molecular mechanisms of chickpea respiration and growth during stress using integrated multi-omics analyses

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Chickpea is one of the most economically important pulse crops in Australia with around 1 million Tons produced in 600,000 ha. Yield is often compromised due to terminal drought, resulting in up to 50% yield loss globally. Early vigour has been identified as agronomically adaptive in settings where it can reduce water loss via evaporation, contributing to the growth, yield and stress response of the crop. However, the larger biomass of lines with increased early vigour could exacerbate late-season drought stress. Recently, 4 pairs of chickpea near-isogenic lines (NILs) have been developed, differing at a genetic locus associated with early vigour. This study will utilise the NILs to explore the interactions between vigour and stress, through a multi-layered field study encompassing early and late sowing, and tissue sampling across development and inside and edge plot rows to simulate resource competition. Using a multi-omics approach, combined with agronomically relevant data including phenology, growth, and yield, we propose a new project that explores how the interactions of vigour and environment impact chickpea phenotype at the molecular, whole plant, and stand level.

Poster 8

Utilizing the Common vetch (*Vicia sativa*) transgenic hairy method for synthetic biology.

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Common vetch (*Vicia sativa*) is a drought-tolerant legume, that holds significant potential to contribute to sustainable agricultural systems due to its high protein content, drought tolerance, low input costs and nitrogen-fixing capability. However, anti-nutritional compounds like β-cyano-alanine limit common vetch's broader use. We are establishing CRISPR genome editing and transgenic hairy roots to test and edit candidate genes to enhance the crop's productivity. Within two weeks, hairy root's induced by *Rhizobium rhizogenes* strain K599 allow for the rapid testing of genome editing constructs, test promoter activity or reporter genes, and assess the engineering of root metabolic and secondary compound pathways. Using a GUS reporter, we showed the cell division cell 45 promoter (VsCDC45) is very strongly expressed in the dividing root meristem cells and in the lateral root primordia. We are also testing the expression patterns of the *V. sativa* Ubiquitin 10 (VsUBI10), AtEC1.2e1.1p, and Cauliflower Mosaic Virus 35S (CaMV35S) promoters in transgenic hairy, vetch roots. In parallel, we are testing the genome editing efficiency in hairy roots of candidate translational enhancer proteins to either Cas9 or Cas12a. We envisage that our rapid hairy root transgenic system will accelerate the testing of DNA-encoded parts, devices, and nano-machines in vetch.

Poster 9

Using optogenetics to investigate the energetics of anion transport in Arabidopsis roots.

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Plant ion homeostasis involves energy-consuming transport cycles, where passive leaks, generally via ion channels, counteract active transport. An example is Cl^- or NO_3^- uptake via proton symport with potential concurrent anion efflux via anion permeable channels. Using light-activated ion channels derived from some species of algae expressed in Arabidopsis, we can now investigate the effects of passive ion leaks using specific wavelengths of light to activate the channels in a graded way at specific times and frequencies. Our aim here was to examine how a light-activated anion leak across root membranes, affects root respiratory demand. The hypothesis being tested is that collapsing the membrane potential across the plasma membrane in root cells of Arabidopsis expressing light activated GtACR1 anion channel will require energy to re-establish the resulting gradients, leading to an increase in respiration rate. The increase in respiration rate may be used to estimate the energetics of maintaining a negative membrane potential and an anion gradient. A system was established to first measure the trans-root voltage and to examine the change in this voltage when GtACR1 was activated with the appropriate wavelength of light using microprocessor-controlled LEDs. Using grafted roots of GtACR1 expressing plants on wildtype scions we could successfully grow the plants under normal light conditions to investigate the roots expressing GtACR1. Light (blue or green) that had a saturating effect on changing the trans-root potential caused whole root respiration rate to increase (and decrease in darkness) by about 10% over time (10-15mins). Wildtype and null controls showed no responses.

Poster 10

Pass the Salt - Is Sodium Accumulation Advantageous in Water Limited Environments?

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67% of Australian agricultural area is at risk of transient salinity, inhibiting plant growth and causing estimated agricultural losses of $519 million per annum. The Portuguese wheat landrace Mocho de Espiga Branca (Mocho) has been found to accumulate high levels of sodium (Na^+ in leaves and shoots, whilst maintaining healthy growth, indicating novel Na^+ tolerance mechanisms not found in commercial wheat. High Na^+ accumulation was found to be caused by a naturally occurring SNP in TaHKT1;5 that results in increased flux of Na^+ from root to shoot. The aim of this work is to further elucidate the mechanisms behind the increased salt tolerance and investigate whether increased leaf Na^+ content leads to greater performance in water limited environments.

To investigate mechanisms behind increased sodium tolerance, a Mocho x Gladius recombinant inbred line (RIL) population was characterised under salt stress and control conditions, identifying 465 novel loci relating to growth performance. To determine whether enhanced ion accumulation in the shoot helps plants to grow better in low to moderate water conditions, a selection of RILs were grown in a moderately saline field. Three RILs were identified which accumulated >10x leaf Na^+ with 5-28% yield improvement when compared to the parent Gladius. This poster will introduce current and future work, further investigating the potential benefits of Na^+ accumulation in water limited environments and the tolerance mechanism behind it.

Poster 11

Regulation and suppression of oxalate accumulation in duckweeds and water spinach

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Oxalate is an organic acid that accumulates in plants, reducing their organoleptic quality and has been linked to kidney stone formation and reduced nutrient availability in humans [1]. The reduction of oxalate in foods will increase their range of potential applications and improve their suitability as vegetable protein sources. Two promising plants for use as vegetable protein sources and as crops for controlled environment agriculture (CEA) due to their essential amino acid composition are duckweeds (*Lemnaceae*) and water spinach (*Ipomoea aquatica*), both of which are high in oxalate. My project aim is to use comparative transcriptomics and RNA-sequencing on high and low oxalate accumulating duckweed lines to identify candidate genes for oxalate metabolism. The enzyme activity of these genes and their effect on oxalate accumulation will be tested using transient gene expression and subsequently the expression of these genes will be manipulated and their homologous in water spinach. This project aims to produce a no or low oxalate accumulating duckweed clone and water spinach cultivar for use as vegetable protein sources and as crops grown in CEA. This research could also resolve some of the genetic regulatory mechanisms associated with OA metabolism in plants.

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Poster 12

Characterising the foliar cuticular wax composition of grain crops

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Terrestrial plants face a constant range of unique biotic and abiotic stresses in their environment and have developed fine-tuned mechanisms to deal with these. Resistance against many of these stresses begins at the cuticle, the waxy outer layer covering the surface of aerially-exposed tissues that is primarily involved in water retention, UV protection and physical defence against pathogens and herbivory. Anchored to the plant cell wall, the cuticle is primarily composed of the water-insoluble polyester cutin, which forms an extended network in which a range of long chain aliphatic compounds, collectively called cuticular waxes, form various crystalline and amorphous bodies both within and on top of the cutin network. The chemical composition of these cuticular waxes is highly species-specific, with great differences in functionality, carbon chain length and heterogeneity common. Recently, cuticular waxes have been linked to some host-recognition processes in pathogenic fungi, and as such, investigation into the specific surface chemical cues that fungal spores detect to promote infection is growing. With fungal diseases costing the Australian grains industry more than $900 million annually, understanding the cuticular wax composition and hence surface chemistry of economically important grain crop leaves will be the first step towards understanding the triggers of fungal germination and hence reducing the incidence of plant disease. Developing novel technologies that interfere in this host-recognition process by fungal pathogens will be paramount in ensuring food security in the context of climate change and growing world populations. In this work, the chemical composition of foliar cuticular waxes from various grain crops are probed using a GCMS-based approach, with their morphological wax structures investigated by SEM.

Poster 13

Antioxidant Effect of Hardwood Pyrolysis Products in Plants

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Agriculture and forestry biomass waste is an abundant source of material composed of substances such as cellulose and lignin, which can be processed into agricultural and other commercial products. Pyrolysis is a process that involves the anaerobic heating of such biomass between 380-700°C to yield a liquid extract. These extracts are rich in volatile compounds and have been used in agriculture as biostimulants and germination accelerators. One of the possible key signalling compound families within this extract are Karrikins (KARs), of which 6 isoforms are known to exist (KAR_1–KAR_6). They share a similar structure and signalling pathway to strigolactone, a plant phytohormone that regulates many aspects of plant development. KARs have also been shown to alleviate plant abiotic stress by enhancing antioxidant enzyme activity. In this research, we use specific pyrolysis conditions to thermally degrade three different Australian hardwood species. By using *N. tabacum*-based plant-pathogen system designed to induce rapid and quantifiable oxidative stress, we have demonstrated that KAR_1 but not KAR_2 provided protection to plant cells, indicating that there is structural specificity to the antioxidant effect induced by individual KAR isoforms. Additionally, the pyrolysis extracts from three different Australian hardwood species demonstrated a similar protective effect to that of Kar_1. With the increased frequency and severity of abiotic stress events, these extracts may contribute to an economically viable and sustainable means to extend the tolerance or enhance the resilience of broadacre crop and horticultural plants to the increasingly harsh growing conditions predicted in the future.

Poster 14

A tough nut not to crack: A non-destructive method of quality determination in hemp

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Industrial hemp (*Cannabis sativa L.*) is a non-psychoactive diecious crop grown primarily for its seed oil, stalk fibers, and flowers, which are respectively used in food, textiles and pharmaceuticals. Due to the historical illegality of the psychoactive varieties of cannabis, industrial hemp research is still in its infancy and much of the knowledge base that we rely on in traditional crop species (eg. wheat, barley, and rice) is absent. One such knowledge gap is regarding seed quality; whilst anecdotal evidence suggests hemp seed quality is correlated with color and maturity, analytical methods for accurate seed quality determination are usually destructive. These analyses calculate heart to hull ratio, quantifying the most economically valuable part of the seed crop, the hemp heart. However, seeds are often left non-viable and therefore cannot be utilized by breeders. In response, we are developing a non-destructive method to determine hemp seed quality. Using X-ray Computer tomography, which creates a 3D model of hemps seeds using the density of both the heart and the hull, it is possible to determine seed quality metrics such as grain fill and heart size. As a part of this method development, we have collaborated with the Australian Plant Phenomics Network to develop a bioinformatics pipeline that performs seed scanning and image processing to extract quality metrics. To specifically accommodate hemp seeds, we designed, and 3D printed a seed holder so individual seeds can be tracked across the various process. This method aims to provide a quantifiable, non-destructive measurement of hemp quality at a fraction of the time it would require for traditional measurements, giving farmers a reliable, time efficient evaluation of their crop.

Poster 15

The Genetic basis of stamen orientation in *Brassica rapa*

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To promote higher genetic diversity and greater fitness, plants have developed various adaptive mechanisms to promote outcrossing, such as self-incompatibility, herkogamy, heterostyly and stamen orientation. In the Brassicaceae, there are two stamen orientation morphs, extrorse and introrse, and in *Brassica rapa*, extrorse stamens is controlled by a single major, dominantly inherited gene called *Anther Orientation 1* (*AO1*). Using a combination of genetic mapping using bulked segregant analysis (BSA) of F_2 plants, RNA-seq expression analysis to detect filament expression of genes, whole genome sequencing of extrorse plants from *Brassica spp.* and biochemical prediction of gene functions, identified a single candidate gene. Translational fusion fluorescent reporter constructs of either the extrorse or introrse *AO1* encoded proteins to cerulean and transient expression in *Nicotiana benthamiana* leaves showed the extrorse translational fusion protein was localised to the cell periphery and nucleus as opposed to the introrse translational fusion was only localised to the cell periphery. In future experiments I will determine the precise cellular location at the cell periphery of the translational fusion proteins.

Poster 16

Evaluation of gRNA on-target prediction for plant genome editing

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The CRISPR guide RNA (gRNA) is responsible for directing Cas effectors to the target DNA sequence, undergoing cleavage. The gRNA is a crucial factor underpinning CRISPR/Cas targeting specificity and on-target genome editing efficiency. Different gRNA target sequences have shown vast differences in their *in planta* activity. Extensive research has revealed complicated sequence and structural features, genomic and epigenomic contexts that influence the on-target performance of a gRNA. Many *in silico* models, with the majority utilizing machine learning-based algorithms, have been developed to predict gRNA on-target performance. However, the algorithms were mostly trained using datasets from animal systems and have only been evaluated in thereof. The performance of these useful tools for plant applications remains controversial from past work. Designing efficient gRNAs has still been a difficult aspect of the plant genome editing workflow and the plant science community has been left in the dark. Yet, genetic transformation and tissue regeneration is laborious and inefficient in many plant species. It is, therefore, important to optimize the process of gRNA design to maximize CRISPR genome editing efficiency *in planta*. Here, we systematically evaluated developed *in silico* gRNA on-target prediction tools for plant genome editing and have further identified several well-performing gRNA on-target prediction tools for plant applications.

Poster 17

Molecular genetic analysis a biological control bacterium A. vitis GTI-5813 for controlling grapevine crown gall disease

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Grapevine crown gall disease (CGD) is characterized by tumour or cancer formation on the trunks, stems or roots of grapevines, and is particularly problematic on young plants. The primary causative agents of CGD are *Allorhizobium vitis* in 90% of cases and *Agrobacterium tumefaciens* in the remaining 10% of cases. Currently, there is no effective chemical treatment exists for CGD and the non-tumorigenic strain *A. vitis* strain F2/5 has shown promise as a biological control agent due to its ability to inhibit tumour formation by tumorigenic *A. vitis* strains. However, F2/5 can induce necrosis. Research by Zheng and Burr [2] revealed that grape tumor inhibition is linked to two nonribosomal peptide synthetase (NRPS) genes, F-avi3342 and F-avi5730, and one polyketide synthase gene, F-avi4330, with deletion of one of these genes resulting in loss of grape tumor inhibition (GTI) capacity. Furthermore, an Sfp-type phosphopantetheinyl transferase (PPTase) gene, F-avi5813, was identified as a post-translation modification enzyme crucial for catalyzing carrier protein domains of PKS and NRPS genes. Our laboratory has developed a novel derived strain from F2/5 strain, GTI-5813, with a targeted CRISPR-induced mutation in F-avi5813. Through experimentation with carrot slice pathogenicity disc assays, GTI-5813 has exhibited the capacity to suppress tumor formation without inducing necrosis on grape shoots. Based on these results, we propose that GTI-5813 may have the potential to inhibit tumor development on grapevines while also preventing grape necrosis.

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Poster 18

Characterisation of AtNHX8 suggests a possible role for lithium in dry seeds

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Lithium (Li^+) is a nonessential trace element in plants. Most studies of Li^+ in plants have focused on its toxicity, as a more toxic analogue of sodium (Na^+). AtNHX8 was identified as a putative Li^+H^+ antiporter with a role in Li^+ tolerance in *Arabidopsis thaliana* seedlings. However, Li^+ has a low abundance in the environment, hence it is unlikely that the actual function of AtNHX8 in plants is related to Li^+ tolerance. Using yeast as a heterologous expression system, we explored the Li^+ and Na^+ transport properties of the protein encoded by multiple natural alleles. Although small differences in Na^+ transport were observed, the results suggested that AtNHX8 is a pH-dependant Li^+ specific transporter across natural accessions, with minimal relevance for Na^+ transport. Analysis of *AtNHX8* expression in different organs, at different developmental stages, showed that *AtNHX8* has highest expression in dry seeds, which is consistent with published transcriptomics data. In addition, we observed that AtNHX8 promotes the accumulation of Li^+ in seeds: *Atnhx8* knockout mutants in high Li^+ accumulating genotypes showed a reduction in seed Li^+ content. While high expression of *AtNHX8* in dry seeds suggests a role during germination, it remains unknown whether it is related to its function in promoting Li^+ accumulation in seeds. Overall, the Li^+ specificity of AtNHX8, its function in promoting seed Li^+ accumulation and its unknown function during germination suggest that Li^+ may have a specific role in the seed.

Poster 19

Altering the strigolactone D53 signaling pathway improves the nitrogen use efficiency of barley

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A major yield-limiting factor in Australia is low soil fertility. To compensate for poor soils, growers often rely on application of nitrogen-fertilizers, which is costly and unsustainable. A cost-efficient and environmentally friendly approach to overcome this is to introduce smart cereal cultivars which can grow with less fertiliser. Recent advances in the field of plant growth regulators have identified strigolactone as an important hormone that controls plant branching, by preventing continued branching in poor soil conditions, hence favouring the nourishment of already developed branches. Barley plants with a mutated strigolactone *d53* signalling gene *d53a-153, d53b+1, d53b-30* were created using site directed nucelogensis-1 gene editing at the University of Adelaide. The performance of these lines was assessed under three nitrogen conditions in a replicated greenhouse experiment to determine their nitrogen use efficiency (NUE). The results revealed major differences between some of the mutants and the wildtype in respect to tillering, flowering, yield and seed morphology. The results revealed that some of the mutants can produce more branches (a factor of yield) under low nitrogen condition compared to the wildtype barley. Therefore, these findings suggest that barley plants with a mutated *d53* gene have more efficient use of resources under sub optimal condition.

Poster 20

Developing functional genomic resources to accelerate Vicia sativa (Common Vetch) improvement.

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According to United Nations statistics, the global human population is projected to increase by nearly 50% to 9.5 billion by 2050. To meet the growing demand for protein in animal feed the pasture and intensive livestock industries require affordable and sustainable protein sources. Similarly, to meet the growing demand for protein for human consumption, affordable and sustainable protein sources are required to be produced on limited arable land and decreasing global water. Common vetch (*Vicia sativa*) is a versatile legume used for soil coverage to reduce water loss, reduction in weed populations, nitrogen fixation, green and brown manure. Common vetch is very well-adapted to poor agronomic conditions, particularly the arid Australian climate and vetch seeds are rich in protein (24–32%) and contain essential unsaturated fatty acids and vitamins. However, common vetch production remains limited, comprising less than 5% of global legume crop production, primarily due to the presence of two anti-nutritional compounds, β-cyano-alanine (BCA) and its dipeptide derivative, γ-glutamyl-β-cyano-alanine (GBCA, Nguyen, *et al.,* 2020). These anti-nutritional compounds are toxic and cause excitotoxicity in monogastric animals such as poultry, pigs, and humans, and thus limiting the usage of common vetch in animal feeding and food markets.

To accelerate common vetch improvement we built a (1) high-quality chromosome-level reference genome (1.65 Gbp, n=7), assembled using Oxford Nanopore, Illumina, and Hi-C data, with 98% completeness and annotated 53,218 protein-coding genes (Xi *et al.,*2022), (2) and whole genome re-sequenced 285 wild accessions (Xi *et al.,* unpublished), (3) implemented rapid transgenic hairy root transformation using *Rhizobium rhizogenes* (Nguyen *et al.,* 2022), (4) optimized a stable *Agrobacterium tumefaciens*-mediated transformation method that achieves a median transformation efficiency of 7 % within 16 weeks (Wang *et al.,* unpublished), and (5) finally developed and implemented a Cas12a and Cas9 CRISPR-based genome editing toolkits (Wang *et al.,* unpublished). Together these resources will accelerate common vetch improvement to enhance the agricultural value of common vetch as a safe, sustainable plant-based protein source for the human food market.

Poster 21

Characterization of water sensing in crop plants using barley as a model system.

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Due to climate change, drought is expected to increase this century, reducing the availability of arable land for food crops. For plants to adapt to drought, roots can alter their root system architecture (RSA) and execute plasticity in branching patterns to efficiently access the available soil water. The suppression of lateral root (LR) formation in the absence of water, termed xerobranching, is an important adaptation for optimizing root foraging for critical soil resources such as water. It is revealed in *Arabidopsis*, that xerobranching is regulated by hydraulic fluctuation and the radial movement of hormones, mainly abscisic acid (ABA). Xerobranching has been reported in a few plant species, however; there is limited information in cereal crops, such as barley *Hordeum vulgare* L. The project will aim to understand xerobranching in barley and identify the molecular mechanism controlling root plasticity. Barley is an important crop in both Australia and the UK for feed and malting. Here, we have developed and optimized a paper roll assay by creating an airgap for measuring xerobranching in the seminal roots (SR) of Clipper, Sahara, Sloop, and Sloop SA varieties from the OzBarley panel. We demonstrate that LR formation is repressed in the airgap when roots are not in contact with moisture. Using this system, we will utilize the genetic diversity of the OzBarley collection, which includes over 200 varieties with diverse genotypic backgrounds, to screen for xerobranching and other root traits. Genome-wide association studies (GWAS) will be used to identify genetic markers and possible genes for ABA synthesis. This study will potentially lead to understanding the molecular mechanisms controlling xerobranching and root plasticity in barley, an ABA-dependent mechanism during drought stress that may be beneficial in breeding drought-resistant barley varieties.

Poster 22

GABA deficiency does not alter photosynthetic CO2 assimilation in Arabidopsis

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The fixation of carbon dioxide (CO_2) from the atmosphere is an integral process underpinning photosynthetic carbon and energy gain of plants. Intriguingly, CO_2 is known to be released into the cytosol as a side product of GABA synthesis, which occurs during a bypass reaction of the mitochondrial-based Krebs cycle. However, a putative connection between GABA production and CO_2 for photosynthesis from the atmosphere had not been investigated. Here, we show that GABA deficiency caused by the knockout of *Glutamate decarboxylases* (*GADs*) does not result in altered photosynthetic CO_2 assimilation. Gas exchange measurements in the GABA-depleted transgenic lines *gad2-1* and *gad1/2/4/5* did not reveal any changes in net CO_2 uptake and in the maximal rate of Rubisco carboxylase activity (V_{cmax}) and as of photosynthetic electron transport (J_{max}). This outcome is consistent with findings demonstrating that GABA deficiency is not linked to promoted plant growth, neither at standard conditions nor in response to long-term CO_2 exposure. A metabolome analysis revealed drastically elevated xylose concentrations in leaf material of the GABA-deficient mutant lines. In addition to the finding of numerous differentially expressed genes that are functionally enriched for functions in cell wall remodelling, the data indicates a link between GABA disruption and sugar salvage during wall recycling processes, thereby affirming that the elevation in monosaccharide content in the *gad(s)* mutants is independent of photosynthetic activities.

Poster 23

Tomato CslM2a play an essential role in pollen development and saponin biosynthesis

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The *cellulose synthase-like* (*Csl*) gene superfamily is a cellulose-synthase gene group identified in plant cell walls. While most Csl family members have been implicated in the backbone synthesis of a range of hemicellulose polysaccharides, the function of some members remains unknown [1]. *CslM* genes are eudicot-specific members of this superfamily. Tomato *CslM1* and *CslM2* have different expression patterns in developmental tissues, both active in young fruits but poorly expressed in mature fruits. Previous research has uncovered that both genes may be involved in the biosynthesis of type II arabinogalactan linkages in the plant cell wall, however, the glycosylating sugar is unknown. Several studies have shown that *CslM2* genes are further involved in saponin biosynthesis, adding a glucuronic acid unit at the C-3 position of triterpenoid aglycones during biosynthesis [2,3]. This work delved more into the role of *CslM2* genes using CRISPR/Cas9 gene editing and tomato as a model plant. *CslM2a* edited lines showed a male sterile phenotype, named nop (no pollen), accompanied by growth defects and parthenocarpy. Cell wall antibody labelling of developing anthers showed decreased pectin Rhamnogalacturonan-I and Arabinogalactan protein epitopes in the pollen mother cell and tapetum cell walls, possibly contributing to the pollen abortion. Also, α-tomatine and other minor tomato steroidal glycoalkaloids (SGA) were absent in the *CslM2a* edited lines. An additional biochemical test is needed to uncover the specific UDP-sugar(s) used by CSLM2a proteins. Collectively, this study shows the essential role that tomato *CslM2a* play in pollen development and saponin biosynthesis while highlighting galactose as a probable candidate nucleotide sugar for tomato *CslM2a* activity in SGA biosynthesis. With its established glucuronosyltransferase activity in triterpenoid saponins, this positions CSLM proteins as a dual-function, promiscuous group among the CELLULOSE SYNTHASE-LIKE proteins.

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Poster 24

The effects of rhizobial nodulation and AM fungal colonisation on the nutritional quality of grain legume crops

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Deficiencies in nitrogen (N), phosphorus (P), zinc (Zn) and iron (Fe) in agricultural soils can greatly harm crop yield and quality, while reducing nutrition for human health [1]. Pulse crops are vital in sustainable agriculture due to their ability to fix atmospheric N through symbiosis with rhizobial bacteria [2]. Arbuscular mycorrhizal (AM) fungi also benefit legumes by acquiring essential nutrients like P, Zn and Fe for the host plant [3]. However, the presence of phytic acid (PA), the storage form of P in seeds, chelates with Zn and Fe, reducing their bioavailability for humans [4]. There is limited research on their impact of rhizobia and AM fungi on PA accumulation, micronutrient bioavailability, and protein content in legume crops.

Firstly, the genetic variation in PA and protein content among 21 field pea cultivars was determined and found that while PA was highly variable amongst the cultivars, protein content was less so. Secondly, a fully factorial experiment assessed nutrient accumulation and bioavailability in the seeds of five field pea cultivars, comparing plants with and without *R. irregularis* inoculation. Results indicated significant differences in root AM colonisation among the field pea cultivars. The root dry weight (RDW) and P concentration in 'Kaspa' significantly increased with AM fungal inoculation. However, AM-inoculated plants did not show any differences compared to uninoculated plants regarding rhizobial nodulation, aboveground biomass, seed PA or protein content, or the bioavailability of Zn and Fe.

This study suggests that AM fungi may play a limited role in enhancing aboveground traits and seed quality in field pea. It highlights the complexity of interactions between AM fungi and field pea cultivars, indicating that while AM fungi can improve phosphorus uptake, their impact on micronutrient absorption is likely more intricate.

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Poster 25

Investigating the role of RNA 5-methylcytosine in post-transcriptional gene regulation in plants

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RNA 5-methylcytosine (m^5C) is a critical post-transcriptional modification involved in regulating RNA metabolism, processing, and translation. Found in both prokaryotic and eukaryotic mRNAs, as well as various non-coding RNAs, m^5C levels have been shown to be involved in responses to abiotic stress conditions, such as extreme heat. Recently, a study uncovered a specific role for RNA m^5C methyltransferase in promoting rice adaptation to high temperatures and enhancing mRNA translation [1]. This finding contradicts previous results in *Arabidopsis thaliana*, where RNA m^5C was linked to reduced mRNA translation. Therefore, the precise role of m^5C in translation remains unclear.

To address this, we conducted transcriptome-wide bisulphite-RNA sequencing (BS-RNA-seq), identified over 800 transcripts which either increased or decreased RNA m^5C under heat shock. Four transcripts, which exhibited significant m^5C increases, were selected for further analysis. To test m^5C's effect on translation, we constructed a luciferase activity analysis system by cloning short (80–100 nt) sequences flanking the m5C sites from these transcripts into a Firefly luciferase reporter plasmid. These plasmids were transiently expressed in *A. thaliana* RNA m^5C methyltransferase mutants, *trm4a*, *trm4b*, *trm4a/b*, and *rcmt9*. Our agroinfiltration and dual-luciferase assay showed that three of the four m^5C -containing sequences increased Firefly luciferase mRNA translation in wild-type *A. thaliana*, but decreased translation in *trm4a*, *trm4b*, *trm4a/b*, and *rcmt9* mutants. One sequence had no effect in either wild-type or mutant lines. After mutating the methylated cytosines in these sequences to guanine, we observed decreased mRNA translation in wild-type *A. thaliana* and no effect in the mutants. These findings are consistent with prior results in rice [1]. Unexpectedly, we also identified a translational enhancer with effects independent of RNA m^5C.

This study demonstrates that luciferase activity analysis is a valuable tool in plants for discovering RNA sequences that modulate translation positively or negatively.

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Poster 26

Unlocking the Value of Lysine for Sustainable Agriculture

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As the global population is set to approach 10 billion by the year 2050, strategies to increase the production and nutritional value of crops are of major significance [1,2]. Lysine is an essential proteinogenic amino acid and is crucial for a myriad of biological functions in all organisms [3]. Yet, it is one of the most limiting amino acids in cereal crops [4]. As such, efforts are being made to exploit lysine biosynthesis in plants for the benefit of agriculture, either for inhibition as a novel herbicide mode of action or for enhanced production to biofortify crops [5,6]. In plants, lysine biosynthesis is tightly regulated via the inhibition of the enzyme dihydrodipicolinate synthase (DHDPS) [7]. However, the exact mechanisms underpinning the regulation of DHDPS in plants remains unclear. In this study, we use a combination of in-solution bioanalytical techniques such as mass photometry and small angle X-ray scattering to shed light on the regulation of the lysine biosynthesis pathway via the inhibition of the enzyme dihydrodipicolinate synthase (DHDPS). We found that lysine mediated significant structural changes to DHDPS and thus propose a new mechanism of allosteric regulation by lysine. We then demonstrated how this mechanism can be exploited for herbicide development by investigating the potential of lysine analogues as herbicide scaffolds. In doing so, we discovered the first lysine biosynthesis inhibitors with both pre-emergent and post-emergent efficacy against weeds, including *Lolium rigidum* (annual ryegrass). Thus, this study provides insight into the mechanism of regulation of lysine biosynthesis and highlights the potential for exploiting this mechanism in the pursuit of herbicides with novel modes of action.

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Poster 27

Understanding salt uptake and accumulation in plants

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Salinity is a global problem and arable land area is decreasing due to human induced salinisation. To gain a better understanding of the fundamental mechanisms of salt homeostasis, we aim to generate new information about the biophysical and biochemical factors influencing the uptake, transport and accumulation of salt in plant organs and tissues, using grapevine rooted leaves as a simple root-to-shoot model. We are comparing *Vitis vinifera* cv. Cabernet Sauvignon and salt-tolerant and salt-sensitive rootstock cultivars grown in a hydroponics system, and observed that the leaf spatial distribution of sodium (Na^+), potassium (K^+) and chloride (Cl^-) is dependent on the strength of salt treatment, treatment period, and grapevine genotypes. Interestingly, although salinity is commonly considered to reduce plant K accumulation, Cabernet Sauvignon was able to maintain stable leaf K levels during moderate salt stress (50 mM NaCl), and the leaf K content increased overtime under high salt stress (100 mM NaCl). We will employ transcriptomics, anatomy and hydraulic data to gain further insights into the leaf spatial accumulation of salt and related ions. Data will be incorporated into a biophysical model to quantify the movement of salt and water through the plant, which could be used for predicting functions of tissues, ion transporters, and energy costs in salt stressed plants.

Poster 28

Pectin Glycosyltransferases: Driving Advances in Bioenergy and Plant Cell Wall Research

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Pectin plays a critical role in plant cell wall integrity, structure, and functionality. The pectic domain of Rhamnogalacturonan II (RG-II) is often described as the most complex glycan on the planet, but the structure is highly conserved in all plant cell walls. Loss of a single glycosyl residue is lethal, leading to challenges in identifying the biosynthetic pathway, as well as exploring function. RG-II is also the major reason why Boron is required for plant growth, since dimerisation of RG-II is driven by the formation of a borate diester between the apiose units on side chain A.

Here, building on our recent work (Zhang et al. 2024) we are implementing a bioinformatics pipeline to identify candidate pectin biosynthesis genes (glycosyltransferases, GTs). Here, we overcome issues of lethality by using gene edit to induce null mutations during callus formation, and maintaining the lines in tissue culture, thereby avoiding the reproductive phase. Cell wall polysaccharides from knockout lines of these candidate genes will be extracted and composition and fine structure analysed. To support established structural methods, such as H1-NMR, we will develop a new form of PACE (Polysaccharide Analysis by Carbohydrate Gel Electrophoresis; Pidatala et al. 2017) making use of a suite of recently described bacterial glycosyl hydrolases that can sequentially degrade RG-II (Ndeh et al 2017). This will be paired with heterologous expression of the candidates GTs to enable substrate-specificity assessment, such that binding affinity methods can be used to validate GT function.

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Poster 29

Glycosyltransferases in grapevines post-smoke exposure: Understanding and modifying their activity and selectivity

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Bushfire smoke is a major threat to the Australian wine industry, which has incurred financial losses of $1.4 billion since 2003. The economic losses include the destruction of vineyards and equipment, alongside the inability to produce quality wine because of 'smoke taint'; i.e., the negative sensory attributes perceived in wine made from smoke exposed grapes attributed to volatile phenols (VPs) and phenolic glycoconjugates (PhGs). Mono-, di-, and tri-saccharides of guaiacols, cresols and syringols have been identified in smoke-affected grapes and wine.

Mitigation strategies employed in the winery have proven ineffective at removing PhGs from wine. Sensory studies have found PhGs can be hydrolysed by saliva to release VPs and contribute to smoky flavour during tasting, but the release rate is determined by their structural composition. However, the biochemical response of grapevines to smoke has not yet been comprehensively studied.

PhG formation in grapevines is catalysed by the addition of sugar moieties from an activated sugar donor to VPs by glycosyltransferase enzymes (GTs). To date, one *Vitis vinifera* GT, UGT72B27 has been reported to catalyse the formation of several phenolic monoglucosides *in vitro*. However, the GTs involved in the synthesis of the longer sugar moieties have not yet been identified.

In this study, we tested whether the model plant *Arabidopsis thaliana* can similarly form phenolic disaccharides after smoke exposure. Next, we used transcriptomics to profile gene expression in smoke exposed *A. thaliana* leaves. Using a candidate gene approach, we combined these data with in-house and publicly available datasets on smoke-exposed grapevine material to identify putative GTs with a role in PhG formation. We are now expressing these genes heterologously, along with their *V. vinifera* homologues, to test their enzymatic activity on different substrates. Identification of novel GTs may lead to the development of new mitigation strategies, either *in planta*, or during processing.

Poster 30

A potassium release mechanism via a water and cation conducting plant aquaporin

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Plant aquaporins (AQPs) were initially characterized as a family of membrane-localized proteins facilitating water transport. However, some plant AQPs exhibit diverse functionalities beyond water transport. The aquaporin AtPIP2;1, an abundant plasma membrane intrinsic protein in *Arabidopsis thaliana*, facilitates the transport of univalent cations and water. Despite this, the mechanisms governing the selectivity of AtPIP2;1 for cations and water remain incompletely understood. We have shown that the regulation of water and cation transport via AtPIP2;1 is finely controlled by phosphorylation/dephosphorylation at four conserved serine residues within loop B, D, and the C-terminal domain. Concurrent modifications at these specific sites can serve as a 'selectivity switch,' modulating the preference between cations and water. When heterologously expressed in *Xenopus laevis* oocytes, AtPIP2;1 demonstrated selectivity of K^+ > Rb^+ > Cs^+ > Na^+ > Li^+ > TEA > choline > NMDG. Significantly reduced K^+ efflux from roots was observed in *atpip2;1* mutants compared to wild-type controls, demonstrating AtPIP2;1's dual transport capacity in plants. These findings shed light on the intricate regulation of plant water transport and offer potential avenues for enhancing water use efficiency under varying water stress conditions.

Poster 31

Crosstalk Between GABA and ROS in Regulating Potassium Transport and Enhancing Stress Resilience in Arabidopsis thaliana

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γ-Aminobutyric acid (GABA), a non-protein amino acid, has emerged as a critical endogenous signal in plants, enhancing water-use efficiency, reducing stomatal pore opening, and conferring drought resilience in *Arabidopsis thaliana*. Recent studies highlight a strong link between GABA metabolism and reactive oxygen species (ROS) generation, especially under stress conditions like high salinity. Both GABA and ROS play essential roles in maintaining potassium (K) homeostasis, which is vital for managing toxic sodium (Na) accumulation. However, the specific mechanisms through which GABA and ROS coordinate K2 transport remain unclear. This study investigates the crosstalk between GABA and ROS in regulating K^+ efflux in *Arabidopsis* via outward-rectifying K^+ channels, specifically the Guard-cell Outwardly Rectifying K^+ (GORK) and Stelar K^+ Outward Rectifier (SKOR).

Initial experiments involved cloning and expressing GORK and SKOR channels in *Xenopus laevis* oocytes, followed by validating K-transport modulation. Preliminary two-electrode voltage clamp (TEVC) data demonstrated that 10 mM hydrogen peroxide (H_2O_2) activates SKOR channels within 10 minutes. Moreover, co-application of 10 mM GABA and 10 mM H_2O_2 significantly enhanced SKOR-mediated K^+ currents, indicating a potential synergistic effect of GABA and ROS in promoting K^+ efflux under stress. These findings establish an initial framework for understanding the GABA-ROS signalling interaction in plant ion regulation, providing insights that could inform future strategies for improving crop resilience under adverse environmental conditions. Future work will further delineate this interaction to clarify GABA's role in balancing ROS and enhancing plant tolerance to stress.

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