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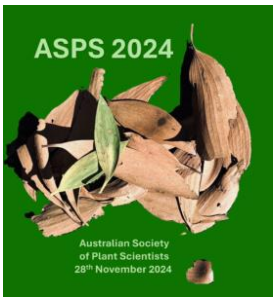

ASPS 2024 Melbourne node – La Trobe University City Campus – level 3 360 Collins St

8:30 Registration open, coffee available

Session 1

8:45 Welcome – Dugald Reid

8:50 **Nicole Thomson** The LEUNIG-SEUSS-WOX Regulatory Complex Governs Cell Patterning and Identity during Arabidopsis Embryo Development.

9:05 **Ronan Broad** The FUN Times are Rolling: Removing Environmental Suppression of Nitrogen Fixation in Legumes

9:20 **Simone Currie** Transcriptional regulation of cannabinoid biosynthesis in Cannabis sativa

9:35 **Francis Kuang** Multidisciplinary Approach Toward Novel Inhibitors Of Ethylene-Forming Enzyme in Plant

9:45 **Bhavna Hurgobin** Mapping the organ-specific accessible chromatin landscape of Cannabis sativa using multi-omics

9:55 **Sofya Gvaramiya** Transcriptional regulation of terpene pathway genes in C. sativa

10:05 **Angelica Rossi** Molecular Farming in Space: Engineering Micro Tom Tomato Trichomes for On-Demand Pharmaceutical Production

10:10 **Onkar Nath** Characterizing the Genomic Diversity of Asian Hemp cultivars

10:15 Coffee break

Session 2

10:45 **Lim Chee Liew** Unlocking the seed germination: insights into gene regulation and cell function during Arabidopsis seed-to-seedling transition

11:00 **Anna Philippova** Circadian-regulated dynamics of translation in Arabidopsis thaliana

11:15 **Marta Peirats Llobet** Uncovering the transcriptome secrets of barley germination: a spatially resolved study

11:25 **Allison van de Meene** Cell wall synthesis and organelle associations in grasses

11:35 **Lucas Auroux** Single-cell 3D spatial gene expression analysis in whole-mount germinating seeds

11:45 **Olivia Doolan** Creating a 3D Atlas of Germinating Barley Grains using Multi-Modal Imaging

11:55 **Yalin Liu** The investigation of evening complex function in wheat

12:00 **Lily Tarry-Smith** Investigating the TaHRZ gene family for iron biofortification and abiotic stress tolerance in bread wheat

12:05 Lunch and Posters:

Muluneh Tamiru Oli Comparative transcriptome analysis provides insight into glandular trichome gene regulatory networks

Anshul Phaugat Enabling plants to thrive in extreme conditions and outer space !!!

Kira Maher Role of nitrogen-fixing cyanobacteria in the growth and nitrogen economy of Australian Cycas

Dinithi Chithrarachchige Insights into drought tolerance of domesticated sorghum, S. bicolor

Thomas Cobbinah Investigating Trichome Development Pathways in Tomato

1:30 Online ASPS awards session

4:15 Posters

5:00 Meeting closes

6:00 Drinks available to purchase at Bang Pop, South Wharf

6:30 Dinner – Bang Pop, South Wharf

The LEUNIG-SEUSS-WOX Regulatory Complex Governs Cell Patterning and Identity during *Arabidopsis* Embryo Development.

N. L. Thomson[1], J. Kaptur[1,2,3], V. I. Strotmann,[4,5] A. Sampathkumar[3], Y. Stahl,[4,5] J. F. Golz[1].

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The establishment of cell patterning and growth during plant embryogenesis is foundational to the development of crops and native plants. While an important driver of patterning during the early stages of embryogenesis is the phytohormone auxin, knowledge of the gene regulatory network that establishes embryonic auxin distribution and pattern formation remains incomplete. My research seeks to bridge this knowledge gap by investigating the potential role of transcriptional co-regulators LEUNIG (LUG), LEUNIG_HOMOLOG (LUH) and SEUSS (SEU) to modulate the activity of WUSCHEL RELATED HOMEOBOX (WOX) transcription factors, key regulators of embryonic patterning.

Utilising an array of molecular techniques, we elucidated the role of the LUG-SEU complex in establishing cell identity and patterning during embryogenesis. Embryos lacking LUG-SEU activity exhibit severe apical-basal defects, including the loss of markers for SAM and cotyledon development, aberrant cell divisions, and abnormal RAM formation; defects linked to disruption of auxin distribution, auxin biosynthesis and/or perception. *In situ* hybridization, promoter-reporter assays, and chromatin immunoprecipitation indicate that LUG-SEU regulates auxin distribution and promotes SAM formation through the direct activation of the *CLASS III HOMEODOMAIN LEUCINE-ZIPPER (HD-ZIPIII)* transcription factors.

Given that the WOX2 module (WOX1/2/3/5) supports SAM initiation through the promotion of *HD-ZIPIII* activity, as well as balancing auxin and cytokinin levels necessary for correct SAM patterning[1], we examined the potential of LUG/LUH/SEU to genetically interact with *wox* mutants. These studies were complemented with advanced protein interaction assays, including FRET-FLIM, which unambiguously identified extensive physical interactions between the LUG-SEU-WOX proteins. Based on these findings, we propose that LUG-SEU modulates WOX activity during patterning and cell identity establishment during the early stages of embryogenesis.

1. Zhang, Z., Tucker, E., Hermann, M., & Laux, T. (2017). A Molecular Framework for the Embryonic Initiation of Shoot Meristem Stem Cells. *Developmental Cell, 40*(3), 264-277.e264. https://doi.org/10.1016/j.devcel.2017.01.002

The *FUN* Times are Rolling: Removing Environmental Suppression of Nitrogen Fixation in Legumes

Ronan Broad[1], Dugald Reid[1]
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To balance the cost of carbon provision to symbiotic nitrogen fixing bacteria, legumes modulate nodule function in response to soil nitrogen levels. This is beneficial in natural conditions, however in an agricultural context it is often desirable to maximize the delivery of fixed nitrogen. The *Fixation Under Nitrate* (*FUN*) gene encodes a bZIP-type transcription factor of the TGA family which acts as a master positive regulator of nodule senescence by directly targeting several pathways to initiate the breakdown of nodules in response to high nitrogen[1]. *Lotus japonicus fun* mutants were shown to have increased leghemoglobin concentration and acetylene reduction under high nitrogen, suggesting an increased capacity to fix nitrogen, however direct nitrogen fixation and phenotypic measurements had not yet been investigated. We have now used ^{15}N isotopic analyses to show that *Ljfun* mutants either fix significantly more nitrogen from the atmosphere or accumulate significantly greater biomass relative to wild type, depending on the growth conditions. We are translating the beneficial *fun* mutant phenotype into two globally important legumes: cowpea (*Vigna unguiculata*) and soybean (*Glycine max*). Phylogenetic analyses identified one *FUN* ortholog in cowpea (*VuFUN*) and two *FUN* orthologs in soybean (*GmFUNa* and *GmFUNb*). Promoter-GUS constructs showed that *VuFUN* has highly specific nodule expression. Moreover, RNA-Seq analysis of cowpea nodules in response to high nitrogen has revealed that the *FUN* transcriptional network is highly conserved in cowpea. Finally, a range of CRISPR/Cas9 vectors have been stably transformed into cowpea targeting the promoter regions and coding sequences of *VuFUN*, as well as the downstream transcription factor *VuNAC094* which also drives nodule senescence in response to high nitrogen[2]. A range of edits of the *VuFUN* coding sequence in T_0 cowpea plants have recently been detected using deep sequencing.

1 Lin, J. *et al.* Zinc mediates control of nitrogen fixation via transcription factor filamentation. *Nature* **631**, 164-169 (2024). *https://doi.org/10.1038/s41586-024-07607-6*

2 Wang, L. *et al.* A transcription factor of the NAC family regulates nitrate‐induced legume nodule senescence. *New Phytologist* **238**, 2113-2129 (2023). *https://doi.org/10.1111/nph.18896*

Transcriptional regulation of cannabinoid biosynthesis in *Cannabis sativa*

Simone J. Currie[1], John Humphries[1], Muluneh Oli[1], Matthew T. Welling[1], Mathew Lewsey[1] and Monika S. Doblin[1].

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Cannabis sativa synthesises a myriad of specialised metabolites, including terpenes, phenols, alkaloids and in particular phytocannabinoids. An understanding of the molecular pathways and mechanisms regulating the production of phytocannabinoids and how they may be manipulated is of great interest to the medicinal cannabis industry. The production of specialised metabolites is, in part, mediated by transcription factors (TFs) that regulate the expression of genes encoding biosynthetic enzymes. In this study, TFs predicted to regulate structural genes of the MEP, oxylipin, polyketide and cannabinoid biosynthetic pathways were identified through promoter analyses using PlantRegMap[1] and gene co-expression analyses of RNA-seq datasets from divergent medicinal cannabis chemotypes. Candidate TFs were validated using DNA-affinity purification sequencing (DAP-seq), virus-induced gene silencing (VIGS) and transient overexpression. *CsHD-ZIP* was identified as a potential negative regulator of two fatty acid desaturase (*FAD*) genes of the oxylipin biosynthetic pathway. Binding sites were found within the exon of one of these genes via DAP-seq. Downregulation of *CsHD-ZIP* via VIGS resulted in increased expression of one *FAD* gene, as well as the downstream gene olivetolic acid cyclase (*OAC*). Furthermore, accumulation of cannabigerolic acid (CBGA) was elevated in *CsHD-ZIP* silenced plants. Together these results are consistent with *CsHD-ZIP* being a negative regulator of cannabinoid biosynthesis. Transient overexpression of *CsHD-ZIP* is currently being pursued to provide further validation of its mode of action. The combination of techniques used in this study to identify and validate TFs has proved useful in extending our knowledge of gene regulation in *C. sativa*.

[1] Tian, F., Yang, D.C., Meng, Y.Q., Jin, J. and Gao, G. (2020) PlantRegMap: charting functional regulatory maps in plants. *Nucleic Acids Res* **48**, D1104-D1113.

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Multidisciplinary Approach Toward Novel Inhibitors of the Ethylene-Forming Enzyme

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[3]School of BioSciences, The University of Melbourne, Melbourne, Australia

Ethylene plays a pivotal role in plant growth and development, making precise regulation of its biosynthesis crucial for agricultural applications such as fruit ripening and delaying senescence. Ethylene biosynthesis is mediated by two key enzymes: 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase (ACO), with ACO catalysing the final step—the conversion of ACC to ethylene. Despite its central role, ACO remains an underexplored target for ethylene inhibition. This study presents findings from a multidisciplinary investigation into ACO inhibitor discovery, integrating enzymology, structural biology, and plant biology. Leveraging recent structural insights into ACO's substrate-binding mechanism, we identified conserved binding patterns among related enzyme families. This informed the hypothesis that small molecules targeting these conserved features could act as competitive inhibitors. To test this, we developed a high-throughput assay to quantify ACO kinetics and evaluate the inhibitory effects of candidate molecules. Arabidopsis thaliana was used as a model system to validate inhibitory effects and assess ethylene-related phenotypes in vivo. Additionally, we tested a lead compound for its ability to delay cut flower senescence, demonstrating its practical potential. Our results confirm that small molecules targeting conserved regions of ACO act as competitive inhibitors, as validated through both in vitro and in planta studies. This work provides novel insights into the regulation of ethylene biosynthesis and identifies promising candidates for agricultural control of ethylene production.

Mapping the organ-specific accessible chromatin landscape of *Cannabis sativa* using multi-omics

Bhavna Hurgobin[1,2], Onkar Nath[1,2], Sophia Ng[1,2], Muluneh Tamiru-Oli[1,2], Lee J. Conneely[1,2,3], Mathew G. Lewsey[1,2,3]

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[3]Australian Research Council Centre of Excellence in Plants for Space, AgriBio Building, La Trobe University, Bundoora, VIC 3086, Australia

Cannabis is one of the oldest cultivated plants in the world. A key feature of this important crop is the presence of the glandular trichomes, which are a highly specialised organ type that synthesize and store a wide array of secondary metabolites. However, little is known about the epigenetic mechanisms controlling the biosynthesis of these metabolites. A better understanding of these mechanisms would allow for a more optimized and tailored production of these metabolites in cannabis. Consequently, we investigated the epigenomics regulation of gene expression in four distinct organs, namely glandular trichomes (GTs), leaves, stems and roots of cannabis, using a combined approach of RNA-seq, ATAC-seq and ChIP-seq. The application of RNA-seq allowed us to identify organ-specific genes. ATAC-seq (assay for transposase-accessible chromatin with sequencing) allowed us to comprehensively map accessible chromatin regions (ACRs) across the four organs, revealing ACRs that were distinct to each organ. In the case of GTs, a portion of these organ-specific ACRs were associated with genes involved in the production of key secondary metabolites. Integration with ChIP-seq (chromatin immunoprecipitation sequencing) data generated for the GTs revealed the presence of the histone marks, H3K4 trimethylation (H3K4me3) and H3K56 acetylation (H3K56ac), which are positively correlated with actively transcribed genes. Some of these histone marks were found to be GT-specific, further validating the findings from the ATAC-seq analyses. Overall, this multi-omics study provides a valuable resource for dissecting the epigenetic basis of organ-specific development and secondary metabolism in cannabis. The ACRs and associated genes identified offer valuable insight into the highly specialised function of GTs, paving the way for targeted manipulation to enhance secondary metabolite production in this important crop.

Transcriptional regulation of terpene pathway genes in *C.sativa*

Sofya Gvaramiya[1,2], Muluneh Tamiru-Oli[1,2], Bhavna Hurgobin[1,2], Mathew Lewsey[1,2]

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Specialised metabolites in plants consist of diverse classes of compounds. Among them, terpenes are the largest group with over 80,000 members that have diverse structures and functions, particularly in plant physiology and interactions with environment. Terpenes have become of great interest because of their broad applications, for example, in cosmetic, pharmaceutical and food industries. Two major biosynthetic pathways involved in terpene production (mevalonate and 2-*C*-methyl-d-erythritol-4-phosphate pathways) and a plethora of terpene synthases are known. But very little is known about the transcriptional regulation of terpene biosynthesis in plants. In this study, we aimed to find transcription factors (TFs) – the key regulators of terpene biosynthesis on the example of cannabis. We sequentially analysed time-series RNA-seq data through differential expression analysis, weighted gene co-expression network analysis and gene regulatory network prediction. From these we selected 10 putative candidate TFs that can play role in terpene biosynthesis. Consequently, predicted candidates were tested with DNA affinity purification sequencing (DAP-seq) to map the transcription binding sites (TFBS) of candidate TFs and check the accuracy of network analysis. Preliminary results indicate that TFBS of some candidates are found in promoter regions of genes involved in both terpene biosynthetic pathways. Functional characterisation of the selected TFs *via* virus-induced gene silencing (VIGS) is in progress.

Molecular Pharming in Space: Engineering Tomato Trichomes for On-Demand Pharmaceuticals

Angelica Rossi, Dr. Lee Conneely, Sonya Gvaramiya, Prof. Mathew Lewsey
La Trobe University, ARC Centre of Excellence in Plants for Space

Long-duration space missions demand innovative strategies for in-situ, on-demand production of essential resources, including pharmaceuticals. Transporting medications from Earth presents several challenges, such as weight and volume constraints, degradation over time, and reliance on resupply missions.

Molecular pharming through space agriculture offers a promising solution, particularly by using tomato trichomes as biofactories. The Micro-Tom cultivar is ideal for space missions due to its compact size and rapid life cycle. This study focuses on type VI glandular trichomes, located on Micro-Tom stems and leaves, which provide a platform for producing high-value specialized metabolites.

The methodology employs synthetic biology and metabolic engineering tools, starting with the identification of trichome-specific promoters to drive gene expression exclusively in trichomes. Genes associated with the biosynthetic pathway for medically relevant compounds are introduced via Agrobacterium-mediated transformation. The compounds produced in trichomes will be analysed using Gas Chromatography-Mass Spectrometry and High-Performance Liquid Chromatography. This analysis will profile the naturally occurring compounds in Micro-Tom tomatoes, alongside those produced via genetic modification, to demonstrate the potential for altering the metabolic profile to enable pharmaceutical production.

This research offers a sustainable solution for on-demand pharmaceutical production in space, reducing reliance on Earth-based resources while improving resource efficiency. Beyond space exploration, these findings could help redefine Earth-based pharmaceutical production, enabling cost-effective and environmentally sustainable methods, particularly for resource-limited settings.

Characterizing the Genomic Diversity of Asian Hemp cultivars

Onkar Nath[1], Matthew T. Welling[1], Laura Steel[1], Mathew G. Lewsey[1], Kim Johnson[1], Anthony Gendall[1]

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Cannabis sativa is a versatile crop with significant economic and ecological importance. While there has been a surge in cannabis research, Asian hemp genotypes remain underrepresented in genetic resources. To address this gap, we sequenced the genomes of two low-THC industrial hemp cultivars of Asian origin and their F1 hybrid. The first cultivar is characterized by a unique cannabinoid profile, producing equal quantities of pentyl (THC, CBD) and propyl (THCV, CBDV) cannabinoids. This unusual chemotype provides a valuable resource for understanding the genetic basis of cannabinoid biosynthesis. The second cultivar, a traditional Chinese landrace, is renowned for its high content of linoleic acid (LA) and alpha-linolenic acid (ALA) in its seeds. The two cultivars also vary by flowering time behaviour, with the first being a late-flowering cultivar. To generate high-quality phased genome assemblies, we employed PacBio Revio HiFi sequencing and assembled the genomes using hifiasm. The resulting assemblies were annotated by transferring annotations from the reference *Cannabis sativa* cs10 genome. The resulting contigs were assembled into approximately one thousand contigs, with an N50 length of over 65 Mb and a total genome size of about 836 Mb. The annotation resulted in the assignment of more than twenty-nine thousand genes to the assemblies. These assemblies will provide valuable insights into the genetic diversity and evolutionary history of *Cannabis sativa*, and will enable the identification of functional variants associated with traits of agronomic and medicinal importance. By understanding the genetic basis of these traits, we seek to advance our understanding of cannabis genetics and provide valuable insights for future breeding and cultivation strategies.

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Unlocking the seed germination: insights into gene regulation and cell function during Arabidopsis seed-to-seedling transition

Lim Chee Liew [1 2] , Yue You [3 4] , Lucas Auroux [1] , Marina Oliva [5] , Marta Peirats-Llobet [1] , Sophia Ng [1 6] , Muluneh Tamiru-Oli [1 6] , Oliver Berkowitz [1 6] , Uyen Vu Thuy Hong [1] , Asha Haslem [1] , Tim Stuart [7] , Matthew E. Ritchie [3 4] , George W. Bassel [8] , Ryan Lister [5 9] , James Whelan [1 6 10] , Quentin Gouil [1 3 4] , Mathew G. Lewsey [1 5 6]

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Seeds are crucial to global food production, providing 70% of resources and initiating the lifecycle of most crops through germination. Uniform germination is essential for optimal plant spacing and harvest timing. Despite the significance of this process, the regulation of seed germination remains poorly understood, hindering advancements in improving seed properties. Traditional bulk-tissue 'omics approaches have identified key transcription factors that influence gene expression and germination progression. However, seeds are complex structures with diverse tissues and cell types, each exhibiting distinct functions and gene expression changes that are context-dependent during germination. To gain deeper insights, we have employed single-cell transcriptomic to analyze germinating seeds in the model plant Arabidopsis. Time-series analyses were conducted at three intervals post-stratification: 12 hours (mucilage excretion), 24 hours (seed coat rupture), and 48 hours (embryonic emergence). This approach allowed for the identification and annotation of individual cell types within the embryo and their transcriptional changes during the first 48 hours of germination. We discovered a common transcriptional state at 12 hours before a dynamic transcriptional landscape where cells undergo extensive reprogramming, transitioning through various states to fulfill functional requirements during germination. Unique gene regulatory programs and specific transcription factors were identified for different cell types. This research offers unprecedented insights into the gene regulatory networks that operate during seed germination, enhancing our understanding of cell function during seed-to-seedling transition. Ultimately, these discoveries hold promise for developing strategies to improve the seed-to-seedling transition, ensuring uniform germination at optimal times.

Circadian-regulated dynamics of translation in *Arabidopsis thaliana*

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In plants rhythmic changes of various environmental cues such as light or temperature are anticipated and synchronized with biochemical processes through the intrinsic timing mechanisms of circadian clocks[1]. In this study, we employ polysome analysis to explore the changes in global translation levels during both diel (light/dark) and circadian (light/light) cycles in 14-day-old *Arabidopsis thaliana* seedlings. We detected gradual changes in the global translational level over the diel cycle. Polysome loading was upregulated during the day and was downregulated during the night. Furthermore, our data also revealed gradual changes in the global translation level over the circadian cycle. We found that polysome loading increased and decreased in the 'subjective' day and night phases, respectively. Thus, our data suggest that the global translation level might be governed by the circadian clock. We then directly monitored diel and circadian dynamics of protein synthesis rates by puromycin labelling. During our study, we also identified novel regulatory elements (upstream open reading frames (uORFs)) that are potentially involved in the translational regulation of circadian and daily rhythms. Using site-directed mutagenesis, luciferase reporter assay system and transient transformation in *Arabidopsis thaliana* we verified functions of predicted regulatory elements and, therefore, shed more light on the translational regulation of circadian and daily rhythms.

References:

1. Saini, R., Jaskolski, M., and Davis, S.J. (2019). Circadian oscillator proteins across the kingdoms of life: structural aspects. BMC Biol 17, 13.

Uncovering the transcriptome secrets of barley germination: a spatially resolved study

Peirats-Llobet M.[1], Staka Z.[2], Yi C.[1], Liew L.C.[1], Berkowitz O.[1,3], Narsai R.[1], Lewsey M.G.[1,3] and Whelan J.[1,2]

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Seeds, which provide a major source of calories for humans, are a unique stage of a flowering plant's lifecycle. During seed germination the embryo reactivates rapidly and goes through major developmental transitions to become a seedling. This requires extensive and complex spatiotemporal coordination of cell and tissue activity. Existing gene expression profiling methods, such as laser capture microdissection followed by RNA-seq and single-cell RNA-seq, suffer from either low throughput or the loss of spatial information about the cells analysed. Spatial transcriptomics methods overcome those limitations by coupling high throughput analysis of gene expression with the ability to record the spatial location of each individual region analysed. We developed a spatial transcriptomics workflow for germinating barley grain to better understand the spatiotemporal control of gene expression within individual seed cell types. More than 14,000 genes were differentially regulated across 0, 1, 3, 6 and 24 hours after imbibition. This approach enabled us to observe that many functional categories displayed specific spatial expression patterns that could be resolved at a sub-tissue level. For example, individual aquaporin gene family members, important for water and ion transport, had specific spatial expression patterns over time, as well as genes related to cell wall modification, membrane transport and transcription factors. Moreover, we developed a user-friendly web app (https://spatial.latrobe.edu.au/) to make exploring the data and differentially regulated genes easier and more accessible. Together, our data provides an unprecedented spatially resolved cellular map for barley grain germination and specific genes to target for functional genomics to define cellular restricted processes in tissues during germination.

Cell wall synthesis and organelle associations in grasses

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Plant roots actively grow into the soil in search of water and nutrients. In dynamically growing regions of a root, cell wall synthesis occurs at a rapid rate to allow expansion of cells and root growth. The cell wall synthases involved are known to be produced in the Golgi with activity either in the Golgi apparatus or the plasma membrane; however, little is known about the overall cytoplasmic organellar organisation as it relates to cell wall development.

In this study, cell wall labelling using fluorescence was correlated with electron microscopy to identify the locations of organelles as it relates to specific cell wall polysaccharides in Barley and *Brachypodium* roots. Elongating regions of growing roots were sectioned, fluorescently labelled with cell wall antibodies, and subsequently imaged using fluorescence microscopy. These sections were then processed and imaged using electron microscopy. The associations of Golgi bodies, endoplasmic reticulum and mitochondria was correlated with regions of deposition of cell wall polysaccharides including cellulose, heteroxylans and (1,3;1,4)-beta-glucan showing relationships between organelles and cell wall synthesis.

Single-cell 3D spatial gene expression analysis in whole-mount germinating seeds

Lucas Auroux[1], Lim Chee Liew[1] and Mathew G. Lewsey[1,2,3]

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Spatial transcriptomics technologies have made enormous progress in terms of outputs and resolutions, helping scientists comprehend the complexity and heterogeneity of multicellular organisms. Due to the cost of commercially available kits and technical difficulties for in-depth coverage which requires multiple consecutive tissue sections, the application and development of these technologies to obtain high-resolution 3D whole-mount plant data is lagging behind. A cost-effective and high-resolution technique to analyse in-depth and in detail where and when exactly transcripts are expressed in native spatial cellular context is needed. PHYTOMap is a targeted multiplexed FISH technology developed for Arabidopsis roots that allows single-cell and spatial gene expression analysis in 3D whole-mount tissue in a transgene-free manner. We optimised this spatial transcriptomics technology for Arabidopsis seed by manual dissection of embryos, optimising sample clearing and retention, probe concentration for optimal imaging, and subsequent confocal microscopy to generate higher resolution images. We applied PHYTOMap for the first time in seeds, allowing us to validate our previously published single-cell transcriptomics data of germinating seeds. It confirms cell state-specific gene expression at subcellular resolution within the native 3D tissue context of germinating embryos. This allows us to precisely localise and quantify the level of transcripts during germination to build accurate co-expression and gene regulatory networks. This will help us to better understand the establishment of unique functions of different cell types and their spatial interactions during the highly dynamic cell states transition happening in germinating seeds.

Creating a 3D Atlas of Germinating Barley Grains using Multi-Modal Imaging

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In 2023, 142.59 million metric tons of barley grain was harvested globally, making it the fourth most cultivated crop worldwide.[1] It is primarily used in animal feed and a small quantity is consumed directly by humans. Alternatively, barley is a key ingredient in the beverage industry, used in the production of many beers and spirts. In 2023, scotch whisky contributed £5.6bn to the UK economy in exports, making barley not only an important crop but also a profitable one.[2] Germination is a pivotal point in plant life cycles. The transformation from dormant grain to plant is a complex biochemical process. Several factors contribute to the success of germination, including morphology, nutrient distribution and spatial transcription, which are amongst the most important, together with environmental conditions. Broadening our understanding of these complex process can help us develop a resilient and sustainable crop in the future.

We are using a combination of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), Micro Computed Tomography (µ-CT) and Spatial Transcriptomics [3] (obtained at La Trobe University, Melbourne) to establish a multi modal approach. Serial sections of germinated grains, embedded in paraffin, were analysed using an Elemental Scientific ImageBio 266 Laser coupled to a PerkinElmer NexIon 350 MS. LA-ICP-MS was used to map the distribution of endogenous metals (Mg, Ni, Mn, Cu, Fe, Mo, Zn), each metal linking to a number of key biological functions. A Brucker SkyScan 1272 µ-CT scanner was used to investigate morphological changes which occur during the first 24 hours after imbibition.[4] The LA-ICP-MS and µ-CT data will be combined with transcriptomic data obtained at La Trobe University to establish a 3D cell atlas of a germinating barley grain, developing an in-depth understanding of this key determining factor of a plant lifecycle.

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The investigation of evening complex function in wheat

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The circadian clock is a biological time-keeping mechanism that regulates key physiological processes, influencing growth, development, and stress responses in plants. While extensively studied in *Arabidopsis thaliana*, the role of circadian clock genes in agriculturally significant crops, particularly bread wheat (*Triticum aestivum*), remains underexplored. The evening complex (EC) is core component of plant circadian clocks and is comprised of ELF3, ELF4 and LUX. This research aims to investigate the function of EC genes, and their involvement in leaf senescence—a critical process affecting yield and grain quality. Utilizing Australian hexaploid wheat cultivars and near-isogenic lines (NILs), we aim to unravel the circadian mechanisms of EC regulating leaf senescence timing.

Our study focuses on three key questions: (1) expression of EC genes across developmental stages in cultivars and NILs; (2) the composition and function of the EC in wheat protoplasts, employing split luciferase to detect protein-protein interactions and transcriptional regulation assay to determine the transcriptional activity of EC components on potential target genes; (3) the role of *ELF3* in regulating senescence, explored through RNA-Seq and ChIP-seq analyses in NILs to identify circadian-regulated transcripts in senescent leaves.

Preliminary findings indicate differential expression of *TaELF3* alternative transcripts in wheat cultivars, with *ELF3α* expressed at higher levels than *ELF3β*, suggesting distinct regulatory roles. This research can advance our understanding of the circadian clock's role in wheat, offering insights into genetic and molecular mechanisms underlying senescence and their potential applications in crop improvement.

Investigating the *TaHRZ* gene family for iron biofortification and abiotic stress tolerance in bread wheat

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Crops adapt to low iron (Fe) soils through a complex network of transcription factors that regulate Fe uptake, translocation, and storage. The *Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zinc finger protein* (HRZ) is a master negative regulator within the plant Fe homeostasis network and knockdown of *OsHRZ* expression increases tolerance to Fe deficiency and grain Fe concentrations in rice (*Oryza sativa* L.). This study describes the identification, annotation, and characterisation of six *TaHRZ1* and *TaHRZ2* homoeologs in the bread wheat (*Triticum aestivum* L.) genome[1]. *In silico* phylogenetic and protein analyses demonstrated that TaHRZ proteins possess Fe binding and polyubiquitination activity, and segregate into two groups: *TaHRZ1* and *TaHRZ2*. Reverse transcription PCR analyses revealed unique tissue expression profiles for each *TaHRZ* homoeolog, and rapid upregulation of all *TaHRZ*s under Fe deficiency, suggesting the *TaHRZ*s function as master negative regulators of wheat Fe homeostasis. We are now using gene editing to knock-out individual *TaHRZ* homoeologs in bread wheat and assess the effect on grain Fe concentration and plant tolerance to Fe deficiency. Together this research represents a culmination of novel wheat genomic resources and breeding techniques and highlights a novel and possibly transgene-free strategy for future wheat Fe biofortification.

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Comparative transcriptome analysis provides insight into glandular trichome gene regulatory networks

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Several commercially important plant secondary (or specialised) metabolites are produced and stored in glandular trichomes (GTs). The metabolites produced by GTs of cannabis (*Cannabis sativa* L.) and its close relative hop (*Humulus lupulus* L.) have significant pharmaceutical and industrial importance. To understand the transcriptional programs underlying secondary metabolism in GTs, we generated comprehensive transcriptome data across five tissues (trichomes, flowers, leaves, stems, and roots) in cannabis, hop and tomato. Tomato, another GT-producing species, serves as a model to study GT development and biology. Our analysis revealed that approximately 15% of predicted genes in cannabis, 7% in hop, and 20% in tomato displayed organ-specific expression. Transcription factors (TFs) accounted for about 10% of these organ-specific transcriptomes and likely regulate specific organ functions. To identify key regulators of tissue-specific functions, we conducted gene regulatory network (GRN) analysis focusing mainly on GTs. Most genes and TFs in GT-specific GRNs were associated with specialized metabolic pathways, highlighting the species-specific nature of specialized metabolism. We further applied the principle of orthology to GRN analysis and investigated the shared regulatory aspects of GT biology across the three species. The shared components across these GRNs were found to be involved in highly conserved processes, including flavonoid biosynthesis, solute transport, and metabolite storage.

The need of a new dawn for Australian protected cropping and far Beyond!

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Australia's protected cropping industry has adopted advanced technologies such as greenhouse automation, hydroponics, LED lighting, and precision irrigation to maximize crop yield and quality. These technologies enable efficient, soilless cultivation, year-round production, and integrated pest management, which together support the industry's response to local climate challenges and food security needs. Despite these advancements, the sector faces evolving challenges from climate change, resource constraints, and labour shortages.

As Australia seeks sustainable growth, innovations such as vertical farming and Controlled Environment Agriculture (CEA) offer transformative potential for both Earth and future space habitats. These controlled systems create highly adaptable environments that optimize resource use and maximize yield, crucial for producing fresh food in extreme conditions, from urban settings to the confines of space.

For space missions and extraterrestrial agriculture, improvement programs focused on traits suited for closed environments such as compact growth, rapid yield, and resistance to limited resources are crucial. These advancements also hold promise for addressing Earth's sustainability goals. Circular practices, including waste recycling and water conservation, are critical in building resilient, self-sustaining systems that can operate both in Australia and in space.

As Australia and the global community invest in protected cropping research, this "new dawn" must align with ambitions that extend beyond terrestrial farming, creating resilient systems to meet the dual demands of future space exploration and a sustainable Earth-based food supply.

Keywords: Protected Cropping, Climate Change, Space Agriculture

Role of nitrogen-fixing cyanobacteria in the growth and nitrogen economy of Australian *Cycas*

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Australia is home to around 70 endemic cycad species. Cycads are notable among gymnosperms for their symbiotic relationship with nitrogen-fixing cyanobacteria. Cycads house cyanobacteria in specialised coralloid roots, so named because of their coral-like shape. The importance of the nitrogen fixed by symbionts to growth and chemical defence in Australian cycads is largely understudied.

The aim of this project was to document the contribution that cyanobacteria make to the nitrogen economy of *Cycas* species. Two species were compared, using anatomical, morphological approaches, and isotope analysis to determine the location and role of cyanobacteria in the coralloid roots. One-year-old *Cycas ophiolitica* and *Cycas megacarpa* were harvested (n=10), and coralloid roots from each plant were sectioned and analysed with a microscope. Data collected included biomass, leaf number, caudex circumference, coralloid root section surface area, and leaf total nitrogen and nitrogen isotope analysis.

C. megacarpa allocated a greater proportion of biomass to coralloid roots, and was significantly larger than *C. ophiolitica*. Coralloid root sections had greater surface area and size of the cyanobacterial zone in *C. megacarpa* compared to *C.ophiolitica*. *C. megacarpa* also had higher total leaf nitrogen than *C. ophiolitica*, though no differences were observed in nitrogen isotope analysis.

Comparison of these two species at the same developmental stage indicates differences in the role of coralloid roots and cyanobacteria in cycad growth and development. Further investigation into other species, and comparisons at different developmental stages, and the impact on nitrogenous specialised metabolites will further elucidate this role.

Insights into drought tolerance of domesticated sorghum, S. bicolor

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Sorghum bicolor is one of the most highly drought tolerant cereal crops. As such, it is a promising model for identifying drought-tolerant genes. Exploring the gene expression changes due to drought can pave the way for utilising them in breeding programs to develop drought resilient crops. The aim of this research is to identify the genes and networks that are differentially expressed in drought stressed sorghum across multiple tissue types. A differential gene expression analysis was conducted for drought stressed *S. bicolor* variety BTx623 using edgeR. Gene ontology enrichment analysis and Weighted Gene Correlation Network Analysis (WGCNA) were conducted to identify the over-represented functions of the differentially expressed genes and to identify clusters of genes that behave together as a response to drought, respectively. From the results, 56 differentially expressed genes were identified in roots which were mainly involved in physiological functions as well as indirect drought responses such as iron-related, signalling and oxidative stress pathways. Direct drought responsive genes could not be detected in this study despite negative impacts on plant growth. These results show that *S. bicolor* variety BTx623 is a highly drought resilient sorghum variety and drought tolerance was mediated by expression changes in the roots. Further investigations are needed involving stronger drought intensities and longer exposure times to further identify drought-responsive genes in BTx623.

Investigating Trichome Development Pathways in Tomato

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The discovery that several specialised metabolites produced by glandular trichomes (GTs) have potential pharmaceutical applications has sparked a surge in research. Most of these studies have focused mainly on understanding the biosynthesis pathways leading to the production of these metabolites. In the current study, we set out to investigate the genetic regulation of GT initiation and development using tomato (*Solanum lycopersicum*) as a model species. To this end, we applied the hormone methyl jasmonate (MeJA), a known promoter of GT development, and the antibiotic neomycin that inhibits MeJA activities, then collected data on trichome density. We also generated RNA sequencing data to identify genes that play a role in GT initiation and development. Application of MeJA alone significantly increased GT density, while neomycin reduced GT density. The combined application of MeJA and neomycin significantly reduced GT numbers compared to the MeJA-treated plants. However, the GT density of MeJA and neomycin-treated plants was at some point higher than the control plants, suggesting neomycin inhibits MeJA activity only partially. Our preliminary transcriptome analysis identified several genes that are differentially expressed between the control and neomycin-treated plants. Interestingly, some of the genes that were downregulated in neomycin-treated plants were also among the genes differentially expressed between the MeJA and control plants, with most of the genes upregulated in MeJA-treated plants. These observations suggested neomycin negatively affects the expression of several genes that are induced by MeJA, and some of these genes are predicted to play a role in plant defence. As defence mechanisms in tomato involves enhanced trichome densities and production of metabolites, we are investigating the possibility that some of these genes might have a role in trichome initiation and development.