

ASPS 2025

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Abstract Booklet

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Monday 24th November

Session 1 - The Future of Plant Science

Session 2 – Plant Organelle and Energy Biology

RN Robertson Award Talk – Michelle Watt

Evolution of C_4 Photosynthesis in the Australian Grass Genus *Neurachne* Involves Three Cell Types

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Keywords:
C_4 photosynthesis, C_4 evolution, *Neurachne*, immunolocalisation, electron microscopy

Abstract:

C_4 photosynthesis has evolved independently over 66 times from the ancestral C_3 pathway, representing one of the most remarkable examples of convergent evolution. The C_4 carbon-concentrating mechanism minimises photorespiration by spatially separating photosynthetic carbon assimilation (PCA) and photosynthetic carbon reduction (PCR), typically across two specialised cell types; mesophyll and bundle sheath cells, with the latter surrounding the vascular bundle, in what is known as Kranz anatomy.

The Australian grass genus *Neurachne* is particularly valuable for studying C_4 evolution, as it the only known grass genus that contains distinct, closely related C_3, C_3-C_4 intermediate, and C_4 species. The two independently evolved C_4 species, *N. muelleri* and *N. munroi*, both exhibit “Neurachneoid”-type Kranz anatomy, in which two sheath layers surround each vascular bundle: an inner mestome sheath containing the PCR machinery and an outer parenchymatous sheath separating these cells from the mesophyll. Remarkably, the leaves of C_3 and intermediate *Neurachne* species displays a similar three-cell arrangement, raising questions about its function and significance in C_4 evolution.

To investigate how the C_4 cycle operates across this three-cell structure and its evolution, the subcellular localisation of key C_4 photosynthetic enzymes (PEPC, PPDK, NADP-ME, and Rubisco) were examined and quantified at the ultrastructural level in C_3, intermediate, and C_4 *Neurachne* species. The findings from these analyses provide new insights, not only into the evolution of C_4 photosynthesis in *Neurachne,* but also Kranz anatomical forms that support C_4 biochemistry providing insights into the evolution of a three-cell C_4 syndrome.

NHX8 and *HKT1* regulate seed lithium accumulation independently of sodium regulation in *Arabidopsis thaliana*

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Keywords:
Lithium, sodium, ion transport, trace elements, salinity

Abstract:

Sodium (Na^+) transport to reproductive tissues is limited by High-Affinity Potassium (K^+) Transporter 1 (HKT1) proteins across plant species[1]. In *Arabidopsis thaliana*, only a single *HKT1* gene is present. However, our Genome-Wide Association Study (GWAS) for seed Na^+ content revealed no significant associations. As lithium (Li^+) is commonly used as a chemical analogue of Na^+[,2], we extended our analysis to seed Li^+ content. Strikingly, GWAS for seed Li^+ content identified two candidate genes, *HKT1* on chromosome 4 and Na^+/H^+ Exchanger 8 (*NHX8*) on chromosome 1. NHX8 has previously been characterised as a putative Li^+/H^+ antiporter[3]. We uncovered the mechanism of seed Li^+ accumulation regulation by *NHX8* and *HKT1*, revealing a regulation distinct from that of seed Na^+ accumulation. Natural variation in *HKT1* alleles influences leaf Na^+ concentrations via differential expression, yet all alleles consistently limit Na^+ accumulation in seeds. In contrast, the impact of *HKT1* on seed Li^+ accumulation is determined by ion selectivity. *NHX8* is required for achieving high seed Li^+ accumulation. While *NHX8* is mainly a Li^+ transporter across accessions, it has a limited Na^+ transport capacity, which can affect seed Na^+ accumulation, but to a lesser extent than *HKT1*. Together, our findings reveal a unique regulation of seed Li^+ accumulation, laying the foundation for exploring the roles of Li^+ in plants beyond its equivalence to Na^+. This work also highlights new biotechnological opportunities, as selective Li^+ transport could be harnessed for Li^+ extraction and/or remediation.

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Anthocyanin – An alternative to conventional reporter systems: Towards a combinatorial strategy for novel regulatory networks and modified metabolic pathways in *Nicotiana benthamiana*

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Keywords:
Plant reporter systems, Anthocyanins, *Nicotiana benthamiana*, Transgene-free genome editing, CRISPR/Cas9 technology

Abstract:

Plant reporter systems play a fundamental role in agricultural biotechnology, enabling gene expression tracking, stress response monitoring, and breeding program acceleration to drive crop improvement efforts.[1] However, the current plant reporter systems rely heavily on transgenic approaches, which pose significant limitations for field applications and commercial deployment.[2] To address these challenges, there is a pressing need to develop non-transgenic methods for real-time monitoring of metabolic pathway regulation under field conditions. We are undertaking a transgene-free genome editing approach to engineer a novel reporter system based on the anthocyanin biosynthetic pathway. Anthocyanins, plant pigments from the flavonoid family, are responsible for the vibrant colours of many flowers, fruits, and leaves.[3] In our innovative reporter system, differently-hued anthocyanins will function as visual indicators to monitor the gene expression of selected metabolic pathways. Using bioinformatic analyses, we identified and characterised flavonoid hydroxylase genes in *Nicotiana benthamiana* which contribute to the different anthocyanin shades. We then silenced these genes using CRISPR/Cas9 technology, generating stable transformants that exhibit a spectrum of anthocyanin shades. These colours will be linked to metabolic pathways of interest through CRISPR/Cas9-mediated homologous recombination. This novel reporter system, utilizing endogenous anthocyanins, will provide readily observable visual cues to detect and assess the activation/ deactivation of metabolic pathways, while generating beneficial phenotypes to provide proofs of concepts in relation to gene regulatory networks. Overall, this non-transgenic reporter system offers a practical and efficient tool for overcoming the constraints of existing transgene-based, in-field plant monitoring systems, and advancing agricultural research towards sustainable crop development.

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From Past Stress to Future Strength: Transgenerational Resilience in Grapevines

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Keywords:
clonal propagation, drought adaptation, water use efficiency, genetic variation, natural priming

Abstract:

Clonal propagation preserves grapevine (*Vitis vinifera L.*) genotypes and is widely used in perennial crop production. However, the long-term responses of clonally propagated grapevines to drought stress remain unclear. Across successive vegetative generations, physiological traits linked to growth, water use, and stress adaptation may shift through osmotic adjustment and epigenetic modifications.

This study examined drought-related physiological variation among Shiraz grapevines across three lineage generations (G1, 36 years; G2, 24 years; G3, 12 years), derived from two historic mother vines (G0) of distinct clones, SARDI 6 (99 years, irrigated) and SARDI 7 (115 years, dry-grown), in the Barossa Valley, South Australia. Vines were located within an 8 km radius, with G1 and G2 in the same block under comparable conditions. Over three growing seasons (2022–2025), we assessed soil and vine water status (ψ_{pd}, ψ_{stem}, ψ_{leaf}), leaf gas exchange (A_n, g_s), intrinsic and agronomic water use efficiency (WUE_i, WUE_{ag}), carbon isotope discrimination ($\delta^{13}C$), leaf proline concentration, and yield components.

Intermediate-aged vines (G2) consistently expressed higher WUE_i and WUE_{ag} than older or younger generations, suggesting more effective stomatal regulation of water status. Yield traits, including bunch weight, were also greatest in G2 vines. Clonal differences were evident in osmotic adjustment: SARDI 6 accumulated more proline, while responses in SARDI 7 reflected its long-term unirrigated history.

These results demonstrate that both clonal origin and generational age shape physiological drought responses. Identifying lineage-specific traits provides valuable guidance for breeding and propagation, supporting vineyard resilience under future

water-limited climates.

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A NAC transcription factor regulatory network coordinates mitochondrial biogenesis in Arabidopsis

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Keywords:

Mitochondria, Proteasome, Ubiquitination, Protein turnover.

Abstract

Mitochondrial biogenesis in eukaryotic cells requires the import of over a thousand nuclear-encoded proteins, which are synthesized in the cytosol and translocated into mitochondria [1]. The translocase of the outer mitochondrial membrane (TOM) complex serves as the central entry point, selectively recognizing and facilitating the import of these precursor proteins [2]. Despite its essential role, the regulation of TOM complex abundance remains poorly understood.

Using both forward and reverse genetic approaches in *Arabidopsis thaliana*, we identified components of the ubiquitin-proteasome system that modulate TOM complex protein abundance. Specifically, we show that the 26S proteasome subunit REGULATORY PARTICLE NON-ATPASE1A (RPN1A) and a putative E3 ubiquitin ligase, SUPPRESSOR OF PPI1 LOCUS1-LIKE1 (SPL1), play critical roles in this process. Altered abundance of these regulators impacts mitochondrial protein import efficiency and overall respiratory activity, underscoring a functional link between proteostasis and mitochondrial performance.

We further demonstrate that this regulation is governed by a hierarchical transcriptional cascade. The ER-bound NAC transcription factor ANAC017[3] induces ANAC053, which, together with ANAC078, activates *RPN1A* expression, thereby influencing TOM complex levels. This two-tiered cascade integrates transcriptional and post-transcriptional regulation to modulate mitochondrial import machinery in response to cellular cues. Collectively, our findings uncover a regulatory circuit that connects transcription factor signaling and the ubiquitin-proteasome pathway to mitochondrial protein import, offering new insights into the control of organelle biogenesis.

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Chloroplast Calcium Dynamics: Linking Photosynthesis and Stress Responses under High Light

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Keywords:
high light stress, chloroplast, calcium signaling, reactive oxygen species, endoplasmic reticulum

Abstract:

Chloroplasts can sense environmental fluctuations via Ca^{2+} signaling. Environmental triggers, such as light changes, physical damage and heat waves, can induce distinct Ca^{2+} signatures in chloroplasts, which may help rebalance photosynthesis and stress responses under fluctuating conditions. Previously, we investigated Ca^{2+} responses to high light (HL) exposure in *Arabidopsis thaliana*, a biphasic increase of Ca^{2+} concentration was detected in the chloroplast stroma by the genetically encoded calcium indicator YC3.6 and confirmed using a newly established stroma-localized R-GECO1 (NTRC-R-GECO1) [1]. Chemical inhibition of photosynthetic electron transport, microscopy-based Fv/Fm experiments, and measurement of the reactive oxygen species (ROS)-redox balance with roGFP-based reporters and Singlet Oxygen Sensor Green (SOSG) chemical dye suggested that photodamage contributes to the stromal Ca^{2+} response. Pharmacological inhibition with cyclopiazonic acid (CPA) suggested that the HL response depends on a Ca^{2+} exchange between the endoplasmic reticulum (ER) and chloroplast stroma. This study implicates a novel Ca^{2+}-mediated acclimation mechanism to HL stress. We have now established an artificial-microRNA-based screening system to identify the channels and transporters regulating chloroplast Ca^{2+} responses. Through this approach, we hope to modulate the chloroplast Ca^{2+} dynamics and elucidate its role in photosynthesis and stress responses, potentially improving photosynthesis efficiency in the context of climate change.

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Engineering Non-Suicidal THI4 Variants to Enhance Thiamin Biosynthesis and Plant Energy Efficiency

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Keywords:
Thiazole synthase, suicide enzyme, thiamin biosynthesis, chloroplast

Abstract:
Thiamin (vitamin B1) is an essential nutrient for both plants and humans [1]. In plants, its biosynthesis depends on a unique "suicidal enzyme", thiazole synthase (THI4), which irreversibly inactivates itself by donating a sulphur atom from a cysteine residue to form the thiazole ring of thiamin [2, 3]. This self-inactivation leads to rapid THI4 turnover, making the pathway highly energy-intensive and limiting crop yield [4]. Interestingly, some plants, such as wheat, oat and barley, also express non-cysteine versions of THI4 [3]. My research explores the possibility of reengineering THI4 into a non-suicidal, catalytically stable enzyme to improve thiamin production and plant performance.

Using Arabidopsis as a model, I am analysing whether a non-cysteine version of THI4 could function in plants. To this end, I characterized a THI4 knockout mutant (*tz-1*), which is unable to survive beyond three weeks without thiamin supplementation. The mutant displayed various pleiotropic phenotypes, all of which were rescued by complementation with THI4 or supplementation with thiamin or thiazole, confirming that the phenotype was specifically due to THI4 loss. Next, I engineered several non-cysteine THI4 variants, including versions from wheat, oat, and barley, as well as a modified Arabidopsis variant in which the catalytic cysteine was replaced by histidine and fused to a His-tag for downstream affinity-based purification. Early phenotyping and thiamin quantification revealed a partial but noticeable restoration of growth in the mutants carrying alternative THI4s. These results highlight the potential of non-cysteine THI4 variants to reduce THI4 turnover, lowering energy costs and improving crop productivity.

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Setting Down Roots: Michelle Watt's Research Story for the 2025 RN Robertson Lecture

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Michelle has researched plant roots her whole career, starting in Canada in the early 1990s with Professor Margaret McCully and discovering the role of water in rhizosheath formation. She moved in 1996 with her family to Australia and did her PhD on phosphorus regulation of proteoid root development and physiology with Professor John Evans, working in the RN Roberston Building of the Australian National University, and collaborating with Drs. Peter Ryan and Gamini Keerthisinghe. At CSIRO Black Mountain with research funding from 2001 to 2015 from the Grains Research and Development Corporation (GRDC), the Australian Centre of International Agriculture Research (ACIAR) and Bayer, Michelle and team members including Drs. Anton Wasson and Sarah Rich integrated root research into conservation farming and physiological breeding of wheat in Australia and India under the mentorships of Drs. John Passioura, Richard Richards, John Kirkegaard and Rana Munns. Excited by the possibility of quantifying root dynamics in soil, Michelle took up a Directorship at the Helmholtz Research Centre Juelich with Professor Uli Schurr and University of Bonn in Germany to use deep phenotyping technologies including MRI and PET to trace water and carbon of roots and rhizospheres (2015-2019). Now at the University of Melbourne (2020-) and supported generously by the University of Melbourne Botany Foundation, Michelle holds the Adrienne Clarke Professorial Chair of Botany, is a Node Leader in the Australian Research Council Centre of Excellence in Plants for Space (P4S), and teaches plant biology and biotechnology to students. Her team and colleagues including Dr. John Golz and Professors Berit Ebert, Doug Brumley, Harvey Millar and Matt Gilliham, co-supervise students and postdocs in engineering of root-microbiome dynamics, building multiple functions into roots to optimise plant harvest index, water efficiency and carbon sequestration, for Earth and space. Michelle has also enjoyed advisory roles as a Southern Panel Member for the GRDC (2021-2024) and a Member of the ARC College of Experts (2022-), as well as advising early career scientists on promotions cases and scholarships. Societies for plant biology in Canada (CSPS) and Australia (ASPS), and in roots internationally (ISRR), have provided Michelle with networks, friendships and fun throughout her career.

Synthetic Genomics: From Bacteria to Yeast and Plants

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Keywords:
Synthetic biology, robotics automation, genome engineering

Abstract:

The ability to redesign and construct genomes is transforming biology, enabling us to uncover fundamental genome-phenotype relationships while pushing the boundaries of what life can be. I will discuss how robotics-accelerated synthetic biology is driving the creation of new-to-nature genomes across bacteria, yeast, and plants, opening new frontiers in both basic research and biotechnological innovation.

Harnessing Plant SynBio for Climate-Resilient Crops and Light-Driven Biomanufacturing

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Keywords:
Synthetic biology, metabolic engineering, biofuels and biomaterials, plant transformation

Abstract:
Plant synthetic biology is rapidly expanding our ability to engineer complex traits yet progress still lags behind microbial systems. Slow and genotype-dependent transformation pipelines, limited chassis diversity, and a lack of standardized regulatory parts constrain design-build-test-learn (DBTL) cycles. Overcoming these technical bottlenecks is key to realising the full potential of plant synthetic biology. In Australia, an increasingly variable climate is intensifying challenges across both broad-acre cropping and horticulture, from drought and frost to pathogens and invasive weeds, eroding productivity and resilience. To meet these pressures, we need faster, more predictable tools for in-crop genome editing and synthetic circuit design that enable rapid iteration and field-ready deployment. At the same time, advances in metabolic pathway optimisation are transforming plants into light-driven on-demand biomanufacturing platforms for sustainable fuels, bioplastics, and high-value compounds. These provide an opportunity for in-situ production without reliance on global food chains, whether you're a remote community on Earth or even in space. Here I will discuss progress, major challenges, and how our growing plant synbio community is building the tools to seize these opportunities.

Developing synthetic gene circuits in moss for on-demand biomolecule production in space

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Keywords:
Synbio, gene circuits, moss, biotechnology, space

Abstract:

Implementing gene circuits in the moss *Physcomitrium patens* (Physco) for targeted gene expression has many applications in healthcare and space exploration. While constitutive expression of transgenes to produce biomolecules may be useful in some cases, energy and resources are typically used inefficiently which may damage overall plant health. Instead, gene circuits allow for spatiotemporal control of gene expression, so transgenes are limited to certain tissue or cell types and in response to external stimuli, such as light, temperature, and chemical induction[1]. One form of gene circuit uses site-specific recombinases to permanently alter gene regulatory components such as promoters or terminators. By driving recombinases with various inducible promoters, recombinases can be precisely controlled to rearrange gene regulatory components and in turn, activate or repress a transgene. My research focusses on testing different components required to construct gene circuits in Physco, with the goal of building gene circuits capable of synthesising useful biomolecules in space.

As longer-term space missions are being planned, availability of common therapeutic products is a major concern for maintaining astronaut health[2]. Constructing effective gene circuits in Physco for space applications requires a combination of suitable gene circuit parts that can efficiently alter gene expression as well as a responsive induction method. In my research, I have been comparing various promoters, 5' untranslated regions, terminators, and multiple recombinases as well as inducible systems. Our lab's goal is to build a platform for precisely controlled, renewable production of therapeutic biomolecules to sustain the health of astronauts on space missions.

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From SynBio to the Field: A Flagship Gene-Editing Breeding Program Delivering MLN-Resistant Maize for Africa

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Keywords:
Gene editing, Virus resistance, Synthetic biology, Maize, Food security

Abstract:

Maize lethal necrosis (MLN) is a dual-virus synergistic disease that poses a serious threat to the food security of smallholder farmers in sub-Saharan Africa. Backed by the Bill & Melinda Gates Foundation, CIMMYT joined forces with Corteva, USDA and other partners to take on this challenge. Within five years, we identified two independent genetic solutions to combat this devastating disease. The first is a mutated peptidase that disrupts viral sanctuary formation. The second is a novel eukaryotic translation initiation factor 4E (eIF4E) mutant which, while preserving normal plant growth, has lost the capacity to support viral protein translation. Using gene-editing technologies, we successfully introduced these traits into elite maize varieties. In Kenyan field trials, the edited lines demonstrated complete resistance to MLN without any yield penalty. These breakthroughs, embodied in **the first gene-edited crop planted on African soil**, represent a major step toward safeguarding the livelihoods of millions of smallholder farmers across the Global South.

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Innovation of photoprotection facilitated evolutionary success of grasses

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Keywords:
Photosynthesis, photoprotection, productivity, angiosperms, grasses, light stress

Abstract:

As climate change intensifies, developing crops that retain high productivity under environmental stress is essential for food security. A core challenge is how plants balance harvesting enough light to power photosynthesis with protecting themselves from excess light that can damage Photosystem I (PSI). Most photosynthetic organisms, from cyanobacteria to gymnosperms, have two major photoprotective systems mediating this trade-off: flavodiiron proteins (FLVs), which provide rapid PSI protection but waste electrons, and the PGR5–PGRL1 pathway, which is slower but likely more energy-efficient. However, angiosperms, which in the beginning of their evolution mostly occupied shaded forest understories, have lost FLVs and rely on PGR5–PGRL1.

Grasses (Poaceae) diverged from other angiosperms in the early Miocene (~20 MYA) and transformed terrestrial ecosystems by colonising open environments with high and fluctuating light. This ecological transition likely required a new fast photoprotection system. We identified a grass-specific PGRL1 paralog that enables ~5 seconds faster PSI protection during sudden increases of irradiance. We propose that this paralog partially replaced the lost FLV function, endowing grasses with both fast and slow photoprotection systems. This evolutionary innovation likely facilitated success of grasses in high-stress open habitats and contributed to the rise of grasslands, which today cover nearly 40% of Earth's surface.

Tuesday 25th November

Session 4 – Plant Nutrition & Development

Peter Goldacre Award Talk – Stephanie Watts-Fawkes

Ammonium facilitator transporters (AMF) mitigate ammonium accumulation in plants

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Keywords:
ammonium, AMF, nutrient transport

Abstract:
The plant Ammonium Facilitator (AMF) transport proteins represent a distinct branch of the DHA2 Drug:H^+ antiporter family, previously associated with multidrug resistance and H^+-dependent exchange of amino acids, siderophores, and glutathione in yeast. We have characterized the functional activities of Arabidopsis and Rice AMF homologs to define their role in cellular ammonium management. AMF genes are ubiquitously expressed at low levels across most plant tissues with their encoded proteins found at the endoplasmic reticulum (AtAMF1;1), the tonoplast (AtAMF1;2, ZmAMF1.1) and the plasma membrane (AtAMF1;3, OsAMF1, MtAMF3, ZmAMF1.2). When expressed in Xenopus laevis oocytes, AtAMF1-3 and OsAMF1) facilitated NH4+ currents, consistent with previous functional analysis (Chiasson et al 2014, PNAS). Disruption of AtAMF2 and AtAMF3 (amf2amf3) led to early shoot senescence, accumulation of shoot NH4+, altered seed development and a disruption in seed N delivery. Shoot NH4+ accumulation in the amf2amf3 mutant can be reduced with the native expression of either the tonoplast AtAMF2 or the plasma membrane AtAMF3. The tonoplast AtAMF2 increases cell tolerance to toxic concentrations of NH4+ (>100 mM) when expressed in a plasma membrane K+ transport yeast mutant (CY162), that also shows hypersensitivity to elevated concentrations of NH4+. Collectively, these results demonstrate that AMF transporters are essential components of plant NH4+ homeostasis, acting across

multiple cellular compartments to mitigate NH_4^+ toxicity and regulate nitrogen fluxes to developing tissues.

Mining novel genes to improve heat stress tolerance in Barley (Hordeum vulgare)

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Keywords:
Barley, MADS-box, floral development, high temperature, reproductive resilience

Abstract:

Barley is an economically important cereal crop in temperate regions, yet its reproductive development and grain yield are highly vulnerable to elevated temperatures. Floral meristem identity and organ formation are governed by MADS-box transcription factors, which act as master regulators of reproductive development. While their developmental functions are well established, their roles in stabilizing reproduction under heat stress are only beginning to be defined.

Recent studies have identified two SEPALLATA-like genes, HvMADS1 and HvMADS8, as central components of temperature-responsive floral regulation. HvMADS1 was shown to maintain spike architecture at elevated temperatures, with loss-of-function mutants producing excessive branching and abnormal inflorescences. This regulatory activity was linked to the modulation of HvCKX3, connecting cytokinin metabolism to meristem activity. HvMADS1 also promotes awn elongation and lemma expansion, acting in association with APETALA2 to regulate downstream targets such as HvSHI and HvDL, thereby influencing grain size and yield. In parallel, HvMADS8 was demonstrated to ensure floral meristem determinacy under heat stress. Mutants developed multiple pistils lacking ovules, leading to severe fertility defects. Mechanistic analyses revealed that HvMADS8 exhibits enhanced promoter binding at high temperature, directly activating D-class genes required for ovule initiation and reproductive stability.

Together, these findings demonstrate that HvMADS1 and HvMADS8 are not only essential developmental regulators but also pivotal in maintaining reproductive success under elevated temperatures. Understanding how temperature reshapes their regulatory activity highlights new molecular targets for breeding climate-

resilient barley cultivars with stable fertility and yield.

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Photoperiod influences the productivity and grain quality of a double podding chickpea (*Cicer arietinum*).

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Keywords:
Chickpea (*Cicer arietinum*), grain quality, SINGLE FLOWER LOCUS (SFL), photoperiod

Abstract:

Chickpea (*Cicer arietinum*) is an important and affordable source of protein as well as starch and dietary fibres globally, with long traditions in food cultures from the Mediterranean, South and Southeast Asia and East Africa. As consumers become more concerned with the nutritional and sustainability credentials of their food there is great potential for expansion in the production of chickpeas in countries such as Australia. Most varieties of chickpea produce a single pod per reproductive node, however *single flower locus (sfl-d)* mutants have been described which can produce two pods per node[1], a trait with potential to stabilise or increase yield. The extent of double podding at reproductive nodes varies in field trials where it has been suggested to positively correlate with moderate soil moisture stress in rainfed systems[2–4]. In this study glasshouse trials were conducted to explore the physiology of double podding nodes in controlled environments to allow comprehensive assessment of grain quality through proximal composition and multi-omics analysis. We present findings that the production of double podding nodes is responsive to photoperiod and not mild soil moisture stress, and propose that availability of photoassimilates may be driving productivity at reproductive nodes in *sfl-d*. Detailed analysis of grain quality by node type will be discussed in the context of yield and productivity, including the likely implications for the inclusion of this trait in breeding programs.

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Optimising Phosphorus Utilization: Integrating Root Biological, Genetic, and Microbial Strategies for Sustainable Crop Production

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Keywords:
Phosphorus efficiency, root architecture and anatomical traits, rhizosphere microbes, acid phosphatase, genetic regulation

Abstract:

Phosphorus (P) deficiency is a major limitation to crop productivity worldwide. Our studies integrate multiple research efforts to explore plant root responses, genetic regulation, microbial interactions, and adaptive mechanisms under P-deficient conditions, providing insights for improving P-use efficiency in crops. This presentation begins with an overview of plant nutrient acquisition strategies, followed by our recent work on P efficiency in narrow-leafed lupin, and case studies on how root traits and plant–microbial interactions enhance P uptake under low P conditions. Using our high-throughput root phenotyping platform, we were able to characterise root trait variability in large set of genotypes in major crop species, including narrow-leafed lupin, chickpea, soybean, wheat, barley, rice, and canola. Genotypes with adaptive root traits enhanced P-use efficiency, enabling targeted breeding for low-P environments. Our studies revealed that long-term P fertilization altered rhizosphere microbial communities and root-secreted acid phosphatase (APase) activity, and regulated P-starvation-responsive (PSR) genes and defense pathways. Transcriptome and biochemical analyses identified ethylene signaling as a key regulator of P-deficiency responses, modulating P redistribution and root development, offering new targets for breeding P-efficient wheat cultivars. These findings collectively contribute to a deeper understanding of plant adaptive strategies to P limitation, paving the way for sustainable agricultural practices and enhanced crop productivity under P-deficient conditions.

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Arbuscular mycorrhizal fungi affect plant zinc nutrition – from genes to plate

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Keywords:
arbuscular mycorrhizal fungi, phytate, ZIP transporter, zinc

Abstract:

Zinc (Zn) is an essential micronutrient for plant and human health, and soil Zn deficiency is widespread in crop-growing regions. Arbuscular mycorrhizal (AM) fungi can contribute to plant Zn uptake via the mycorrhizal pathway of Zn uptake by colonising the host plant's roots, and have been suggested as potential contributors to crop Zn biofortification. Until recently, there were several unknowns surrounding AM fungi and Zn. We were lacking fundamental information on: (i) the quantity of Zn provided by the mycorrhizal pathway, (ii) the molecular mechanism(s) underlying mycorrhizal Zn uptake into the host plant, and (iii) the effects of AM fungi on the bioavailability of Zn in crops (for human nutrition). Using radioactive Zn and specialised soil hyphal compartments, we quantified the amount of Zn arriving through the mycorrhizal pathway in three crop species, and found that up to a quarter of shoot Zn was contributed by AM fungi[1,2]. We then generated a transcriptomic dataset from *Medicago truncatula* roots to identify and then characterise, for the first time, a ZIP transporter that is likely performing the role of Zn uptake between fungus and plant[3]. More recently, we have extended work on plant Zn nutrition to human Zn nutrition, by determining the effects of AM fungi on the accumulation of the anti-nutrient phytic acid and Zn bioavailability, in a collection of food crops. This work showed that AM fungi can increase the bioavailability of Zn in, e.g., sorghum and bread wheat[4], but negatively impact Zn bioavailability in rice[5], barley and lentil grain.

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Wednesday 26th November

Session 5 – New Technologies & AI in Plant Science

Annals of Botany Travelling Speaker – Lena Mueller

Session 6 – Beneficial Plant–Microbe Interactions

More than just a good chat: applying Large Language Models to plant science challenges.

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Abstract:

Without a doubt Large Language Models (LLMs), like ChatGPT and Claude, have revolutionized learning, teaching, planning and coding over the last few years. While this is useful as an assistant for plant scientists, there are a great deal of ways that LLMs can help solve big and challenging problems when used to their full potential. Here we will take a short (and very accessible!) tour of LLM capabilities beyond the usual chat, including RAG models, reasoning, nucleotide embeddings, and agentic collaboration. We'll dive into some concrete examples of using LLMs in the context of plant biology.

Accurate phenotyping of foliar diseases in barley using a high-throughput machine-learning based image analysis

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Keywords:
Digital phenotyping, image analysis, machine learning, barley net blotch

Abstract:

Advances in genomics have led to the development of various tools, including high-quality reference genomes, extensive marker databases, and genomic breeding platforms. Genotyping has become highly efficient and refined, and yet, phenotyping has not progressed at the same rate and is a bottleneck limiting the power of genetic analysis and genomic prediction. Phenotyping for disease resistance has remained reliant on traditional disease scoring, which is biased towards selection for major resistance (*R*) genes and missed the nuanced expressions of quantitative resistance. To maximise the potential genetic gain in breeding for quantitative disease resistance, selection efficiency must be achieved through high-throughput, quantitative, and accurate phenotyping that can capture as much phenotypic variation to obtain a higher resolution between genotype-phenotype interaction, and host-pathogen dynamics.

The digital phenotyping platform, the Compolytics® Macrobot NextGen imaging robot at La Trobe University, AgriBio, a GRDC investment for the Net Blotch Consortium aiming to enhance the genetic outcomes for net blotch resistance in barley, enables high throughput imaging of disease inoculated detached leaves. To maximise the phenotyping capacity of this platform, a machine-learning based model was developed to precisely and quantitatively measure disease expression of net blotch and barley scald on detached leaf segments based on the amount of green, chlorotic, and necrotic tissues, together with 10 RGB indices as indicator of leaf health. The implementation of this model will enhance the detection of quantitative disease resistance in diverse germplasm resources and mating populations for subsequent development and careful curation of resistant cultivars.

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Pixels to Phenotypes: An RGB Pipeline for Scalable Trait Extraction, Per-Leaf Tracking, and Cross-Species Analysis

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Keywords: plant phenotyping, imaging systems, plant traits, deep learning, cross-species generalisation

Abstract:

Image-based high-throughput phenotyping is capable of capturing millions of images indoors and in the field using different imaging systems. The current bottleneck in phenomics shifts from data collection to rapid, robust analysis with powerful AI models. Manual, observer-based assessment of plant traits cannot handle unavoidable leaf occlusions or photometric variability. A purpose-built RGB pipeline addresses these limitations by (i) separating plants and leaves from soil and tracking them across time-series image sequences with benchmark AI models, and (ii) automatically extracting numerous rosette- and leaf-level traits that characterise geometry and growth dynamics through a custom user interface. The pipeline is evaluated on the largest repository of top-view RGB images of *Arabidopsis thaliana*, collected in controlled environmental settings using the cutting-edge phenotyping equipment. Rosette and individual leaf instances receive precise annotations through a user-friendly tool to support advanced model training and validation. The pipeline outperforms existing tools, achieving nearly 97% segmentation mask overlap for rosette and leaf segmentation and 95% multi-object tracking accuracy across early, intermediate, and mature rosette growth stages. The same approach is going to extend to major crops such as maize, potato, and sunflower, supporting continual learning and generalisation to species not seen during training phase. Reliable plant segmentation and per-leaf tracking under varied environmental conditions enable consistent time-series trait extraction for stronger decisions in plant research and breeding. In summary, the pipeline accelerates the genomics-to-phenomics workflow and helps identify valuable traits faster, at scale, and with higher precision.

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Novel approaches to characterise and manipulate flowering genes in horticulturally important tree crops

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Keywords:
Flowering, tree crops, CRISPR, RNAi, nanoparticles

Abstract:

Flowering is a complex process regulated by many different environmental signals and endogenous signalling pathways. Much of what we understand about the genetic pathways controlling flowering has come from studies in model species such as *Arabidopsis thaliana*, although studies in non-model species indicate that many of the genetic pathways regulating flowering are highly conserved within angiosperms. Tree crops are highly valuable to Australia, accounting for half of the horticulture industry value. However, little is known about the genetic pathways that control flowering in these tree crops.

Our previous research through the National Tree Genomics program identified key flowering genes in several nationally important tree crops and characterised their function by overexpressing them in *A. thaliana*. However, to test the function of these genes within the tree species themselves we are now, as part of the Genetics for Next Generation Orchards program, exploring new approaches such as CRISPR gene editing and nanoparticle-mediated RNAi. We have successfully created CRISPR-edited lines in citrus and preliminary results have shown that nanoparticle-mediated RNAi is possible in tree crops, observing a significant reduction in expression of endogenous genes in several tree crop species. Here, I will present our latest research on these technologies and their applications for manipulating horticulturally important traits such as flowering in tree crops.

Decoding genome awakening during seed germination using single-cell analysis, multiomics and spatial transcriptomics

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Keywords:
Seed germination, Single-cell genomics, Spatial transcriptomics, Multiomics integration, Gene regulatory networks

Abstract:

Understanding how plant genomes transition from a dormant to a germinated seed remains a fundamental challenge with critical implications for crop improvement. In Arabidopsis, we have optimised protocols and integrative analysis for single-cell, multiomics, and spatial transcriptomics technologies to investigate genome regulation of seed germination. Using single-nucleus RNA-sequencing, we profiled transcriptional dynamics during the release of seed dormancy, uncovering previously uncharacterised cell state transitions. Among 25,000 nuclei, we identified distinct trajectories where embryonic and endospermic cells undergo transcriptional remodelling, and revealed coordinated transcriptional responses across multiple tissue types. Our findings suggest that the endodermis cells display a distinctive pattern in their cell states during those early stages, indicating tissue-specific transcriptional responses, supporting the existence of a germination "decision-making centre" within the radicle vasculature[1]. To link chromatin accessibility and gene expression, we established robust single-nucleus multiomics workflows (snATAC-seq + snRNA-seq) capable of profiling 5,000-8,000 nuclei. This integrated approach enabled identification of accessible chromatin regions, transcription factor binding motifs, and their regulatory interactions during genome activation. Chromatin immunoprecipitation confirmed direct transcription factor targets

throughout germination. To complement temporal dynamics with spatial context, we adapted a targeted single-cell 3D spatial gene expression technology[2]. We validated our single-cell transcriptomics data and revealed subcellular transcript localisation within native tissues. Together, these approaches enabled reconstruction of comprehensive gene regulatory networks orchestrating germination and early cell fate decisions. Our methodological innovations advance understanding of the seed-to-seedling transition and have direct agricultural applications for improving germination uniformity and timing - critical targets for crop breeding[3,4].

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Control of arbuscular mycorrhiza symbiosis by peptide hormones

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Keywords:
Arbuscular mycorrhiza symbiosis, peptides, receptors, CLAVATA signalling

Abstract:

Arbuscular mycorrhiza (AM) symbiosis is a mutually beneficial interaction of plants with soilborne *Glomeromycotina* fungi. The host controls the symbiosis through complex signaling networks between colonized root cells and other tissues of the plant. Several genes encoding secreted peptides of the CLE family are upregulated in *Medicago truncatula* roots during AM symbiosis. The AM-induced *MtCLE53* is a negative autoregulatory signal restricting AM fungal root colonization in concert with the shoot-acting receptor-like kinase *SUNN* (1). We recently found that the pseudokinase *CORYNE,* known to form a complex with SUNN, is also a negative regulator of AM symbiosis (2). However, our data indicate that CORYNE is only partially required for MtCLE53 signaling, suggesting *CORYNE* integrates multiple signaling pathways to modulate AM symbiosis. Our research further shows that CORYNE is part of an as-yet-unknown receptor complex that perceives the novel symbiosis regulator MtCLE16 (3). *MtCLE16* is induced in colonized cortex cells and promotes AM symbiosis by dampening host stress and defense responses. We hypothesize that MtCLE16 acts as a local signal amplifier promoting symbiosis. Intriguingly, some AM fungi also produce signaling peptides resembling those of plants, including CLE-like peptides. Functional investigation of the CLE-like peptide produced by the AM fungus *Rhizophagus irregularis* revealed that it signals through an as-yet-unknown host receptor complex involving CORYNE to manipulate host responses for microbial benefit. Together, our data suggest that AM symbiosis is regulated by at least two distinct CLE signaling pathways with opposite effects on the interaction, and that AM fungi mimic symbiosis-promoting CLE peptides for their own benefit.

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The regulation of nodulation by CLE and RGF signalling peptides in legumes

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Keywords: Autoregulation of Nodulation, biological nitrogen fixation, legume, root development

Abstract:

Symbiotic interactions between legumes and rhizobia bacteria result in biological nitrogen fixation, enabling the host plant to thrive in nitrogen-deficient soils and produce protein-rich seeds consumed globally. Prior to nitrogen fixation, a series of coordinated external and internal molecular signalling events initiate and guide the formation of root nodules. These nodules house the rhizobia and form the physiological interface where fixed nitrogen is exchanged for photosynthetically derived carbon [1].

To prevent nodules and rhizobia from becoming a metabolic burden, host plants regulate both nodule number and spatial distribution through local and systemic signalling pathways [1]. Central to these pathways are mobile peptides that act in a temporally and spatially specific manner to optimise nodulation according to the plant's environmental conditions [2].

Among these peptides, members of the CLAVATA3/ESR-related (CLE) and Root Meristem Growth Factor (RGF) families have emerged as key regulators. CLE peptides are well characterised in Autoregulation of Nodulation and nitrate-regulation of nodulation, whereas RGF peptides are established regulators of root development [3] and have more recently been implicated in nodulotaxis [4]. Using systematic phylogenetics and functional characterisation, including molecular approaches and synthetic peptide application studies, we identified conserved, legume-specific CLE and RGF peptides that negatively regulate nodule development in soybean, along with orthologues in other legumes.

Collectively, these findings have identified new molecular targets for improving symbiotic efficiency across multiple legume species. Exploiting these targets could enable the development of legume varieties with optimised nodulation and reduced dependence on synthetic fertilisers.

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Engineering Legumes for Enhanced Symbiotic Nitrogen Fixation the FUN Way

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Keywords:
Legumes, symbiotic nitrogen fixation, nodule senescence, cowpea, genome editing

Abstract:

To balance the cost of providing carbon to symbiotic nitrogen fixing bacteria, legumes regulate nodule function in response to soil nitrogen levels. While this is beneficial in natural ecosystems, in an agricultural context it is often desirable to maximise symbiotic nitrogen fixation. The *Fixation Under Nitrate* (*FUN*) gene encodes a TGA-family bZIP transcription factor which acts as a master regulator of nodule senescence by directly targeting several pathways to initiate the breakdown of nodules in response to high nitrogen[1]. In *Lotus japonicus*, *ljfun* mutants exhibited increased leghemoglobin concentration and acetylene reduction under high nitrogen, suggesting an increased capacity to fix nitrogen. Isotopic and phenotypic analyses confirmed *Ljfun* mutants either fix significantly more nitrogen from the atmosphere or accumulate significantly greater shoot biomass relative to wild type, depending on growth conditions. We are now translating the beneficial *fun* mutant phenotype into two globally important legumes: cowpea (*Vigna unguiculata*) and soybean (*Glycine max*). Promoter-GUS and RNA-Seq analyses in cowpea revealed highly specific nodule expression and conservation of the *FUN* transcriptional network. Utilizing CRISPR/Cas9 genome editing, we have produced a range of independent *vufun* knock-out mutants in cowpea, with early results showing increased shoot biomass and total nitrogen content under moderate soil nitrate conditions relative to wild type. In addition to ongoing molecular and phenotypic characterisation in controlled environments, we are preparing for the intentional release of the *vufun* mutants to assess their agronomic performance under field conditions.

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Do Beneficial Root Symbioses Impact Root Hydraulic Conductance?

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Keywords:
Arbuscular mycorrhiza; Root hydraulic conductance; *Solanum lycopersicum* L.; *Pisum sativum* L.; Optical dendrometer.

Abstract:
Arbuscular mycorrhizal (AM) symbiosis is known to increase plant nutrientacquisition and improve drought tolerance. Most studies have focused on aboveground traits, leaving the underlying mechanisms unclear. One possible mechanism underlying AM-induced drought tolerance is enhanced root hydraulic conductance (K_r), which describes how effectively plant roots take up water from the soil and transport that water to the root collar. Previous research reports conflicting effects of AM fungi on K_r, with suggesting both enhancement and no influence. In contrast, almost nothing is known about the impact of nodulation on water transport and drought physiology.

To address these gaps, we employed an optical dendrometer to monitor dynamic plant water potential and estimate K_r. This method enables continuous, non-invasive monitoring of stem water potential, offering real-time insights into xylem water transport and short-term hydraulic responses [1].

We examined the influence of AM on K_r in tomato and pea using soil sterilisation or mycorrhizal-deficient mutants. To study the influence of nodulation, we compared K_r in wild-type pea, supernodulator mutant, and nodulation-defective mutant. Strikingly, we found AM colonisation did not enhance K_r in either species. Furthermore, nodulation status had no effect on K_r in pea. These findings indicate that AM symbiosis does not influence drought tolerance by improving soil-to-root water transport and there is no trade-off between nodulation and root water uptake capacity. Future work could explore alternative mechanism by which AM symbiosis enhance drought tolerance.

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Beyond the root: Linking root morphology, mycorrhizal symbiosis, and exudation through Root Economic Space in phosphorus-stressed eucalypt species

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Keywords:
Root economic space; mycorrhiza; eucalypts; root traits; carbon allocation

Abstract:

How plants allocate carbon between leaves, roots, and symbionts is central to understanding their ecological strategies. The root economic space (RES) provides a framework to capture belowground carbon allocation along two dimensions: a strategic axis (acquisitive vs. conservative root morphology) and an investment axis. Mycorrhizal fungi are expected to interact with both axes by modifying carbon demand and nutrient return, but their influence on RES remains unclear in eucalypts.

We conducted a glasshouse experiment with twelve Eucalyptus species inoculated with a mixed arbuscular and ectomycorrhizal fungal community. Root traits were quantified alongside measurements of biomass, photosynthesis, leaf mass per area (LMA), carboxylate exudation, and non-structural carbon (NSC) as an indicator of belowground energy reserves.

Results revealed substantial interspecific variation along the strategic axis, with some species exhibiting acquisitive traits (high SRL, fine absorptive roots) and others conservative traits (low SRL, thick roots). Mycorrhizal inoculation rarely altered SRL, suggesting that intrinsic strategies are stable. Instead, inoculation primarily modified the investment axis by changing total root length, surface area, and biomass. Several species also increased carboxylate release and root investment without shifts in morphology, highlighting a decoupling of strategy and investment.

Our findings show that species identity defines root strategies, while mycorrhizal

fungi predominantly regulate carbon allocation along the investment axis of RES. Integrating leaf and root economic spaces provides new insight into plant−fungus−nutrient interactions.

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Thursday 27th November

Session 8 – Plant Biotic & Abiotic Stress Signalling

Session 9 – Teaching, Outreach & Indigenous Learnings

Session 10 – Frontiers in Genetics and Molecular Biology

The physiological basis of a catastrophic tipping point in grape berries in response to heatwaves

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Keywords:
grape berry, cell death, hypoxia, respiration, heatwaves

Abstract:
Late-season cell death and shrivel in grape berries, common in varieties like Shiraz and Cabernet Sauvignon, can decrease yield and quality. This phenomenon is driven by hypoxia at high temperatures and water stress (1,2). Our goal was to investigate the links between berry temperature, respiration, oxygen diffusion, and ethanol accumulation to develop models that predict heat stress in grapes.

Internal oxygen measurements confirmed large hypoxic regions correlating with reduced cell vitality. Using X-Ray microCT imaging, we identified a complex gas diffusion pathway from lenticels on the pedicel into the berry. We discovered this pathway becomes partially blocked late in ripening, a condition more severe in Shiraz than in other varieties. This blockage restricts oxygen supply, leading to widespread hypoxia. To quantify the changes in diffusion resistance, detailed 10x 3D print models of the pedicel and entrance to the berry were constructed. This allow measurement of oxygen diffusion through the region of interest that could be approximated and scaled back to the actual size.

A key consequence of hypoxia is the accumulation of ethanol inside the berry and its release as a volatile, both of which correlate with increasing temperature. This response differs significantly between varieties and has a distinct temperature "tipping point." Beyond this point, the exponential increase in berry respiration dramatically accelerates ethanol production.

Based on these physiological findings, we constructed non-linear mathematical models that successfully predict the onset of cell death and ethanol release. These models provide a tool for forecasting grape berry stress under high-temperature conditions.

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Genetic control of seed germination in Barley (Hordeum vulgare) under changing salinity: new players and targets for genetic improvement

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Keywords:
Germination, CRISPR-Cas9, Barley, Salinity stress, Genetic Improvement

Abstract:

Germination is a critical phase transition in a plant's life cycle. Unlike dormancy mechanisms, the processes controlling germination in non-dormant seeds are main little known. We investigated putative barley orthologs of an Arabidopsis gene family previously identified in the Masle lab as critical to the initiation and progression of germination under salinity and osmotic stress. The profound structural, compositional, and regulatory differences in germination between Arabidopsis and cereal/grass seeds raised questions about the relevance of these genes outside Arabidopsis, particularly in major staple crops. To assess the functional conservation and adaptive importance of our target genes, we engineered CRISPR-Cas9 transgenic lines edited in one or two members of the putative orthologous barley genes and isolated a set of homozygous mutants. Some mutations and combinations of mutations delayed and slowed germination under salinity stress, but unlike Arabidopsis, so too constitutively, under optimal environmental conditions. Their analysis revealed that this was primarily controlled by the seed husk, through properties determined during development on the mother plant, some anatomical, others yet to be identified, and involved a novel transcription factor not previously known to play a role in seeds. These findings open new avenues for the genetic improvement of crop performance and adaptation to climate change.

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Metabolites as markers for heat stress tolerance

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Keywords:
Stress tolerance, *Triticum aestivum*, Metabolites, Biomarker

Abstract:

Metabolomics is a powerful tool in understanding how plants respond to abiotic stresses such as heat, drought and salinity (1). Due to their sessile nature, the metabolomic profile must adapt to maintain cellular homeostasis and compensate for the plants surrounding environment. Through comparative metabolomic profiling we have been developing biomarkers for heat tolerance in wheat (*Triticum aestivum*) genotypes from diverse breeding backgrounds. The endogenous GABA content varied 10-fold amongst the collection and responded to temperature treatments in a genotype dependent manner. Metabolomics was performed using Gas Chromatography Mass Spectrometry on 10 genotypes exposed to temperatures between 17ºC and 37ºC for 2 hours. It was observed that a 5ºC temperature increment was sufficient in shifting the metabolite profile. A correlation analysis revealed that the relative abundance of 42 of 132 detected metabolites were significantly impacted by temperature. Phytol strongly accumulated in all genotypes exposed to heat stress but was this was less pronounced in heat tolerant genotypes. Amino acids tended to accumulate, while sugars and TCA intermediates were metabolised under increasing temperature. Phenotypic characterisation of the diversity collection is ongoing and must be completed before final validation of potential metabolite biomarkers of heat tolerance and integration into plant breeding programs.

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‘Resistance that stacks up’ - Diversifying rust resistance in cereals

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Keywords:
Genetic resistance, rust pathogens, diverse mechanisms, NAC transcription factors, gene pyramiding

Abstract:

Staying ahead of the arms race against rust diseases in cereal crops is essential to maintain and preserve food security. The methodological challenges associated with conventional disease resistance breeding are major bottlenecks for deploying resistance (*R*) genes in high-yielding crop varieties. Advancements in our knowledge of plant genomes, structural mechanisms, innovations in bioinformatics, and improved plant transformation techniques have alleviated this bottleneck by permitting rapid gene isolation, functional studies, directed engineering of synthetic resistance and precise genome manipulation in elite crop cultivars. Most cloned cereal *R* genes encode canonical immune receptors which, on their own, are prone to being overcome through selection for resistance-evading pathogenic strains. However, the increasingly large repertoire of cloned *R* genes permits multi-gene stacking that, in principle, should provide longer-lasting resistance. This presentation will give an overview of how my lab has applied genomics-enabled developments to unravel diverse rust resistance mechanisms using barley as a cereal model crop. The mechanistic diversity of leaf rust resistance alleles characterised in my lab over the past few years including *Rph1, Rph7, Rph15, RphHma5* and *RphCaspian* will be discussed in the context of their applicability for resistance gene engineering in relevant elite high-yielding barley cultivars in collaboration with the Plant SynBio platform at La Trobe University. Our research is

also leading to the development of breeder-friendly tools and informing new genetic frontiers in plant immunity that can be directly applied to enhance plant breeding outcomes for durable disease resistance. It is expected that the lessons learnt from using the barley pan genome and leaf rust model pathosystem can be extrapolated to crops species with more complex genome structures.

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Proteomic analysis of lettuce grown the International Space Station to assess plant adaptive responses to microgravity induced watering challenges

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Keywords:
Microgravity, Lettuce, Abiotic stress, Hypoxia, Proteomics

Abstract:

The microgravity environment encountered by plants grown in space habitation presents a critical watering challenge, owing to altered fluid and gas exchange dynamics[1]. Gravity-dependent convection, responsible for passive gas mixing and reoxygenation of water is absent in microgravity, imposing a waterlogging effect and subsequently root hypoxia[2]. NASA has identified a fine balance is required between overwatering and underwatering in microgravity, to avoid either hypoxia or drought stress, respectively. Understanding how plants perceive and respond to microgravity-induced abiotic stress is critical to optimise plants for space cultivation[3].

In 2025, NASA astronauts from Expedition 73 conducted an experiment aboard the International Space Station (ISS) using red romaine lettuce (*Lactuca sativa* cv. Outredgeous) grown in the Advanced Plant Habitat. Flooding, drought and wilting stress was imposed in triplicated 3-week grow-out experiments, with parallel ground control experiments conducted at the Kennedy Space Centre.

Lettuce subjected to drought and wilting exhibited reduced growth and biomass in both ISS and ground controls, while flooded plants appeared phenotypically unaffected. Proteomic analysis of leaves and roots returned from the ISS and performed at the University of Western Australia will be presented to show the parallel changes that occurred in energy metabolism, hypoxia, and abiotic stress responsive pathways, in response to the watering regimes. The collective influence on the lettuce proteome from ISS environmental factors including microgravity, the absence of fluid convection and elevated CO_2 will also be presented relative to matched ground controls.

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Independent and combined heat and drought impact *Vicia faba* development, grain yield and protein.

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Keywords:
Drought, Heat, Yield, Protein, Faba bean.

Abstract:

Vicia faba L. (faba bean) is an annual grain legume, grown in rotation with cereals and canola across Southern, medium rainfall zones in Australia. There is growing interest in Australian faba entering the high value processing market for its high protein content and established role in rotations. However, heat and drought are often combined stressors that significantly impact yield and nutritional composition in grain legumes [1,2]. For faba to establish and maintain a role in the processing industry, a developed understanding of yield and protein variation under abiotic stress is needed. This work aims to investigate key developmental processes that contribute to final yield and protein dynamics in faba bean under independent and combined heat and drought. Two controlled environment rooms with gravimetric Phenospex DroughtSpotter systems held 192 pots at the Australian Plant Phenomics Network, in The Plant Accelerator facility, Adelaide. Treatment conditions (Control, Drought, Heat, Heat and Drought) were applied from flowering until maturity. During the treatment period, water use, SPAD, reproductive development and biomass data was collected. At maturity, yield parameters were collected and grain protein determined with nitrogen combustion. Understanding development under heat and drought stress will highlight key pathways that determine yield and protein outcomes in Australian faba bean. Results will help identify strategies for breeding high yielding, and high protein faba, promoting grower and processor profit, as demand for plant protein grows.

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Invited Speaker

Revecca Kakavanos-Plew

Abstract:

The benefits of an interactive simulation in tertiary teaching of Mendelian genetics concepts

Students studying first year Biology and second year Genetics courses are expected to understand foundational Mendelian genetic concepts (Monohybrid and dihybrid crosses; Mendelian inheritance laws) as part of their health/biomedical programs. Having taught these courses over many years, the investigators have routinely observed students struggling to grasp these conceptually challenging and complex concepts, particularly those focused on dihybrid crosses. This has been documented through formal anonymous student feedback in these courses, and/or the poor outcomes achieved by students in answering questions around Mendelian genetics in their summative assessments.

Research consistently reports that students struggle to apply the principles of Mendelian inheritance often leading to misconceptions at a tertiary education level (Smith and Knight, 2012; Haske-Ittah and Yarden, 2018; Maghfiroh et al, 2024). As part of this project, we have developed an interactive simulation to help students better visualise, engage with and understand the complex Mendelian genetic concepts in real time, in a user friendly environment. This aligns with both constructivist (Piaget, 1952; Vygotshy, 1978) and cognitive load (Sweller et al, 2011) pedagogical theories. Although several Genetics based digital tools do exist many are designed for general use (Rutten et al, 2012) or are not aligned to tertiary curriculum and specific course outcomes (Dirks et al 2014). Simulations have also been used at the University of South Australia in microbiology (Mahdi et al, 2022) and immunology (Costabile and Turkanovic 2024) and support the benefits of simulations on student learning.

This research project (HREC ID 207233) evaluates the benefits of the interactive simulation we have developed in a second year Genetics course (BIOL2016) via two ways. The first involves comparing student responses pre- and post- simulation in a paper based anonymous survey with Likert style and free-text questions assessing: student knowledge and understanding of Mendelian genetics concepts, the perceived level of difficulty and confidence levels in answering questions on this topic as well as general feedback on the simulation.

To assess student short term and long-term gains following the use of the simulation, we will also compare student performance on Mendelian inheritance questions in a summative assessment between students that completed the simulation (2025) to previous cohorts where the simulation was not available (2023/2024). All data will be pooled and analysed for changes in mean responses using appropriate statistical tests.

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Declare of Beware: Declarations of generative AI use undergraduate biochemistry and genetics assessments.

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Keywords:
Generative AI, Assessment design, Practical Assessment, Discovery Learning

Abstract:
Generative Artificial Intelligence (GenAI) is reshaping higher education, creating opportunities for pedagogical innovation while challenging academics to redesign traditional written assessments. Although these technologies enable personalised learning and more efficient feedback, they also raise concerns about academic integrity, the authenticity of student work, and the development of critical cognitive skills (Francis et al., 2025; Lye & Lim, 2024). While current debates often focus on integrity and policy responses, there is growing recognition that GenAI demands deeper reflection on learning outcomes, pedagogy, and graduate preparedness for a changing workforce (Chiu, 2024). Earlier studies indicate that although most students are familiar with GenAI tools, their views on ethical use vary widely, and many call for consistent institutional guidance (Johnston et al., 2024).

This study explores a “declare or beware” approach to student use of GenAI in written practical and discovery learning assessments across three undergraduate Biochemistry and Genetics courses delivered across two teaching periods in 2025. A total of 273 assessment submissions were analysed, and 214 declarations recorded (78.4% compliance). Of these, 63.1% reported GenAI use—most frequently ChatGPT (70.3%) and Grammarly (30.4%). Compliance averaged 82.7% although varied from 56.3-100% across the different assessments analysed. In the first teaching period, 6.7% of declarations failed to specify which GenAI tools were used, however, introduction of a purposefully designed declaration template in the second semester, eliminated this issue.
These results suggest that GenAI use is becoming embedded in assessment preparation. Transparent disclosure fosters responsible engagement and supports the development of AI literacy, interdisciplinary learning, and authentic assessment design.

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A year of Plants for Space education and engagement

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Keywords:
Education, Engagement, Space Horticulture, Sustainability,

Abstract:

The ARC Centre of Excellence in Plants for Space (P4S) operates at the intersection of plants, people, technology, and sustainability, fostering critical and creative thinking. P4S uses the lens of space to frame sustainable futures in a new context – which aims at alleviating environmental anxiety, and encourage optimistic and innovative thinking, unconstrained by biological and societal norms[1].

One of Plants for Space's missions is a future ready workforce and society – which focuses on providing our researchers with skills to put their work in broader societal context, and translate and showcase their work to a wider public, as well as enthusing students (primary, secondary and tertiary) and the general public about STEM and science as a human endeavour.

In 2025 P4S was in their second year of operating, which provided a range of educational and engagement activity – here we will explore what the year has provided in learnings on the best form of 'scientific storytelling', and what lessons can be taken away from two years of operating as nationwide Education and Engagement Team within an ARC Centre of Excellence.

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Seeding Ideas – exploring an Indigenous food production system with first-year students

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Keywords:
Native grains, object-based learning, Indigenous knowledge systems

Abstract:

The University of Sydney's "One Sydney, Many People Indigenous Strategy" (2021-2024) and the national "Indigenous Strategy" by Universities Australia (2022-2025) highlight the importance of introducing First Nations knowledge into the curriculum. An immersive practical was designed to incorporate Indigenous content into first-year biology. At the beginning of the practical, students are introduced to basic knowledge of plant taxonomy, plant anatomy and broad-acre crop production systems in the *Poales*, highlighting plant traits that are important for successful modern agricultural crops, and to provide the framework for discussions on native Australian grains in the modern context.

During the practical, students consider the traits of wild Australian cereals and shrubs which were traditionally used for food, determined from the literature and discussions with Gamilaroi knowledge holders. Students observe a variety of florets and seeds under the dissection microscope, which facilitates learning about plant and seed structures, and stimulates thoughts on potential challenges for processing of seeds. Subsequently, students compare hand-threshing florets of developed crops (wheat, barley) with native grass seeds (e.g. *Astrebla* sp.), which highlights Indigenous ingenuity for food production[1]. Students then make, and compare, the flavour of flatbreads of native-grass-seed-and-wheat flour mixes and 100% wheat.

To complement the hands-on activities, students explore benefits and challenges of

growing native grasses and developing an Indigenous-led native grains industry in group discussions. Throughout the practical, cultural guidance is provided by a Gamilaroi staff member. The practical emphasises the importance of grasses for food, different knowledge systems, Indigenous Cultural Intellectual Property and deep connection to Country.

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Embedding First Nations science in undergraduate science curriculum

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Keywords:
First Nations knowledge; science curriculum; agriculture; food science; undergraduate

Abstract:

There is a growing urgency to diversify food production systems amid increasingly variable and extreme climate conditions, compounded by pressures from a growing global population[1]. Producing high quality food sustainably in a model based on only a few staple crops and production systems is becoming increasing risky. Educating current and future students on the potential presented by the adoption of native plant species into mainstream food supply is essential[2]. To achieve this, students must graduate with appropriate knowledge to engage with First Nations peoples and the agrifood sector. The first step to achieving this is upskilling academic staff to sensitively embed First Nations native food knowledge into the curriculum.

Guided by a framework for curriculum development for First Nations Australians Science[3] and a sensitive, reciprocal collaboration with a First Nations business, Red Centre Enterprises and community Elders, this presentation will describe the first year of an initiative in the School of Agriculture Food and Wine. This curriculum framework exceeds typical agricultural innovation, unveiling a blueprint for genuinely sustainable living, deeply rooted in Australia's First Peoples' wisdom. The project aimed to deliver professional development to targeted academics to encourage development and delivery of First Nations science curriculum. Diverse courses from agricultural, food and wine science undergraduate programs spanning several year levels were identified for First Nations curriculum inclusion. We will showcase how First Nations science can be embedded in these undergraduate science courses by describing several successful case studies and reporting preliminary data from staff and student experiences.

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Working with Reflection bot Riff to improve reflection and the student experience in WIL

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Keywords:
Reflective practice, experiential learning, artificial intelligence, work integrated learning

Abstract:
Reflective practice is foundational to experiential learning and the development of professional identity in higher education (Gibbs, 1988; Warhurst, 2025). However, sustaining meaningful reflection during Work Integrated Learning presents challenges, particularly when students face long hours, geographical isolation and limited access to academic mentoring or peer discussion. Reinforcing metacognitive strategies is pivotal in promoting independent learning (Kuiper, 2002). And, few learners are naturally reflective, but most can learn, or improve reflection in practice (Edwards, 2010). *CrushPad*, a mobile-first reflection app for Bachelor of Viticulture and Oenology students at Adelaide University, has been conceived and is currently being implemented to explore how artificial intelligence can scaffold reflection, cognition, and well-being during placements supporting independent learning outcomes.

Artificial intelligence in education is an emerging field in educational technology however it remains unclear how to harness its capabilities to meaningfully impact teaching and learning (Harper, 2023). *CrushPad* integrates structured prompts, mood tracking and AI-assisted reflection integrating Stanford d.School's Riff tool. In its current ideation and prototyping phase, the project investigates how conversational AI can enhance depth of reflection, promote growth-mindset thinking, and help students articulate their professional and experiential learning across a ten-week placement.

The initiative aims to contribute to educational scholarship in two ways: (1) by developing and testing ethical, context-sensitive approaches to AI in reflective pedagogy and (2) by generating data to support research on metacognition and professional identity formation within STEMM disciplines. Early feedback suggests that AI-guided reflective dialogue can enhance student engagement and cognitive self-awareness while offering actionable insights into student learning and well-being.

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Establishing a new paradigm for salinity tolerance in cereals

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Keywords:
Salinity tolerance, OzBarley, Genome Wide Association Study (GWAS), field trials

Abstract:

Salinity tolerance consists of several sub-traits. All plants exclude >95% of soil Na^+ from their tissues, but the majority of research has focussed on enhancing Na^+ exclusion from leaf blades. While improvements in this sub-trait have been successfully introduced into some crop species, its adoption into bread wheat or barley has been lacking, as its influence on improving yield has been inconsistent/detrimental. With recent evidence[1,2,3] finding no negative correlation between leaf Na^+ concentration and yield in bread wheat and barley, it is hypothesised that exclusion of Na^+ from reproductive tissue is a key determinant of yield.

Accessions in the OzBarley population accumulate a large range of grain Na^+ accumulation, despite accumulating similar levels of leaf Na^+. The reason why these barley accessions accumulate altered grain Na^+ concentration is known, they have a different allele of a Na^+ transporter, *HvHKT1;5*. However, the tolerance mechanism which enables them to maintain their yield under salinity is unknown.

Here we present evidence showing different alleles of *HvHKT1;5* are linked to grain Na^+ accumulation but not leaf Na^+ accumulation in the OzBarley population. We also present preliminary results of a genome wide association study which has identified novel loci on chromosomes 1H, 2H and 3H linked to salinity tolerance in a field grown OzBarley population. We will also present the progress of dissecting the floral tolerance mechanism in a selection of OzBarley lines grown under different salinity

stresses in the greenhouse.

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Polymeric Nanocomplexes for Delivering Genetic Material into *Arabidopsis* Pollen

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Keywords:
Nanoparticles, gene delivery, pollen, Arabidopsis

Abstract:

Efficient delivery of nucleic acids into plant male gametophytes represents a promising strategy for introducing CRISPR for precision genetic manipulation. Here, we report the development and application of a polymeric nanocomplex platform capable of delivering both plasmid DNA and mRNA into *Arabidopsis thaliana* pollen grains. As a proof of concept, we used a nanocomplex approach to deliver a transient plasmid encoding a nuclear localized superfolder green fluorescent protein (NLS:sfGFP:NLS) driven by the pollen-specific *Lat52*[1] promoter. This enabled targeted expression of sfGFP in both sperm and vegetative nuclei, facilitating visualization of nuclear compartments within the pollen. Similarly, nanocomplex delivery cytoplasmic eGFP mRNA led to detectable cytoplasmic fluorescence, confirming effective uptake and translation of exogenous transcripts in pollen cells. Importantly, viability and germination assays demonstrated that nanocomplex-treated pollen retained normal physiological function, indicating that the delivery process does not compromise pollen integrity. GFP, a widely used reporter protein, provided a robust and non-invasive means to monitor gene expression and intracellular targeting in real time. Together, these results demonstrate the potential of polymeric nanocomplexes as a versatile, transgene-free tool for introducing functional nucleic acids and proteins into plant male gametophytes. This platform offers new opportunities for transient gene expression studies, and potentially heritable genome editing without tissue culture bottlenecks[2,3]. Ultimately, this strategy could accelerate the development of improved plant varieties, supporting more efficient and sustainable agriculture.

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Let there be light: controlling daily rhythms of protein synthesis

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Keywords:
Circadian clock, translation, TOR, light, energy

Abstract:

Protein synthesis requires vast amounts of cellular energy to maintain. Therefore, this must be managed according to changing availability and demands across the daily cycle and for plants, this is intrinsically linked to light availability and photosynthesis. We have used a combination of polysome profiling, puromycin labelling, Ribo-seq and reporter assays to measure the dynamics of protein synthesis across daily cycles. We found that the circadian clock contributes to drive daily rhythms of translation and is required to anticipate the onset of light and increase protein synthesis in the morning, which is mediated by activity of TOR, a global regulator of translation. We also used the Ribo-seq dataset to identify genes that are strongly translationally regulated, including circadian clock genes, and verified these using reporter assays. Thus, our study indicates both circadian regulation of translation and translational regulation of the circadian clock.

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

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Keywords:
Herbicides, aromatic amino acid biosynthesis, repurposing, weeds

Abstract:

The utility of current herbicides is becoming increasingly threatened by the rapid evolution of herbicide-resistant weeds. 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 antibiotic effectiveness could be re-purposed as herbicidal scaffolds to fast-track the identification of new herbicides. We explored the herbicidal efficacy of an aromatic amino acid (AAA) biosynthesis inhibitor that targets dehydroquinate synthase (DHQS) in bacteria. By determining the crystal structure of *Arabidopsis thaliana* DHQS and performing enzyme kinetic assays we confirmed that plant and bacterial DHQS enzymes possess a high degree of structural conservation, and the re-purposed inhibitor retains micromolar potency against plant DHQS. Then, upon subsequent discovery that the re-purposed inhibitor exhibits herbicidal efficacy in-soil, we improved the herbicidal potency of the inhibitor through the synthesis and characterisation of 42 analogues. Our lead analogue displays plant specificity, having no impact on common soil-dwelling bacteria, and demonstrates potent post-emergent herbicidal activity against one of Australia's most problematic weed species, wild radish. Mode of action studies using transcriptomics has afforded further insight into plant consequences to treatment with the lead analogue and the mechanisms mediating plant demise, including disruption to AAA biosynthesis. Overall, this study demonstrates that re-purposing inhibitors and targeting DHQS are viable approaches to expedite the identification of chemical scaffolds with new molecular targets to combat rising herbicide resistance.

Poster Abstracts

Evidence for cell-specific stress signalling driven by specialised chloroplasts in C_4 photosynthesis

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Keywords:
Chloroplast signalling, C_4 photosynthesis, transcriptomics

Abstract:

Robust communication between chloroplasts and the nucleus is critical for plant environmental acclimation. These chloroplast-nuclear or "retrograde" signalling pathways relay information from the chloroplast via reactive oxygen species (ROS), signalling metabolites, and proteins to the nucleus to induce transcriptomic remodelling necessary for stress acclimation[1,2]. However, to date, chloroplast signalling pathways have been studied largely at the whole leaf level, neglecting the fact that chloroplast function is not homogenous across all cell types of the leaf. Such differences in chloroplast function are especially pronounced in plants performing C_4 photosynthesis, where extreme specialisations in mesophyll (M) and bundle sheath (BS) chloroplasts may fundamentally impact their capacities for chloroplast signalling[3]. Here we systematically investigate potential cell-specialisations of chloroplast signalling in the model C_4 grass *Setaria viridis*., using cell type-enriched transcriptomics to profile M- and BS-specific responses to combined high light and heat stress (HL+H). Co-expression and gene regulatory network analyses highlighted the M-enriched upregulation of ROS scavenging and xenobiotic detoxification transcripts strongly linked to the 3'-phosphoadenosine 5'-phosphate (PAP)- and β-cyclocitral-dependant chloroplast signalling respectively. This was further evidenced by M-specific accumulation of these chloroplast signals under HL+H. In contrast, BS responses to HL+H were defined by a unique regulatory network of AP2/ERF transcription factors implicated in rapid retrograde signalling via triose phosphate[4]. Taken together, these results suggest that cell type-specialisations of chloroplast biochemistry and photosynthesis are indeed linked to concurrent specialisations in chloroplast signalling, highlighting a novel opportunity to improve the stress resilience of crops through the cell type-specific manipulation of these pathways.

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Genome-wide association study to identify genetic determinants of symbiotic nitrogen fixation under drought in *Lotus japonicus*

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Keywords: Symbiotic Nitrogen Fixation, Drought, GWAS, Rhizobium.

Abstract:

Legumes fix atmospheric nitrogen through a symbiotic relationship with rhizobium, supporting legume growth and enhancing soil nitrogen for subsequent crops. However, abiotic stresses such as drought disrupt this process, negatively impacting nitrogen fixation and overall yield [1]. The presence of genetic variation in nitrogen fixation response to water deficits among legume cultivars presents an opportunity to enhance drought tolerance in nitrogen fixation. Integrating beneficial variants into modern crop breeding programs could enhance the resilience of legume cropping and reduce the requirements for synthetic fertilizers.

150 wild *Lotus japonicus* germplasm lines [2] were phenotyped for nitrogen fixation under drought and well-watered conditions. Drought stress was induced using gravimetric method, and key traits such as nitrogen fixation by measuring N incorporation into plant biomass, leghemoglobin concentration, nodule number and plant biomass were collected at harvest after drought treatment. The collected data is used in Genome-Wide Association Studies (GWAS) to identify Quantitative Trait Loci (QTLs) associated with drought tolerance. These QTLs will be validated by gene editing using CRISPR and *Lotus* LORE1 mutants. QTLs which are found to be imparting tolerance could provide new approaches to enhance legume productivity and drought resilience.

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Intrinsic molecular and metabolic mechanisms underlying heat and drought tolerance in contrasting wheat cultivars

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Keywords:
Wheat, abiotic stress, panomics, heat/drought tolerance

Abstract:

Heat and drought are two of the most pressing abiotic stresses limiting wheat production in Australia and globally. Improving intrinsic tolerance to these stresses is critical for developing resilient cultivars and safeguarding yield stability. In this study, various wheat cultivars previously described (1) as either drought tolerant or drought susceptible were grown under controlled greenhouse conditions to investigate intrinsic mechanisms contributing to improved stress tolerance. Plants at flowering were exposed to either seven days of severe drought stress, 24 hours of heat stress at 37°C, combination of both stresses, or maintained under well-watered conditions. Plant responses were assessed through 'Panomics' i.e. biomass measurements, malondialdehyde levels as an indicator of oxidative stress, transcription levels of enzymes within the GABA shunt pathway in shoot tissue. While previous studies have largely focused on the impacts of heat and drought stress on yield and grain development, the underlying molecular and biochemical mechanisms contributing to cultivar-specific tolerance remain poorly understood. By linking physiological, molecular, and metabolic responses across various cultivars, this work provides novel insights into the processes that influence performance under stress. The outcomes will contribute to a deeper understanding of intrinsic protective mechanisms and inform breeding strategies for wheat cultivars better adapted to increasingly frequent heat and drought events.

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The impact of *Arabidopsis thaliana* cell wall mutants on the assembly of a synthetic community of bacteria at the rhizoplane

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Keywords:
Cell wall, rhizosphere, SynCom, metagenomics, PGPB

Abstract:

Plant growth promoting rhizobacteria can significantly impact plant health and productivity, particularly during environmental stress. Plant breeding towards fully utilising the potential in the rhizosphere, first requires an understanding of the plant factors that shape microbiota composition[1]. As the external surface of the root, the plant cell wall forms an interface for interactions with rhizosphere microorganisms. This highly dynamic and heterogeneous structure, primarily composed of complex polysaccharides and glycoproteins, forms a key barrier in defence[2]. However, the molecular contributions of the cell wall to determining the selective colonisation of the rhizoplane are largely unknown. We selected a panel of 16 *Arabidopsis thaliana* cell wall mutants affected in polysaccharide biosynthesis pathways or post biosynthetic processing. Using a hydroponics-based system[3], the mutants alongside wild type and positive control *Lotus japonicus*, were inoculated with a 16-member synthetic bacteria community[4]. 16S rRNA amplicon sequencing revealed distinct root-associated communities compared to bulk media, confirming a rhizosphere effect. As previously published, this community differentially colonised the roots of wild type Arabidopsis and Lotus. While most mutants were comparable to wild type, lines with additional root system architecture phenotypes showed significant shifts in beta diversity. Pairwise comparisons between mutants showed some significant differences in bulk media and root community compositions. Notably, two mutants exhibited contrasting abundances of the plant growth promoting *Herbaspirillum seropedicae*. These findings suggest that cell wall phenotypes can modulate root microbiota, with further investigation needed to unpack the mechanisms by which the plant cell wall affects bacteria binding and establishment at the rhizoplane.

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Genetic Dissection of Spike Morphology in Australian Barley Panel: Insights from a Multi-Model GWAS and Haplotype Analysis

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Keywords:
Barley; Spike architecture; Genome-wide association study; Haplotype analysis; Breeding selection

Abstract:

Barley (*Hordeum vulgare* L.) is a globally important cereal crop increasingly challenged by climate variability and shifting agronomic demands. Spike morphology is pivotal in determining yield potential, but its genetic underpinnings remain only partially understood. Here, we leveraged the OzBarley panel with 214 elite Australian barley accessions, integrating high-density markers (65,465 SNPs) with multi-environment phenotyping to dissect the genetic architecture of six spike traits: awn length (AL), grain number per spike (GNS), spike density (SD), spike length (SL), spikelet number per spike (SNS), and seed setting rate (SSR). Genome-wide association studies identified 27 stable quantitative trait loci (QTLs) distributed across six chromosomes, explaining up to 22.52% of phenotypic variance. High heritability estimates (49.02-93.54%) and strong inter-trait correlations ($r = 0.33$-0.89, $p < 0.001$) underscore the stable genetic control. Haplotype-based dissection prioritized two major loci: *QAL.Murdoch.7H.1*, and *QGNS/SD/SNS.Murdoch.4H.1*. A missense SNP (Lys372Asn) in *HORVU.MOREX.r3.7HG0720710* was implicated in the suppression of awn length at *QAL.Murdoch.7H.1*, while a nonsynonymous mutation (Ala127Thr) in *HORVU.MOREX.r3.4HG0336810* at *QGNS/SD/SNS.Murdoch.4H.1* was associated with increased spikelet and grain number. Longitudinal analysis revealed directional selection for favourable haplotypes, *QGNS/SD/SNS.Murdoch.4H.1*-Hap002 and *QAL.Murdoch.7H.1*-Hap004, with frequencies increasing significantly in recent Australian cultivars. Conversely, unfavourable haplotypes introduced during the 1990s were later counter-selected, highlighting refined breeding strategies. Our findings offer critical insights into the genetic regulation of barley spike morphology, thereby linking genomic discoveries to breeding applications.

The OzBarley panel serves as a valuable genomic resource for accelerating marker-assisted selection, facilitating the development of high-yielding barley cultivars tailored to meet future agricultural demands.

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Mystery of the macadamia tree – to be or not to be mycorrhizal

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Keywords:
Macadamia, arbuscular mycorrhizal fungi (AMF), phosphorus, dark septate endophytes (DSE), sustainable agriculture

Abstract:

The nuts of macadamia (*Macadamia integrifolia, Macadamia tetraphylla* and their hybrid) nuts are a uniquely Australian product, representing the only native flora to have been developed into a globally recognised food crop. Production has grown steadily over the past decade, with increasing plantations both domestically and internationally. Macadamias are relatively recently domesticated species, with breeding and cultivation efforts occurring primarily the past century[1]. As such, there are significant gaps in the knowledge regarding their physiology. One key area of uncertainty concerns the mycorrhizal status of the macadamia tree; they are generally understood to be unable to form associations with arbuscular mycorrhizal (AM) fungi, but this has not been comprehensively tested[2].

In this study, we explored whether inoculation of *Macadamia integrifolia* and *Macadamia integrifolia x tetraphylla* seedlings with different species of AM fungi could stimulate root colonisation in pot-based experiments. Three experiments were conducted to assess the mycorrhizal status of macadamia: (1) inoculation of macadamia with six different AM fungal species or mixes of species, (2) growth of macadamia in four natural soils including two macadamia orchard soils, containing native soil microbiome, (3) co-cultivation with five different companion plant species. We found no AM fungal colonisation in the roots sampled from either experiment. However, we did frequently observe the presence of dark septate endophytes. From these experiments, there is no evidence that macadamia can be colonised by AM fungi when using three inoculation methods that reliably stimulate colonisation in AM-compatible plants (pot culture, native soil microbiome and companion plants).

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Optimal prime: Seed priming in *Cannabis Sativa L*. and adapting to challenges in the Australian Environment

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Keywords:
Seed priming, Hemp, Drought, Hydrogen Peroxide, Micronutrients

Abstract:

Cannabis sativa (Industrial Hemp) is an emerging fibre and food crop in the Australian landscape with many states making progress towards implementing the crop on a larger scale. Whilst this is a step in the right direction, there are still environmental challenges facing hemp's continued growth and implementation for plant breeders(1). Seed priming is a well-established method of improving plant performance and response to stress by applying an external factor before germination/sowing(2). As a strategy to improve stress tolerance, gross yield and seed quality, we have investigated redox-priming (hydrogen peroxide), Nutri-priming (micronutrients) and a combination of the two treatments. We investigated if priming improved the cultivar Han NE response to drought stress across multiple generations. As a part of this investigation, we examined several key physiological factors including total grain yield, above ground biomass, harvest index, and water use efficiency. This data was paired with key quality indicators in both leaf and seed tissue such as micronutrient concentration (Fe, Zn, Mn), phenolic content, and antioxidant capacity. This data was taken from both generations with consideration for the difference between male and female plants. Whilst there was a significant improvement in the first generation with some of these traits such as grain yield and harvest index this effect was lost in the subsequent generation.

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Mycorrhizal fungal colonisation can enhance root nodulation but reduce zinc and iron bioavailability in field pea

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Keywords:
Arbuscular mycorrhizal fungi; nutrient bioavailability; phytic acid; protein; field pea

Abstract:

Deficiencies in nitrogen (N) and phosphorus (P) in agricultural soils reduce crop yield, grain quality, while low zinc (Zn) and iron (Fe) in crops impair human nutrition [1]. Pulse crops fix atmospheric N via rhizobia [2], while arbuscular mycorrhizal (AM) fungi enhance plant uptake of P, Zn and Fe [3]. However, grain phytic acid (PA) can chelate Zn and Fe, lowering their bioavailability to humans [4]. The tripartite relationship between rhizobia, AM fungi and field pea can affect pea micronutrient bioavailability but poorly understood, yet are crucial for sustainable diets.

We first assessed PA and protein variation in 21 field pea genotypes. PA levels varied widely, while protein content was relatively stable. Two factorial experiments were then conducted using *R. irregularis*, applied either as a commercial inoculum with added P or as an in-house pot culture (WFVAM10) without added P.

Commercial AM fungal inoculum resulted in 20–55% root colonisation, with no significant differences in measured parameters. In contrast, the pot culture inoculum achieved 50-65% and also enhanced rhizobial nodulation in two pea genotypes. Pot culture AM fungal inoculation consistently increased biomass in field pea, as well as total Zn and Fe uptake per plant. However, the increased pea yield appeared to dilute Zn and Fe concentrations, and ultimately reduced Zn and Fe bioavailability in the AM plants.

Results indicate that the source of AM fungal inoculant and soil P addition strongly influence the function of the plant-fungal association. This work also underscores

the importance of balancing yield and micronutrient bioavailability in legume improvement strategies.

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How a liverwort pushes the safety-efficiency trade-off to extremes

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Keywords:
hydraulic transport, pegged rhizoids, liverworts, convergent evolution

Abstract:

The evolution of hydraulic transport tissue has enabled some plant clades to maintain leaf hydration in drying environments, allowing them to continue photosynthesis where other species cannot. While the physiological strengths, limitations, and variations within tracheophyte xylem are well characterized, much less is known about the multiple independently evolved vascular transport systems in the bryophyte clade. The marchantialean or complex thalloid liverworts have evolved pegged rhizoids, which are thought to internally transport water across the lower external surface of the thallus, but this has not been tested physiologically.
Here we demonstrate that pegged rhizoids in the liverwort *Marchantia polymorpha* transport water very efficiently. Pegged rhizoids can maintain thallus hydration under extreme evaporative demand, but cavitate under low water potential. This system highlights an independently evolved hydraulic transport mechanism, underscoring the adaptive significance of specialized water transport structures in an early land plant lineage.

Comparative genomics-driven insights into *Plasmodiophora brassicae* putative effector functions

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Keywords:
Plant-microbial interactions, plant development, genomics, effectors, Brassica.

Abstract:

Clubroot is a major soil-borne plant disease infecting a broad range of Brassica crops, leading to significant yield loss internationally. In Australia, clubroot disease is a serious problem in Brassica vegetable crops [1]. With the increasing acreage of canola-growing regions, Australia potentially is on its way to face a serious clubroot problem similar to current outbreaks in Canada. Although the Canadian isolate of the clubroot causal agent, *Plasmodiophora brassicae*, was recently sequenced, comparative genomic studies remain limited due to the lack of complete genomes from a diverse geographic range [2].

We have recently addressed this gap by generating the first telomere-to-telomere complete genome assemblies for Australian field-sampled clubroot isolates. We have further sequenced a subset of 13 clubroot samples collected across a wide geographic range to build the high-confidence phylogenetic tree, placing the Australian isolates into an international context [3]. Using a large collection of diverse genomic assemblies, we performed small secreted protein (effectors) prediction and in-depth analysis of the core effectors set shared across highly divergent *P. brassicae* isolates. We further suggest how these effectors may manipulate the plant development and immunity-related signalling to cause disease symptoms. This work provides a foundation for uncovering the molecular

mechanisms underlying clubroot disease and supports future efforts to engineer durable resistance in Brassica crops.

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Harnessing Low-Energy Light: Core Genome Insights into Far-Red Light Photosynthesis in *Acaryochloris marina*

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Keywords:
far-red light photosynthesis, Chlorophyll *d*, energy adaptation, comparative genomics, cyanobacteria

Abstract:
Acaryochloris marina is a marine cyanobacterium that redefines the energetic limits of oxygenic photosynthesis. Unlike other phototrophs that rely on chlorophyll *a* (Chl *a*), *A. marina* uses chlorophyll *d* (Chl *d*) as its primary pigment, extending photosynthetic activity into the far-red region of the spectrum (Miyashita, 1996). This adaptation allows it to thrive in low-light, far-red–enriched niches where photon energy is insufficient to drive canonical oxygenic photosynthesis.

To investigate the genomic basis of this low-energy adaptation, a comparative multi-omics approach was applied across five *A. marina* strains. A pangenome of 13,242 orthologous groups was defined, of which 3,618 were shared across all strains. This core set was refined to 1,115 *Acaryochloris*-specific, constitutively expressed genes by excluding conserved *Chl a*-cyanobacterial orthologs and conditionally expressed genes.

Functional enrichment identified a suite of energy-related adaptations, including expansions in light-harvesting and electron transport proteins. In particular, multiple homologs of *pcb* genes were identified within the core genome, encoding Chl *d*–binding antenna complexes that optimise energy capture from far-red photons (Ulrich et al., 2024). These antennae, combined with unique redox tuning in the photosystems, illustrate how *A. marina* maintains efficient electron transfer with reduced photon energy input (Shen et al., 2024).

Together, these findings provide new insight into the molecular strategies that enable efficient energy capture and utilisation under low-energy far-red light, highlighting *A. marina* as a model for the energetic limits of oxygenic photosynthesis.

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A noncanonical kinase complex mediates pathogen effector recognition and modular immune signaling in wheat

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Keywords:
Disease resistance, Wheat, Host-pathogen interactions, Effector recognition

Abstract:

The lack of knowledge of resistance mechanisms and the host and pathogen *R* and *Avr* genes that modulate pathogen ingress has often led to “blind” selection of disease resistance, and due to fast adaptation of pathogens in one-on-one relationships, rapid breakdown of resistance. Leaf rust, caused by *Puccinia triticina* (*Pt*), remains a major threat to global wheat production. To date, over 80 loci conferring leaf rust resistance (*Lr* genes) have been identified, yet few of these and no corresponding *Avr*s from *Pt* have been fully characterized. This study identified a race-specific all-stage wheat *R* gene *Lr41*, which is among the first known alien introgression from *Aegilops tauschii* (*Aet*) on chromosome 2DS. *Lr41* encodes a tandem kinase confirmed with comparative genomics and transcriptomics, mutagenesis and functional validation. We further showed that *Pt* virulence on *Lr41* was due to variations in a specific genomics region affecting *AvrLr41*. Experimentally confirmed with native- and hetero-expression, this study provided a novel model illustrating direct and unconventional R-Avr interaction in wheat leaf rust resistance. We anticipate the current findings would help understand how wheat

recognizes important pathogens to activate its immune response. We demonstrated that AvrLr41 directly interacts with Lr41, and a potential unconventional tripartite immune mechanism involving the tandem kinase Lr41, AvrLr41 and truncated helper NLR. These findings expand the known functional configurations of resistance modules and underscore the adaptive versatility of immune signaling in wheat.

The importance of the alternative oxidase family in growth and reproduction of *Lotus japonicus*

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Keywords:
Alternative oxidase, Alternative respiration, Growth, Reproduction

Abstract:

Alternative oxidases (AOX) are a family of alternative respiratory proteins found ubiquitously in plants. They act as an alternative terminal oxidase in the mitochondrial electron transport chain (mETC), bypassing complexes III and IV of the classical pathway. As the alternative pathway bypasses proton-pumping steps of the mETC, it is a non-energy conserving pathway[1]. AOX1a is the most studied AOX isoform due to its high transcriptional upregulation in the presence of stress, both biotic and abiotic, where it acts to prevent the overproduction of reactive oxygen species. In all species studied to date, barring legumes, under non-stressed conditions no AOX is detected in mature plants. However, legumes present a unique expression model of AOX, with constitutively high transcript abundance of the *AOX2a* isoform in photosynthetic tissue, but limited expression in roots, while *AOX2d* in highly expressed in roots[2]. This is counter-intuitive as it is energy-wasteful and why it occurs is not yet understood. When all *AOX* isoforms were targeted via antisense knockdown of a consensus sequence in soybean, plant growth and reproduction were severely impaired[3]. It is unknown whether targeting individual isoforms would give the same result. This project uses the naturally occurring long terminal repeat transposon, *Lotus retrotransposon 1* (*LORE1*) in the model legume *Lotus japonicus* to investigate the role of individual AOX isoforms. Knockouts mutants of both *Aox1a* and *Aox2a* (separately) have been developed and grown under non-stressed conditions. Surprisingly, both mutant lines have impaired growth compared to control lines.

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Cannabinoid Modulation in Low-THC Hemp Under Heat and Drought Stress

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Keywords:
hemp; cannabinoid biosynthesis; heat stress; drought stress; genotype-specific responses

Abstract:

Hemp (*Cannabis sativa* L.), comprising low-THC cultivars widely grown for fibre, seed, and emerging commercial uses, also produces a diverse array of cannabinoids that environmental stresses can influence. A key concern is that drought or heat stress may elevate Δ⁹-tetrahydrocannabinol (THC) above regulatory thresholds, jeopardising crop compliance [1, 2]. This study examined the physiological and metabolic responses of two hemp lines (W27, W35; <0.04% THC) to controlled drought and heat stress during flowering. Rooted female clones were grown under controlled conditions and subjected to either moderate drought (reduced irrigation) or heat stress (short: 3 days and long: 2 weeks at 40/30 °C Day/night). Plant height, shoot and root dry mass, stomatal conductance, chlorophyll content, photosynthetic rate, and cannabinoid content were measured. Results showed that drought did not significantly affect plant height and root biomass but slightly reduced shoot biomass. It also suppressed stomatal conductance and chlorophyll content, with stronger effects in W27. Total cannabinoid levels declined under drought, particularly THCA, whereas THC remained comparatively stable. Heat stress caused more pronounced, genotype-specific effects. Long-term exposure significantly reduced shoot and root biomass in W27 but had minimal impact on W35, while short-term heat strongly inhibited photosynthesis in both lines. Cannabinoid metabolism was highly responsive to temperature, with long-term heat stress reducing acidic cannabinoids (THCA, CBDA, CBCA) and increasing neutral forms (THC, CBD, CBC). However, THC concentrations remained below 0.04% under both stresses. Overall, these findings demonstrate that drought and heat strongly influence hemp physiology and cannabinoid profiles, but outcomes depend on genotype, stress type, and duration.

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The relationship between geographic location and performance of the C_4 enzyme phosphoenolpyruvate carboxylase across *Setaria species*

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Keywords: C_4 photosynthesis, *Setaria viridis*, food security, phosphoenolpyruvate carboxylase, A-Ci curves.

Abstract:

Climate change, food insecurity, and limited arable land are major global challenges that demand strategies to enhance crop productivity and resilience. C_4 species offer a promising avenue for improvement due their tolerance to extreme environments, due to their C_4 carbon concentrating mechanism (CCM). PEPC is the first carboxylase within the CCM and catalyses the reaction which converts HCO_3^- and PEP into oxaloacetate. PEPC facilitates a chain of events which ensures a higher concentration of CO_2 around Rubisco than is found in C_3 species.

While previous studies on six natural accessions of *Setaria viridis* have demonstrated that geographic origin influences phenotypic and photosynthetic traits[1], the effect of geographic variation on C_4 PEPC kinetics across *Setaria* species remains unexplored. This study examined five *Setaria* species (*S. viridis*, *S. pallida*, *S. macrochaeta*, *S. kagerensis*, and *S. parviflora*) grown under standardized conditions. Leaf gas exchange (A-Ci curves) and PEPC enzyme assays were used to assess interspecific variation in photosynthetic performance.

Results revealed substantial variation in A-Ci responses among species, with *S. pallida* exhibiting the highest initial slope and *S. kagerensis* the lowest. PEPC activity correlated with the initial slope for *S. pallida* and was significantly higher than all other species, including the model *S. viridis*, while *S. kagerensis* displayed PEPC activity comparable to *S. viridis*. These findings highlight natural variation in C4 photosynthetic traits that may be exploited to improve crop performance. In particular, *S. pallida* and *S. parviflora* represent promising candidates for genetic mining or breeding strategies aimed at enhancing C4 efficiency in model and crop species.

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Understanding the drought responses of *Cicer arietinum* (chickpea) through physiological, transcriptional, and molecular characterisations

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Keywords:
Chickpea, drought stress, transcriptomics, phenotyping, membrane transport

Abstract:

Across Australia's primary Northern *Cicer arietinum* (chickpea) production region, moderate and severe reproductive-stage drought events drive substantial chickpea yield losses [1]. Present laboratory or glasshouse-based "drought" treatments are atypical of agriculturally-relevant drought events [2]. These incongruous stress conditions induce distinct physiological and transcriptional plant responses [3], hindering effective lab-to-field translational outcomes in chickpea. To overcome this translational barrier, a novel glasshouse-based terminal drought method was developed and validated across a drought-sensitive (*sen*) and drought-resistant (*res*) chickpea genotype. Under the imposed drought regime, soil water availability trends successfully mimicked the dominant water stress patterns characteristic of Australian chickpea cropping regions. With declining soil water availability, *sen* and *res* plants exhibited stomatal closure-induced reductions in photosynthetic carbon assimilation alongside alterations in leaf biochemistry and genotype-specific shifts in root:shoot resource allocation. Following the assembly of drought-responsive *sen* and *res* transcriptomes, gene ontology enrichment analysis highlighted both tissue- and genotype-specific trends in oxidative stress responses, abscisic acid signalling, and post-translational protein modification. Across both genotypes, significant drought-induced transcriptional regulation of the aquaporin membrane channel family occurred in leaf and root tissues. The functional screening of differentially-expressed chickpea aquaporins revealed previously unexplored roles in drought-responsive water and hydrogen peroxide (H_2O_2) transport. Under an agriculturally-relevant drought treatment, this multi-faceted characterisation of a drought-sensitive and drought-resistant chickpea genotype provides valuable insight into mechanisms and potential sources of chickpea drought resistance.

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Characterization of mitochondrial LYRM proteins in *Arabidopsis thaliana*

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Keywords:
Mitochondria, LYRM proteins, oxidative phosphorylation, cytochrome *c* maturation.

Abstract:

Mitochondria are essential organelles that produce ATP through oxidative phosphorylation (OXPHOS), a process catalysed by five multi-subunit complexes (CI-CV). The biogenesis of OXPHOS complexes is a highly coordinated process involving dual genetic systems, protein translation, the import and assembly of individual subunits, and protein maturation via the insertion of essential cofactors such as iron-sulfur clusters (ISC) and heme groups [1]. A distinct subclass of assembly factors crucial for these processes is the Leu-Tyr-Arg motif-containing (LYRM) proteins. Following their import into mitochondria, LYRMs interact with acyl carrier proteins (ACPs) or other target proteins to facilitate critical processes such as OXPHOS complex assembly, ISC biogenesis, and mitochondrial translation [2]. In *Arabidopsis thaliana*, we show that B22 (LYRM3), through its interaction with a mitochondrial ACP (SDAP1), is required for CI assembly and activity, and that this function depends on the integrity of the LYR domain [3]. Complementation with mutated versions of B22 lacking a functional LYR domain failed to rescue the CI defect and associated growth phenotypes. In addition, we identified a possible role for LYRM12 in cytochrome *c* maturation (CCM). Loss of LYRM12 leads to growth defects accompanied by reduced levels of both *c*-type cytochromes and other CCM-related proteins. Overall, our findings support a model in which distinct LYRM–ACP pairs act as metabolic checkpoints, ensuring that the OXPHOS complexes, ISC machinery, and other pathways assemble only when substrates are available.

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High temperature leads to a loss of isohydricity in *Pinus radiata*

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Keywords:
Heat stress, isohydry, stem water potential, stomatal conductance, evaporative cooling

Abstract:
Pinus radiata has been characterised as strongly isohydric (Sharma et al., 2024), which means that it tends to close stomata to maintain leaf water potential relatively constant under changing environmental conditions. However, under high temperatures, where leaf cooling may be necessary to prevent overheating, the ability to maintain this isohydric behaviour remains unexplored. Here, we examined the impact of increasing temperature (and associated VPD_{needle}) on whole plant stomatal conductance (g_c), canopy transpiration (E_c), minimal conductance (g_{min}) and stem water potential (Ψ_{stem}) in *Pinus radiata*. g_c declined as temperature increased from 22ºC to 32ºC, resulting a relatively constant Ψ_{stem}. However, beyond this temperature, g_c remained constant, which led to an exponential rise in E_c and pronounced declines in Ψ_{stem}. g_{min} contributed 18% of g_c at 42ºC and showed an exponential rise at temperatures higher than 42ºC. Our findings suggests that temperatures higher than 32ºC may shift stomatal behaviour from isohydry to anisohydry in *Pinus radiata*. This shift may be crucial to avoid overheating and damage of plant tissues but at the same time leads to more negative Ψ_{stem}, thereby increasing the chances of xylem embolism. The observed shift in stomatal response reveals that even in water conservative species like *Pinus radiata*, high temperatures may compromise stomatal regulation of water potential.

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Decrypting the cell-specific signalling pathways and acclimation responses driving stress-resilient C_4 photosynthesis.

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Keywords:
Abiotic stress, single cell transcriptomics, spatial metabolomics, cell-specific gene expression, retrograde signalling

Abstract:

In response to perturbed photosynthesis by environmental stresses, chloroplasts accumulate reactive oxygen species (ROS) to activate signalling pathways that alter nuclear gene expression towards acclimation. However, our understanding of these processes is derived exclusively from C_3 plants, leaving open questions about how specialised C_4 chloroplasts coordinate stress responses across distinct cell types.

Using the C_4 model *Setaria viridis*, we integrated live-cell imaging, genetic manipulation, and 'omics technologies, including single-cell transcriptomics (scRNA-seq), tissue-enriched metabolomics, and spatial metabolomics to dissect cell-specific responses to heat and high light stress. We uncovered temporal and spatial patterns of ROS accumulation across the entirety of a stressed *Setaria* leaf. Further scRNA-seq analyses of isolated leaf cells under control and stress conditions revealed the precise cell types driving the spatial separation of acclimation responses. For instance: (1) epidermal pavement cells specifically upregulated flavonoid metabolism and accumulated UV-protective compounds; and (2) mesophyll, bundle sheath, and vascular cells exhibited cell-specific expression patterns of key ROS homeostasis genes, providing a mechanistic explanation to the ROS accumulation patterns observed in these cell types. Excitingly, cell-specific manipulation of these genes showed improved short-term stress resilience.

Together, these results provide preliminary evidence that specialised C_4 cells and chloroplasts contribute significantly to cell-specific stress signalling communication. This has implications in crop bioengineering approaches, such as transferring C_4 photosynthetic machinery into C_3 crops, which currently overlook incorporating stress signalling networks. Understanding how chloroplast- and cell type-

specialisation intersects with stress signalling responses under C_4 photosynthesis may provide novel strategies for improving crop productivity under challenging environments.

Engineering crop resilience by creating stress protein mimics

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Keywords:
Resilient Crops, Osmotin**,** Drought, Protein Modelling

Abstract:

Domestication has narrowed the genetic diversity of crops, compromising their ability to withstand stressful environments. While wild plants have evolved robust survival strategies, cultivated crops are bred for rapid growth and high yield, often lacking these essential traits. Previously, it was believed that plant stress tolerance is governed by transcriptional regulation of stress-response pathways and physiological adaptations. Here, we demonstrate a more nuanced, alternative strategy employed by resurrection plants, a group of plants capable of surviving desiccation of their vegetative tissue. By analysing computational models of stress proteins from the Australian resurrection plant *Tripogon loliiformis* and contrasting sensitive (IR64) and resilient (APO I) rice genotypes, we discovered that subtle sequence variations in the amino acid sequence of stress tolerance proteins significantly enhance their function. Our models predicted that the osmotin protein from the sensitive IR64 genotype exhibits reduced ligand binding capacity compared to the tolerant APO I genotype. To validate these findings, we conducted glasshouse trials with transgenic rice plants. Our results demonstrated that rice expressing the *Tripogon* and APO I osmotin protein sequences were more stress tolerant compared to IR64 and non-transgenic controls. Further analysis identified three key amino acid differences in the APO I sequence as well as a modified leader sequence. To understand the specific role of these amino acid changes we have created transgenic *Arabidopsis thaliana* plants expressing site-specific mutations in the IR64 osmotin sequence to mimic the APO I sequence. Our research offers a promising avenue for developing more resilient crops by editing the genomes of cultivated crops to mimic the genetic signatures of resilient wild relatives.

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Elucidating the role of BELL homeodomain transcription factors in *Arabidopsis thaliana* leaf development.

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Keywords:
BELL, TALE Homeodomain Transcription Factor, Leaf Shape, Margin, Serration.

Abstract:

Leaves are three-dimensional organs that arise from the shoot apical meristem to facilitate photosynthesis. In most cases, leaves have distinct adaxial and abaxial surfaces that are specialised for light capture and gas exchange. Despite their common function, leaf shape can range from simple, with an undivided lamina, to compound, with leaflets emerging from a common rachis. Leaf margins may be further decorated by iterative outgrowths, termed serrations, that increase their complexity. The morphological diversity of leaves is underpinned by changes to the molecular networks that regulate organ development. By studying these networks within species, we stand to gain insight into the genetics of organogenesis.

In *Arabidopsis thaliana*, serrations arise due to differential growth along the edge of the leaf whereby some regions of the edge grow more than adjacent regions. Altering the timing and distribution of the regions of differential growth results in variations to leaf margin elaboration. The *BELL* family homeobox transcription factor genes, *SAWTOOTH1* (*SAW1*) *and SAWTOOTH2* (*SAW2*), have a role in repressing serration outgrowths[1]. We demonstrate five additional *BELL* family genes are expressed in the developing leaves of *Arabidopsis thaliana*. Mutagenesis experiments indicate that these genes function along with *SAW1 and SAW2* to regulate leaf morphogenesis. Genetic interactions demonstrate that *BELL* homeodomain transcription factors have both non-overlapping and redundant functions in suppressing serration development.

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Variation in rapid transcriptomic cold stress responses of wheat varieties

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Keywords:
Acclimation, frost, stress response, transcriptomics, Australia

Abstract:

Frost is one of the main abiotic stressors impacting crops. Plants have a suite of molecular responses to cold stress that both help to limit immediate damage and stimulate acclimation to future cold stress[1]. However, the flowering tissue of many crops including bread wheat is vulnerable to frost events, particularly around anthesis. In contrast, during the vegetative phase wheat plants are generally considered to be tolerant to cold and frost stress. Understanding cold stress responses at the early vegetative phase could help identify weaknesses or potential breeding targets when compared to the flowering tissue[2]. Using a combination of short read RNA-seq data and long read Iso-seq data to quantify early gene expression responses to a cold shock, similar to dusk conditions prior to a frost event, we explored variation across a diverse set of 16 spring wheat varieties grown in Australia. Across these varieties we find a conserved set of responses that include gene families that downregulate growth, upregulate antioxidant and unfolded protein responses, and support acclimation through cryoprotectant pathways. However, the magnitude of these responses varied across varieties and was strongly positively correlated across nine key gene families (person's $r > 0.8$ for pairwise comparisons). Additionally, the interaction between two main cold response genes (COLD1 and VRN1) could explain between 37% to 54% of the coordinated variation in gene family responses. Understanding a small set of genes that can be master regulators of hundreds of stress response genes is critical to breeding for higher crop abiotic stress tolerance.

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Shortening juvenility in citrus: gene discovery and CRISPR-based genome editing to accelerate breeding

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Keywords:
Citrus, QTL, CRISPR-Cas9, TFL1, epicotyl transformation

Abstract

Citrus species have a prolonged juvenile period, usually lasting between 7 to 15 years, during which flowering is suppressed. This long juvenile period hinders progress in breeding programs. While *FT*-like genes promote early flowering or precocity, *TFL*-like genes suppress flowering and contribute to this extended juvenility. In this study, we explored two approaches that may benefit citrus breeding programs. First, we sought to identify genes contributing to precocity by conducting an individual segregant analysis (ISA). Our newly developed per-sample QTL (psQTL) tool was able to identify QTLs that are putatively involved in precociousness in segregating progenies of a complex early-flowering citrus accession hybridised with *Citrus glauca*. Amongst several candidate genes identified in these QTL regions, we find *FT3* gene, which has previously been reported to have a role in promoting precocity. Second, we conducted CRISPR-Cas9-mediated genome editing of the flower suppressing gene *TFL1* in *C. sinensis* to reduce the extended juvenile period. We generated transgenic *C. sinensis* plants using an optimized epicotyl transformation tissue culture protocol. The editing of *TFL1* was confirmed by Sanger Sequencing. Overall, the results of this study can accelerate citrus breeding by identifying candidate genes for early flowering and creating citrus lines with a reduced juvenile period.

Determining the genetic basis of key agronomic and seed traits in Cannabis sativa L. through Quantitative Trait Loci (QTL) Mapping

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Keywords:
Cannabis, Hemp, QTL mapping, Genetics, Breeding

Abstract:

Cannabis sativa L. has been cultivated over millennia for nutritional, industrial, and medicinal purposes. However, due to a near global moratorium on cultivation and research throughout most of the 20th century, *C. sativa* crop improvement using modern breeding approaches is lagging and genetic loci contributing to key agronomic traits remain largely unknown. Increasing market demand in functional food and carbon-negative construction sectors, coupled with easing regulation in the 21st century, has renewed interest in *C. sativa* as an industrial crop.
In this study, a feminized segregating F_2 mapping population, derived from a low-yielding, tall parent with large seeds and a high-yielding, short stature parent with small seeds, was phenotyped for key agronomic and seed traits, including plant height, stem and pith diameter, hundred seed weight, and seed size. A mid-density Single Nucleotide Polymorphism (SNP) genotyping panel was used to generate a genetic linkage map. Quantitative Trait Locus (QTL) mapping identified several major QTLs for plant height, branching architecture and seed size. Our results provide novel target regions and associated molecular markers and candidate genes for future breeding efforts to improve the performance of industrial *C. sativa*.

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Genome wide association studies of circadian-linked traits in Australian wheat

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Keywords:
Triticum aestivum, circadian clock, GWAS, leaf senescence, grain protein content.

Abstract:

The circadian clock is a biological time-keeping mechanism that coordinates key physiological processes in plants. While extensively studied in *Arabidopsis thaliana*, its roles in agriculturally important crops such as bread wheat (*Triticum aestivum*) remain underexplored. Our lab's previous study found that Australian wheat cultivars display extensive variation in circadian rhythms, and that this variation correlates with key agronomic traits including the timing of senescence and grain protein content (GPC) (1). To investigate the genetic basis of these relationships, we are conducting genome wide association studies (GWAS) using a subset of 244 cultivars from the OzWheat panel grown under controlled glasshouse conditions. Multiple traits such as heading date, flowering date, nitrogen uptake and timing of senescence has been measured (2). Ongoing work includes measuring yield components such as thousand grain weight and GPC. Combined with SNP genotyping data, these phenotypic traits will enable GWAS to identify novel genomic loci associated with senescence and nitrogen use efficiency in Australian wheat. This research aims to uncover genetic variation underlying circadian-regulated traits, providing new insights into the molecular basis of senescence and potential strategies for crop improvement.

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The Role of Inversions and Haplotype Blocks in a Young Self-Fertilising Plant Species

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Keywords:
Plant speciation, chromosomal rearrangements, mating systems

Abstract:

Chromosomal inversions and other recombination-suppressing mechanisms can generate large haplotype blocks, reducing local recombination between haplotypes. These regions restrict gene flow and promote the accumulation of novel mutations, fostering genetic isolation within closely related taxa. In diploid plants, inversions are increasingly recognised for their role in divergence and speciation (Huang and Rieseberg, 2020). Studies in outcrossing species such as sunflower and monkeyflower show that inversions can capture locally adapted alleles that encode temporal or ecological isolation between haplotypes, thereby limiting gene flow (Lowry and Willis, 2010; Todesco et al., 2020). However, the role of inversions and haplotype blocks remains underexplored in self-fertilising species, where reduced effective recombination is already a feature of the mating system.

In this seminar, I present recent findings on chromosomal inversions and haplotype blocks in *Cicer echinospermum* P.H. Davis, a diploid (2n = 2x = 16), self-pollinating legume closely related to chickpea (*C. arietinum* L.). *C. echinospermum* is a relatively young species, diverging from *C. arietinum* approximately 95–127 KYA (von Wettberg et al., 2018), yet the two species have persisted in sympatry since. Variation in interspecific hybrid fertility, alongside irregular meiotic pairing, suggests karyotypic diversity within *C. echinospermum*. I present cytogenetic and population-scale bioinformatic evidence confirming inversions and large haplotype blocks in *C. echinospermum* and explore their potential impact on gene flow. These findings highlight the importance of inversions in shaping gene flow in a self-fertilising species, with implications for species coherence and long-term viability *in situ*.

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Local Auxin Homeostasis Determines Lateral Root Angle in Arabidopsis

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Keywords:
Root System Architecture, Lateral Root Angle, Auxin, Auxin Homeostasis

Abstract:

A primary focus of our research is to understand the key determinants of root system architecture (RSA) with the view of manipulating crop root systems to match the availability of water and nutrients. The angle at which lateral roots exit the primary root is a major determinant of RSA and this is controlled by metabolite hormones (e.g., auxin and cytokinin) and peptide hormones (e.g., C-TERMINALLY ENCODED peptides; CEPs)[1,2]. From an RNA-seq analysis of roots from a CEP receptor mutant with significantly steepened RSA[3,4], we identified differentially-expressed genes from a multigene family that controlled auxin homeostasis and hypothesised that these genes were downstream of CEP-CEP receptor signalling. Genetic and phenotypic analysis of RSA however, showed that these genes were independent of CEP-mediated control of RSA. Further investigations using grafting, in addition to whole-plant and tissue-specific CRISPR knockout, showed that several members of this gene family acted specifically in lateral roots to control RSA. Finally, confocal microscopy with promoter reporters showed that members of this gene family express in tissues where the bend in lateral roots is determined. Our results show that local auxin homeostasis controls the extent of lateral bending which shapes overall RSA in *Arabidopsis*. Since our prior research has shown that the control of RSA in Arabidopsis is directly applicable to crops[5], we propose that the manipulation of these two RSA controlling systems may provide a fruitful avenue to manipulate RSA in crops.

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From green bean to brewed coffee: A Lipidomic perspective on coffee lipid composition

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Keywords:
Green coffee beans, roasted coffee beans, brewed coffee, lipid composition, lipidomic, diterpene esters.

Abstract:
Coffee is a globally traded commodity valued not only for its economic importance but also for its flavour, aroma and health-related benefits. Lipids play an important role in shaping the coffee's organoleptic properties. The lipid profile of coffee differs across green beans, roasted beans and brewed coffee and is influenced by genetics, environment, processing, roasting and brewing. Although coffee lipids have been studied extensively, most compositional analyses remain focused on green coffee beans. As a result, the transformations that lipids undergo from harvest to the final cup are still not fully understood. Recent advances in lipidomics offer new opportunities to characterise the coffee lipidome more comprehensively. However, major lipids (e.g diterpene esters) are inconsistently detected. The study highlights the limitations of the current lipidomic methods and proposes strategies to enhance their application in tracking lipid composition from bean to cup.

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Exploring the carbon budget of chickpea accessions with contrasting carboxylate exudation under mild-phosphorus deficiency

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Keywords:
Carbon fixation, Root exudation, Respiration, Chickpea

Abstract:

Photosynthetically fixed carbon (C) used for biomass construction, respiration and root exudation, can be significantly affected by nutrient deficiency[1]. Root exudation in chickpea (*Cicer arietinum* L.) typically does not respond to phosphorus (P) limitation and may reflect the release of surplus carbon[2]. A multi-channel gas exchange system for whole-plant analysis was established to investigate the daily C budget of two chickpea accessions (ICC 456 and ICC 4814) with contrasting root carboxylate exudation and root size. We quantified C allocation to biomass, respiration, and exudation in chickpea plants at 28 and 36 days after emergence (DAY 28 and DAY 36) under mild P-deficiency.

The results showed shoot and root respiration rates at night in ICC 456, and root respiration during both day and night in ICC 4814, decreased significantly with plant development. Relative to seedlings at DAY 28, root carboxylate exudation rates from seedlings at DAY 36 increased in both ICC 456 and ICC 4814, while root amino acid exudation rates decreased significantly. Root sugar exudation rates were faster in ICC 4814 than in ICC 456. Both chickpea accessions allocated a smaller proportion of C to respiration and root amino acid exudation, while increasing the proportion allocated to root exudation of carboxylates and sugars in their daily C expenditure. Shoot exudation was very little in daily C investment. This study provides a whole-plant perspective of the C expenditure budget and reveals a flexible C allocation strategy during chickpea development, which is significant in maintaining C economy under nutrient deficiency.

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Bacterial Endophytes of Wild Australian Grapevines as a Novel Source of Biopesticides in Viticulture

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Keywords:
Microbiome, Wild Relatives, Biopesticides, Viticulture

Abstract:

Grapevine disease management is a major challenge for the Australian wine industry. Harmful chemical pesticides traditionally used in viticulture pose environmental and health risks and drive resistance in pathogens. Sustainable alternatives are urgently needed.

We are investigating bacterial endophytes, symbiotic microbes within plant tissues, as biopesticide candidates. These microbes suppress pathogens through direct inhibition, immune priming, and competitive exclusion, and their bioactive compounds may be formulated independently for viticultural use.

Our research focuses on the native grapevines *Apocissus antarctica* and *A. hypoglauca*, wild Vitaceae relatives adapted to fungal-rich rainforest environments. Preliminary sequencing reveals distinct endophytic communities with biopesticide potential, with early isolates showing promising antifungal traits. We are characterising these microbiomes through culture-independent and culture-dependent profiling using 16S rRNA amplicon sequencing and culture-based isolation from surface-sterilised *Apocissus* spp. tissue guided by amplicon data, with isolates identified via morphology and Sanger sequencing. High-performing strains undergo whole genome sequencing and metabolite profiling to evaluate their biocontrol potential.

Given their phylogenetic proximity to *Vitis vinifera*, bacterial endophytes may offer functional compatibility with domesticated grapevines. This project aims to uncover novel microbial candidates for sustainable disease management and reduce reliance on harmful, non-sustainable, chemical inputs in Australian vineyards.

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

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Keywords:
crown gall disease, grapevine, biological control

Abstract:

Crown gall disease of grapevines is a major problem affecting grape production in North America (especially California), Australia, Europe, and China, with global losses estimated at over US $2 billion annually. Approximately 90% of grapevine crown gall cases are caused by Allorhizobium vitis, while the remaining 10% are due to Agrobacterium spp. Current biological control agents, such as Rhizobium rhizogenes K84 and its derivative K1026, are ineffective against grape crown gall. A. vitis strain F2/5 was previously shown to control crown gall, but it also caused necrosis in grape tissues. Disruption of the gene F-avi5813 abolished necrosis while maintaining tumour inhibition (GTI+, Zheng & Burr, 2016, US patent 9,717,252 B2). However, market interest in this GMO has been low, and other mutations of F-avi5813 were reported to abolish both necrosis and GTI activity.

We used CRISPR/Cas9 genome editing of A. vitis F2/5 to generate a mutational series of F-avi5813. Mutants displayed varied necrosis and GTI phenotypes in carrot taproot disks, Nicotiana benthamiana leaves, and grapevine assays (Wu et al., 2024). One mutant, F-avi5813-5, retained strong GTI activity comparable to wild-type F2/5 against pathogenic strains K377 and K306, while inducing reduced necrosis. We renamed this strain GTI-5813 for field testing. Preliminary trials showed increased callus formation at grafting unions treated with GTI-5813 compared to F2/5, likely due to reduced tissue damage.

Further multi-site and multi-year trials are required to confirm efficacy and safety before GTI-5813 can be released as a new biological control agent for crown gall in viticulture.

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Rapid domestication - a new approach for accelerating exploitation of wild barley genomes to improve complex traits

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Keywords:
Breeding, genetic variation, re-domestication, yield, wild barley

Abstract:

Genetic variation underpins crop improvement but has been markedly reduced through domestication and breeding, particularly in spring barley, where multiple chromosomes show depleted diversity [1-2]. Wild barley represents a rich reservoir of alleles for enhancing complex traits such as yield and stress tolerance [3]. However, the identification and evaluation of elite × wild progenies are time-consuming and labor-intensive, constraining the use of wild germplasm. We developed a rapid screening approach to assess multiple wild barley accessions under breeding-relevant conditions by selecting key domestication loci in genotypes retaining substantial wild genome content [4]. Controlled and field evaluations identified accessions that enhanced yield and drought resilience. Genetic analyses revealed novel alleles associated with tiller number, yield—including a previously uncharacterized *Vrs1* variant—and phenology. This strategy provides an efficient framework to exploit wild barley diversity for the improvement of elite cultivars and demonstrates a scalable pathway for integrating genebank resources into modern breeding programs.

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VsA2 Variants Confer Seed Coat Colour for Traceable Low-Toxin Vetch Varieties.

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Keywords:
VsA2, seed coat colour, amino toxins, CRISPR-Cas9, supply, *Vicia sativa* (Vetch).

Abstract:

Common vetch (*Vicia sativa*) is a valuable annual legume used in crop rotations within arid Australian farming systems. Despite its agronomic benefits, the grain contains neurotoxic compounds such as β-cyanoalanine (BCA) and the dipeptide γ-glutamyl-β-cyanoalanine (GBCA), which are harmful to monogastric animals including pigs, poultry, and humans. These amino-toxins restrict vetch grain to low-return, yet ecologically important, on-farm roles such as weed suppression, water retention, nitrogen fixation, and soil carbon restoration.

Our research group is developing low-toxin vetch varieties using advanced genome editing and genomics, aiming to unlock higher-value markets for vetch grain. To support future commercialization, it is essential that seed supply chains can reliably identify and maintain the purity of zero-toxin vetch varieties. While biochemical and genomic assays can distinguish toxic from non-toxic lines, they are costly. To address this, we have developed a unique, inexpensive, and visually identifiable seed coat marker trait for use in non-toxic vetch varieties.

From our extensive vetch diversity collection, we identified four distinct seed coat (testa) colour lines: pale white, yellow, green, and tan. As a test case, we performed whole-genome Illumina sequencing on the green and pale white testa lines, aligned the reads to our reference genome, conducted variant calling, and pinpointed nucleotide polymorphisms in conserved genes regulating phenolic compound biosynthesis—key determinants of testa colour.

In the white testa line, we discovered a single amino acid substitution (G153W) in the conserved WD40 domain of the *VsA2* WDR transcription factor, which mediates protein–protein interactions. In the green testa line, we identified a 12-bp in-frame deletion within the same domain, predicted to yield a protein with two WD40 repeats—one fewer than the wild type. Genetic complementation experiments are

underway to confirm the role of *VsA2* in conferring the green and white testa phenotypes. In future applications, our CRISPR genome editing pipeline will enable targeted modification of *VsA2* to generate a reliable, visually distinct marker for supply chain traceability of non-toxic vetch varieties.

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Metatranscriptomic and orthology analyses of tRNA-mRNA dicistronic genes in land plants

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Keywords:
Discistronic tRNA-mRNA, metatranscriptome analysis, evolutionary conservation

Abstract:

Most nuclear-encoded genes in plants are monocistronic, with each mRNA producing a single protein. Recently, however, dicistronic transfer RNA–messenger RNA (tRNA–mRNA) transcripts have emerged as a notable feature of plant transcriptomes. Studies have shown that protein-coding mRNAs can be transcribed together with adjacent tRNA genes, forming continuous transcription units [1]. Grafting experiments with transgenic dicistronic constructs further revealed that these transcripts can move systemically to distant tissues[1]. Although such transcripts are widespread across plant species, their physiological role and molecular function remain unclear. To gain insight into their function and evolutionary origin, we performed a metatranscriptomic survey of publicly available RNA-seq datasets from 15 species spanning bryophytes to angiosperms. We detected abundant tRNA–mRNA dicistronic transcripts across all species, involving 53 tRNA anticodons that cover all 20 amino acids, typically located within 1000 bp of their associated protein-coding gene (PCG). As no conserved function has been established, we conducted orthology analysis of predicted dicistronic PCGs. This revealed that many are part of conserved orthologous groups across land plants, with strong conservation of tRNA isodecoders and their corresponding amino acids. Together these results provide the first evolutionary and expression insight into tRNA-mRNA dicistronic genes in land plants.

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Uncovering the diversity of *Rhizoctonia solani* in Western Australia's grain growing regions: implications on pathogenicity levels in wheat

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Keywords:
Rhizoctonia solani, wheat, cereal crop disease, plant-pathogen interactions, fungal pathogen diversity

Abstract:

Wheat underpins global food security, yet soilborne pathogens, such as *Rhizoctonia solani*, continue to reduce yields and grain quality, with losses exceeding 20% reported in cereals (1). The fungus is divided into 13 anastomosis groups (AGs), making effective disease management challenging due to the pathogen's genetic diversity, host preferences, and variable pathogenicity, which is further compounded by a shortage of well-annotated and phenotyped isolates (2). This study aims to investigate the population diversity and spatial structure of *R. solani* in the cereal-associated AG-8 within Western Australia's grain belt and to quantify how this diversity translates to disease severity in wheat. Representative isolates are being collected across low, medium and high rainfall zones to capture environmental and management gradients. The isolates will undergo genome sequencing and genomic characterisation to define genetic clusters and infer spatial population structure. This data will be integrated with pathogenicity assays in a panel of locally relevant wheat cultivars under controlled conditions, using standardised root disease indices and growth metrics. Genotype–phenotype associations will be modelled alongside environmental covariates to identify variants and lineages linked to higher pathogenicity. The study's outcomes will include a WA diversity map of *R. solani*, evidence for spatial–environmental drivers of population structure, and prioritised isolate sets for mechanistic studies. By linking population genetics to disease outcomes in wheat, this work will inform targeted management, guide resistance screening, and ultimately support the development of durable wheat resilience to *R. solani* in Western Australian production systems.

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Identification and Functional Characterization of RCMT9, a Novel RNA m⁵C Methyltransferase in *Arabidopsis thaliana*

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Keywords:
RNA 5-methylcytosine (m⁵C), *Arabidopsis thaliana*, Epitranscriptomics, post-translational regulation

Abstract:
Epitranscriptomic modifications such as 5-methylcytosine (m⁵C) are essential for regulating RNA stability, processing, translation, and transport in animals and plants (1-3). In plants, m⁵C shows dynamic patterns and is important for root growth, vegetative transitions, heat stress tolerance, and mediates systemic RNA transport(2-7). Evolutionary conserved m^5C methyltransferases/writers include NSUN2, reader proteins include ALYREF, and in animals' demethylases include TET and ALKBH1 (8, 9).

In *A. thaliana*, NSUN2/TRM4B is an established methyltransferase/writer (10, 11), while other writers, like RCMT9, are less well-defined (9). Our work shows *rcmt9* mutants have ~50% reduced global m⁵C and develop enlarged leaves under blue-red light. Agroinfiltration of RCMT9-Cerulean fusions in *Nicotiana benthamiana* leaves showed weak fluorescence, however, after Bortezomib treatment fluoresce was significantly increased indicating proteasomal regulation of RCMT9. Together, our results establish RCMT9 as a key contributor to RNA m⁵C methylation in *Arabidopsis* and suggest its abundance is post-translationally regulated.

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From leaf to cell: the single-cell transcriptomic journey of barley

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Keywords:
Barley leaf, single cell transcriptomics

Abstract:

The plant biology research community currently lacks a comprehensive understanding of how individual plant cells respond to pathogen infection. One promising approach to profile these dynamics is single-cell technology, which is well established in mammalian pathology but still emerging in plant systems. A major obstacle is the need for plant-specific protocol optimisation, particularly due to challenges in the cell extraction process.

This project aims to apply single-cell technology to investigate transcriptional changes at the cellular level in barley leaves infected with the net blotch pathogen. Current efforts focus on optimising the cell extraction protocol, which will lead to the development of a computational analysis workflow.

By enabling transcriptional profiling at single-cell resolution, this approach will help uncover molecular mechanisms involved in pathogen resistance. It also offers the potential to identify rare cell populations and novel genes linked to disease resistance. Despite existing technical challenges, this innovative strategy promises to deliver deeper insights into barley–net blotch interactions at cellular resolution.

Improving chickpea productivity by enhancing resistance to soil acidity

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Keywords:
chickpea, acid tolerance, wild relatives, genome-wide association study, breeding

Abstract:
Chickpea (*Cicer arietinum* L.) is an important grain legume valued for its nutritional benefits and its role in sustainable farming systems through symbiotic nitrogen fixation, which reduces the need for nitrogen fertilisers in rotational crops (Merga & Haji, 2019; Wallace et al., 2016). Australia is the world's leading chickpea exporter, with production concentrated in the northern grain belts. However, efforts to expand cultivation into Western Australia and southern New South Wales are constrained by soil acidity, to which chickpea is highly sensitive (Vance et al., 2021). This challenge is further exacerbated by the crop's narrow genetic base, a consequence of domestication and breeding (Berger, 2006). By contrast, wild relatives provide a reservoir of untapped allelic diversity that can be harnessed to improve adaptation to acidic soils (Sharma et al., 2013). The paucity of acid-tolerant cultivated chickpea germplasm limits expansion into acidic soils; this study tests the potential of wild relatives to overcome this barrier.

In this study, 307 accessions of *C. reticulatum* and *C. echinospermum* from the Australian Grains GenBank (AGG) were screened for low pH tolerance under hydroponic conditions, using net root length as the phenotypic measure. All accessions were genotyped for genome-wide SNPs using the Diversity Arrays Technology (DArT) platform. Genome-wide association mapping identified putative candidate gene(s) linked to tolerance, which will be validated through *Agrobacterium rhizogenes*-mediated hairy root transformation in the commercial variety HatTrick. Subsequent mechanistic studies will elucidate the basis of tolerance, enabling the development of molecular markers for deployment in breeding. This approach will accelerate production of acid-tolerant chickpea cultivars, facilitating expansion into acidic regions and strengthening the sustainability of Australia's chickpea industry.

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Using two complementary spatial transcriptomics techniques to examine the rise of Neurachneoid-type C_4 Kranz leaf anatomy in the genus *Neurachne*

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Keywords:
C_4 evolution, *Neurachne*, spatial transcriptomics, Stereo-seq, laser microdissection

Abstract:

C_4 plants display improved photosynthetic efficiencies over C_3 species in hot, arid, and/or high salinity conditions. C_4 photosynthesis evolved from an ancestral C_3 state independently over 66 times in angiosperm lineages and is a remarkable example of convergent evolution. A C_4 syndrome is achieved through repurposing of C_3 leaf anatomy and biochemistry, enabling a spatial separation of the photosynthetic reactions, typically across two leaf cell types- photosynthetic carbon assimilation (PCA) in mesophyll cells and photosynthetic carbon reduction (PCR) in vascular sheath cells. The stepwise changes underlying C_4 evolution are informed by examining groups comprising closely related species using C_3, C_4, and/or C_3-C_4 intermediate phenotypes. Amongst the grasses, the Australian genus *Neurachne* is the only known group with closely related individual species demonstrating these different photosynthetic phenotypes.

All C_3 grasses possess two vascular sheaths; however, some C_4 grasses possess only one sheath layer. Interestingly, all *Neurachne* species possess two sheath layers. Immunolocalisation of select C_4-associated enzymes in C_4 *Neurachne* leaves suggest PCA takes place in both the mesophyll and outer sheath tissue, with PCR in the inner sheath. To further understand the functioning and evolution of a C_4 pathway in such a three-cell anatomy, this study will examine cell-specific gene expression patterns in a C_3, a C_3-C_4 intermediate, and two C_4 *Neurachne* species. To achieve this, two complementary spatial transcriptomics technologies will be used: laser microdissection, which physically isolates cells of interest, followed by RNA-seq, and the *in-situ* chip-based sequencing technology, Stereo-seq[5], that captures differential gene expression with subcellular resolution.

Nanotechnology enabled DNA free CRISPR gene editing in chickpea

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Keywords:
Chickpea, DNA free gene editing, nanoparticles, nodulation, ROS

Abstract:

Chickpea (*Cicer arietinum*) is the third most widely cultivated legume crop worldwide. Australia's chickpea industry is worth $1.33 billion, leading the export of high-quality seed worldwide [1]. Even though genetically modified chickpea expressing heterologous genes can be generated at low efficiency (<5% transformation efficiency) [2] there is an increasing interest in the deployment of gene-edited (GE), rather than genetically modified (GM), crops as the former mimics natural variation in genome sequences and thus can be released into the market as de-regulated crops [3] in several countries including Australia. In this project we have applied nanoparticles coating ribonucleo-proteins (RNP) as in vitro assembled CRISPR components to transform chickpea pollens which will be used for artificial pollination thus mitigating the need of plant regeneration by tissue culture and using RNP eliminates the insertion of transgene. Nanoparticle mediated genetic delivery in plant cells relies on nanoparticles ability to compact and encapsulate the genetic material and protecting the cargo from degradation while delivering it to the nucleus via endocytosis pathway [4]. The project focuses on the development of such methods by systematically testing two different nanoparticle (NP) categories such as positively charged and neutral nanoparticles that enables the bypass of cell wall barrier, protection of cargo and targeted tissue/cell/organelle delivery [5]. Furthermore, this method will be used to study the function of flavonoids in regulating reactive oxygen species (ROS) while nodulation in chickpea which will provide insights in controlling stress induced coping mechanisms and their impact on nodulation growth which will help understand nitrogen use efficiency.

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Genome-wide association mapping of stomatal traits in wheat diversity panel

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Keywords:
Stomata, Rapid phenotyping, Wheat diversity panel, Genome-wide association study (GWAS)

Abstract:

Stomata are tiny pores located on the surface of plants that have a role regulating carbon dioxide uptake for photosynthesis and water loss through transpiration. They are crucial for plant growth, water use efficiency and adaptation to environmental stress. However, in bread wheat there is still insufficient knowledge in how stomatal traits relate to productivity.

This study aims to investigate stomata variation and identify the genetic loci influencing these traits in wheat. A rapid and automated stomata phenotyping method[1] was used to analyse stomata density and size on both abaxial and adaxial leaf surfaces. The analysis of a diversity panel of 292 wheat accessions[2] selected from hot and dry climates shows large variation in stomata density ranging from 17 to 55 stomata/mm² and stomata, from 1365 to 2559 µm². Strong negative correlations between stomata density and size, with smaller stomata size typically associated with higher density. These observations were consistent across both abaxial and adaxial surfaces.

Genome-wide association studies (GWAS)[3,4] are being conducted to identify genetic loci associated with stomatal traits. Candidate gene analysis will evaluate the genes located within these loci to confirm their role. In parallel, stomatal genes reported in published studies will be determined, their homologs and homeologs in bread wheat identified and compared with GWAS results. The findings will provide new insights into the genetic basis of stomatal traits in wheat and have the potential to guide breeding strategies for improved drought tolerance, water-use efficiency,

and yield.

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Investigating the origins and evolution of stomatal opening pathways

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Keywords:
Stomata, Evolution, Phylogenomics, Light response, Signalling pathways

Abstract:

Stomatal responses to light are critical for enabling efficient photosynthesis and water use in plants (1). The genetic components underlying light-driven stomatal regulation are well-characterised in model angiosperms, particularly *Arabidopsis thaliana* (2). However, there are considerable gaps in knowledge for non-flowering plants. The origins, evolution and conservation of genetic components involved in stomatal regulation provides important insights into stomatal regulation in other plant lineages. This study aims to investigate the evolutionary history of light-responsive stomatal regulatory mechanisms in land plants by leveraging extensive genomic and transcriptomic datasets available, focussing on 350 species that span all major plant lineages, to predict the timing of the duplications from which they arose. Our findings reveal that while some components are conserved across land plants many exhibit complex evolutionary histories, with key components having recent origins. For example, BLUE LIGHT-DEPENDENT H^+-ATPASE PHOSPHORYLATION (AtBHP), HIGH LEAF TEMPERATURE 1 (AtHT1), PROTEIN PHOSPHATASE 1 (AtPP1), CONVERGENCE OF BLUE-LIGHT AND CO_2 (AtCBC) and PP1 REGULATORY SUBUNIT2-LIKE PROTEIN1 (AtPRSL1) and QUICKLY ACTIVATING ANION CHANNEL 1 (AtQUAC1) likely evolved in angiosperms, while BLUE LIGHT SIGNALING 1 (AtBLUS1) and B-CARBONIC ANHYDRASE (βCA) likely originated in seed plants. Ion channels such as SLOW ACTIVATING ANION CHANNEL 1 (AtSLAC1), Shaker K^+_{out} channels, and Shaker K^+_{in} channels have likely been lost in liverworts, mosses and lycophytes respectively. These evolutionary trajectories provide new insights into the origins and evolution of light-responsive stomatal regulation pathways and provide a foundation for candidate gene selection for further investigation in diverse land plant species.

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High Salt? No Stress – Improving Cultivar-Specific Transformation to Investigate Salinity Tolerance

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Keywords:
Salinity, compartmentalisation, editing, transformation, CRISPR

Abstract:

67% of Australian agricultural area is at risk of transient salinity[1], inhibiting plant growth and causing estimated agricultural losses of $519 million per annum[2]. 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[3], 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-D* 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 better investigate mechanisms underpinning increased sodium tolerance, elite cultivars have been transformed using a CRISPR-Cas9 construct to generate *TaHKT1;5-D* knockout mutants, mimicking the Mocho accumulation phenotype. These mutant lines will enable investigation into how sodium is sequestered within different plant tissues, and what mechanisms are present to protect developing floral tissues. Uncovering the molecular mechanisms underpinning ionic tolerance will be vital for climate-proofing bread wheat in an increasingly saline environment.

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Improving pod shatter resistance in canola through gene editing

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Keywords:
Canola – *B.rapa* - pod shatter – gene editing

Abstract:

Brassica napus (canola) is a globally important oilseed crop. However, its yield is often reduced by pod shattering, a natural seed dispersal mechanism common in Brassicaceae family such as *B. rapa* and *B. napus*. Previous studies have shown that this process is regulated by multiple genes.
Recently, a Quantitative Trait Loci (QTL) associated with pod shatter resistance was identified in *B.rapa*, a close relative of canola that shares high genetic similarity. Through the evaluation of these QTL regions, we identified an novel natural mutation in a classic pod shatter related gene, SHATTERPROOF (SHP), which may contribute to pod shatter resistance. Preliminary analysis shows that SHP encodes a transcription factor and the novel mutation has the potential to alter the shape of its protein dimers it forms with itself and other related transcription factors, thereby affecting protein function. We plan to further investigate the function of this mutation in both A. thaliana and canola to test its contribution to pod shatter.
This research will strengthen our understanding of the genetic mechanisms controlling canola pod development and shattering, and potentially provide breeders and growers with a unique mechanism to confer pod shatter resistance.

Exploiting Plant Lysine Biosynthesis: Insights into the Regulation and Inhibition of DHDPS

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Keywords:
Lysine biosynthesis, DHDPS, allostery, mechanism of regulation, herbicide discovery.

Abstract:
Strategies to increases crop production are urgently required in order to feed our growing population[1,2]. Herbicides are integral tools for maximising agricultural outputs; however, the rapid rise in herbicide resistant weeds and a stagnant development of new herbicides over the last four decades is resulting in significant crop loss to weed invasion[3,4]. As such, herbicides with novel modes of action are urgently needed to overcome herbicide resistance.

Lysine is a proteinogenic amino acid crucial for a myriad of biological functions in plants[5]. Thus, lysine biosynthesis represents an attractive herbicide target. In plants, lysine tightly regulates its own production via the allosteric inhibition of the first enzyme in the pathway, dihydrodipicolinate synthase (DHDPS). Despite lysine being a potent inhibitor of DHDPS, the mechanism of lysine-mediated inhibition is unknown. Furthermore, the potential of lysine as a herbicide compound has never been explored. In this study, we sought to understand the molecular determinants underpinning lysine-mediated DHDPS inhibition using mass photometry and small angle X-ray scattering. Using these in-solution techniques, we have observed large lysine-induced structural changes to DHDPS protomers, which we propose to be the

mechanism of regulation for this enzyme. We then exploited this mechanism for herbicide development and generated a series of lysine analogues that also disrupted DHDPS oligomers but permitted herbicidal activity against weed species. As such, this study provides novel insights 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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Deciphering the genetic regulation of inflorescence development in bread Wheat

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Keywords:
Wheat genetics, Wheat functional genomics, Plant physiology, Plant reproductive biology

Abstract:

The project aim is to investigate sugar regulatory genes and biological processes that influence inflorescence development and growth in wheat. To identify sugar regulatory genes that control inflorescence development, I will investigate early flowering (cv. Paragon NILs having photoperiod insensitive alleles of (*Ppd-D1a*)), normal flowering (photoperiod sensitive cv. Paragon, referred as (WT)) and late flowering wheat mutants (null *ppd-1*) from which I predict sugar regulatory genes (*MIPS*, *IMP* & *MIOX)* regulate the identity or determinacy of meristems. Following sugar level measurements and expression analysis of these genes, my next objective would be mutant analysis. I will develop identified gene's knock-out mutants to investigate the function of the encoded proteins (genes), and the influence they have on inflorescence development during spike developmental stages.

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A Rapid CRISPR/dCas9 Platform for Systemic, Targeted DNA Methylation Editing in *Nicotiana benthamiana*

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Keywords:
Epigenetics, DNA methylation, CRISPR, Nicotiana benthamiana, gene regulation

Abstract:

Targeted DNA methylation editing is a powerful approach to investigate and manipulate heritable gene regulation in plants. However, current epigenome engineering workflows rely on *Agrobacterium*-mediated transformation, callus regeneration, and plant propagation, process that typically requires 8–12 months to generate a single edited line [1]. Here we propose a novel system designed to dramatically reduce this timeline by using a *Nicotiana benthamiana* line that constitutively expresses a CRISPR/dCas9-methyltransferase complex. with gRNAs to be delivered transiently via a viral vector(s) [2]. The viral vectors in combination with gRNA mobility sequences spread gRNAs systemically, including to meristematic tissue, thereby activating targeted methylation across the plant. This approach eliminates the need for tissue culture, potentially reducing the time to produce modified, heritable epimutants to less than four months. As a proof of concept, we will target the phytoene desaturase (PDS) promoter to induce DNA methylation and transcriptional silencing, detectable by a characteristic leaf-bleaching phenotype. This rapid, modular system offers a flexible platform for functional studies of methylation epialleles and lays the groundwork for future crop improvement strategies based on epigenetic modification.

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Exploring grapevine genetics for loose bunch architecture- improving genetic resources for complex traits related to disease resilience

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Keywords:
Epigenetics, Genomics, Grapevine clonal selection, Bunch architecture

Abstract:
Grapevine (*Vitis vinifera*) has global cultural and viticultural significance and presents an interesting model for studying epi/genetics due to its long history of clonal propagation. As opposed to sexually reproducing species that undergo epigenetic recombination and reprogramming, grapevine has accumulated somatic mutations through centuries of clonal propagation[1,2]. It is commonly accepted that the wide variation observable in a population of a single variety is explained by both epigenetic and genetic variation however the relative importance of each is not well understood. Improving the understanding of the link between genetic, epigenetic and phenotypic variation is important for the development of clonal varieties. Grapevine selection has focused primarily on wine quality and the resulting clones are poorly equipped for the threats of climate change[3]. A major issue for modern viticulture is Botrytis bunch rot (BBR), a necrotrophic fungal pathogen for which no active defence mechanism or resistance genes have been characterised in grapevine. However, loose bunch architecture has been found to provide passive defence against BBR by reducing humidity in the cluster environment[4]. This project aims to investigate genetic and epigenetic alleles associated with facets of the complex trait of bunch architecture. While the molecular drivers of phenotypic variation among grapevine clones are not well characterised, early reports suggest that the small proportion of SNPs found in grapevine populations does not reflect dramatic change in phenotype observed in the field[5]. Considering the link that the accumulation of DNA methylation has to the domestication of this species[3], there is potential for epigenetic variation to be the source of some of these differences.

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Investigating the Genetic Regulation of Meristem Maintenance and the Floral Transition in *W. australiana*

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Keywords:
Duckweed, Flowering Transition, Meristem, Wolffia australiana

Abstract:

As the world population grows and humanity pursues long-duration Space missions, common challenges linked to limited resources and sustainability arise, demanding innovative solutions. Duckweeds (family Lemnaceae) are minute aquatic monocots with remarkable potential to address these issues due to their rapid growth, minimal space requirements, and nutrient-rich biomass. However, their reliance on clonal reproduction limits genetic manipulation and the genetic mechanisms underpinning meristem maintenance, and the floral transition remain poorly characterised, particularly in the local species *Wolffia australiana*. As part of the Centre of Excellence in Plants for Space, this project investigates key developmental processes in *W. australiana* critical for developing genetics resources to enable targeted improvement of plant traits.

Optimal flowering conditions for *W. australiana* were initially defined by testing different environmental stimuli, including salicylic acid, sucrose and cold treatment, to trigger flowering reliably. Scanning electron microscopy (SEM), light and confocal microscopy were then used to examine flower development, meristem structure and transition. A genome-wide bioinformatics analysis using Orthofinder identified orthologues of key genes involved in meristem maintenance and the floral transition. Candidate gene expression will be validated using droplet digital PCR (ddPCR).

This work will provide fundamental insights into duckweed development for targeted genetic improvement. Results from this project have the potential to deliver sustainable solutions in biotechnology and phytoremediation, addressing the challenges on Earth, while also facilitating life support systems for off-Earth habitation.

Exploring an undefined class of RNA-Binding Proteins as Potential Translational Protectors Under Salinity Stress

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Keywords:
RNA-binding proteins (RBPs), Halophytes, Salinity stress, Translation regulation, Evolutionary conservation

Abstract:
Salinity threatens global food security, affecting 30% of irrigated farmland globally. Glycophytic crops lose 50–80% of yield under moderate salinity (1), whereas halophytes thrive through sodium exclusion, compartmentalisation, or secretion. Remarkably, some halophytes tolerate much higher cytosolic Na^+ than glycophytes yet sustain growth (2).

Translation is highly sensitive to sodium$^+$, which disrupts ribosomal function and suppresses protein synthesis (3). RNA-binding proteins (RBPs) are emerging as key regulators of translation under stress. We identified a conserved, previously undefined class of RBPs across 498 species and 127 families of Viridiplantae, comprising 2,300 orthologs of yeast Stm1 and human SERBP1. These intrinsically disordered proteins are characterised by HABP4_PAI-RBP1 and Stm1_N domains and RGG-rich regions.

A multiple sequence alignment was generated using MAFFT (E-INS-I), and maximum likelihood inference (JTT+R10) was applied to construct a phylogeny. This revealed a fivefold enrichment of this RBP class in halophytes (5% vs. 1% across Viridiplantae). Notably, 38% of halophyte species carried common amino acid variants at two positions otherwise conserved in 90% of orthologs. These species belonged to Brassicaceae, Caryophyllaceae, Arecaceae, and Ostreobiaceae. The variant residues flank the sole structured region of the protein and are known in other eukaryotes to interact with ribosomes (4).

We propose that these substitutions enhance translational resilience under salinity stress. Ongoing work will test the role of this class of RBPs in mRNA stability and ribosome integrity, providing mechanistic insights into how some plants safeguard translation and offering new strategies to engineer salt-resilient crops.

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Exploring the Phenotypic Diversity in Phenology and Drought-Tolerance Traits of *Brassica carinata* as an opportunistic crop for biofuels industry

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Keywords:
Biofuels, oilseeds, phenotypic diversity

Abstract:
Brassica carinata A. Braun, commonly known as “Ethiopian mustard,” is being developed as a low carbon intensity, non-food oilseed feedstock to produce advanced drop-in renewable fuels, protein-rich meal, and bio-products. It is also used as bio-fumigant, to suppress soil-borne pests and pathogens in crop rotations and is reputed to perform well compared to related Brassica species in environments with stress factors such as drought, hot temperatures, and hard frost events. The global biojet market currently has up to 30 billion gallons of unsupplied demand, expected to double by 2040. These market dynamics highlight Carinata’s significant potential in the Australian cropping system. Assessing the main characteristics of *B. carinata* for cropping (phenology ranges, biomass, adaptation) (early vigour, transpiration efficiency, yield) for breeding purposes could contribute significantly to South Australian farming systems. In this work, we assessed the phenotypic variability of 100 genotypes of *B. carinata* available in the Australian Grains Genebank in terms of agronomic adaptivity to Australian farming systems and key traits (phenology, early vigour) for breeding conferring drought tolerance. The experiment was planted on 5 June 2025 at the Waite Campus in an α -lattice design, with subblocks and two replicates (as per Barreto et al., 1996). Data are currently being collected and will be presented in a poster during the conference.

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A Dual-Target Herbicidal Compound with a Novel Mode of Action

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Keywords:
Herbicide discovery, lysine, amino acid biosynthesis, plant enzymes, weeds

Abstract:

Herbicide resistance is a major threat to our natural environment and agricultural industry. The emergence of resistant weeds has been driven by repeated application of the same herbicides, combined with a lack of new herbicides entering the market in the past 30 years.[1] We recently developed the first herbicidal inhibitors of lysine biosynthesis, which represents a novel herbicide mode of action. We showed that the most potent of these inhibitors, MBDTA-2, targets the first enzyme in the pathway, DHDPS.[2] However, the similarity in *in vitro* and *in vivo* potency of MBDTA-2 is unusual given that the full amount of compound applied to a plant is not expected to reach the target enzyme. In the present study we investigated this phenomenon and demonstrated that it cannot be attributed to the inhibitor being modified to a more potent form *in vivo*. We discovered using enzyme kinetic assays that it can instead be explained by MBDTA-2 having a novel, dual-target mode of action through the inhibition of a second lysine biosynthesis enzyme, DHDPR. Enzyme kinetic assays and *in silico* docking revealed that MBDTA-2 exhibits differential allosteric and orthosteric inhibition of DHDPS and DHDPR, respectively. We also demonstrated for the first time that this inhibitor has herbicidal efficacy against the most economically significant weed in Australia.[3] This work has the potential to contribute to the development of dual-target herbicides with a novel mode of action, which may have a reduced propensity to generate resistance in weeds.

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Create Plant Regulatory Networks with AI Agents: A Showcase Using the PSoup Package for Network Translation

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Keywords:
AI, Agents, regulatory networks

Abstract:

The growing complexity of biological systems and the explosion of multi-omics data demand intelligent, transparent, and human-centered automation in scientific discovery. Agentic AI frameworks offer this capability by enabling autonomous reasoning, collaborative workflows, and continuous learning across research domains.

Plant trait regulatory network agents are modular systems of intelligent agents that automates the creation, validation, and scaling of regulatory networks underlying complex plant traits. Built using packages like LangGraph, it provides full traceability of each reasoning step, revealing the agent's thought process and keeping humans in the loop. This transparency transforms automation into an interpretable and auditable reasoning process.

The PSoup Agent integrates literature mining, network construction, perturbation exploration, and simulation matching within a self-improving loop. Using the PSoup package as a showcase, we show how algebraic rules translate a regulatory network, built from literature mining by AI agents, into a mathematical model of shoot branching. The agents then search the literature for perturbation data on specific nodes to test and validate the networks. Tasks that once required several months of manual work can now be completed in few minutes, accelerating model development and hypothesis testing. Beyond PSoup, the same agentic architecture can generate other network types, including gene regulatory networks (GRNs), protein–protein interactions (PPIs), and metabolic networks.

Designed for scalability and adaptability, the network agents demonstrate how AI agents can dramatically speed up network creation, enhance reproducibility, and open new pathways toward Breeding 4.0 and data-driven digital agriculture.

Towards Chemical-Free Weed Control: The Potential of Laser Weeding in Baby Leaf Crops

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Keywords:
Rocket, Seedbank, Shepherd's purse, Spinach, Stinging nettle

Abstract:
Baby leaf crops form an important component of human diets globally, including in Australia, owing to their high nutritional value. Despite their popularity, the presence of toxic weeds in harvested produce can pose serious health risks to consumers, while contamination from nuisance weed species often leads to product rejection by supermarkets, causing significant losses to growers and processors. Unfortunately, there are very few registered chemical herbicides for weed control in baby leaf crops[1]. These issues highlight the urgent need for reliable and sustainable weed management strategies.

This study investigates laser weeding as an innovative, non-chemical weed control technology under commercial field conditions[2]. Field trials are underway in Victoria to compare the effectiveness of laser weed control with conventional chemical and manual weed control in spinach (*Spinacia oleracea* L.) and wild rocket crops (*Diplotaxis tenuifolia* (L.) DC). Stinging nettle (*Urtica dioica* L.) and Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) are the most problematic weeds across trial site. Data on weed control efficacy (%), crop injury, weed contamination at harvest, changes in the soil weed seedbank and crop yield is being collected.

Preliminary results suggest that laser weeding can effectively control weeds, particularly at the early seedling growth stage, with performance strongly dependent on weed species. Minimal crop injury was observed, and the technology demonstrated potential to reduce weed contamination at harvest. Laser weeding appears to be more effective in spinach as compared to wild rocket crop. While promising, the full assessment of weed control, weed seedbank dynamics and crop yields will reveal the potential role of laser weeding in future integrated weed management programs for baby leaf production systems.

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based laser weed control compared to conventional herbicide application across three vegetable production systems. *Pest Management Science*. 81(8), 4922-4936.

Title: Structural insights into Phosphoenolpyruvate carboxylase from C_4 photosynthesis

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Keywords:
Phosphoenolpyruvate carboxylase, Photosynthesis, AlphaFold3, Structural Biology

Abstract:

Phospho*enol*pyruvate carboxylase (PEPC) (EC 4.1.1.31) catalyses the first committed step in C_4 photosynthesis. PEPC facilitates the conversion of phospho*enol*pyruvate (PEP) and bicarbonate into oxaloacetate and inorganic phosphate, thereby enhancing the carbon flux entering the C_4 cycle. An allosteric enzyme, such as PEPC, gets regulated by various metabolite effectors that bind to distinct sites[1]. An example of an essential allosteric activator is Glucose-6-Phosphate (G6P), which induces a conformational change that modifies the enzyme's active site function, allowing for PEPC to increase its substrate binding and catalytic efficiency[2,3]. PEPC has a high degree of sequence homology across different species and amongst the three distinct subtypes of C_4 Photosynthesis. The PEPCK subtype has a six-amino acid-specific deletion towards the C-terminal end of the PEPC enzyme; this deletion was not observed in the other PEPC enzymes of the NADP-ME and NAD-ME subtypes[4]. Tertiary structure prediction tools, such as AlphaFold3, facilitate modelling the unique six-residue PEPCK-specific deletion is located adjacent to the G6P binding site. Small-angle X-ray scattering (SAXS) experiments have demonstrated that G6P induces significant conformational shifts in *Urochloa panicoides* PEPC, indicating enhanced enzyme flexibility. CRYSOL analysis corroborated these findings, with experimental scattering profiles of *U. panicoides* diverging from predicted models compared to those of the other grasses. Collectively, this project highlights the critical importance of conserved allosteric sites in regulating C_4 PEPC. These findings enhance targeted mutagenesis approaches and structure-function prediction, advancing both evolutionary and biotechnological studies in C_4 photosynthesis research.

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Lunar Effects on Agricultural Flora (LEAF): Investigating duckweed heat tolerance and gas exchange

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Keywords:
Duckweed, heat tolerance, stress tolerance, gas exchange

Abstract:

Artemis III will bring humans back to the Moon for the first time in a century. The Lunar Effects on Agricultural Flora (LEAF) payload, designed to be deployed as a growth chamber on the Moon's surface, will study how the lunar environment affects the germination, growth, and stress responses of *Wolffia* (duckweed), *Arabidopsis thaliana*, and *Brassica rapa* (Wisconsin Fast Plants). Alongside ground controls, astronauts will return seedling samples to Earth, allowing the LEAF consortium* to explore effects on gene expression, morphology, metabolism, stress signalling, redox state, and cell walls.

While testing the plants in anticipated pre-launch, launch and loiter conditions, we have found that duckweed fronds can survive for extended periods at up to 40˚C in the dark, followed by rapid resumption of photosynthesis and growth upon return to the light and cooler temperatures. To investigate duckweed's high basal heat tolerance further, we are using a suite of analytical techniques, including RNAseq to measure gene expression across a range of temperatures, chlorophyll fluorescence to determine when photodamage occurs, antioxidant enzyme assays to quantify reactive oxygen species, and membrane thermostability tests. In parallel, we are developing methodology to measure carbon flux in duckweed using a Licor-6800 infra-red gas analyzer with an aquatic chamber. Our research has implications for plant growth and food production in changing climates on Earth as well as sustained long term, space habitation in future.

Exploring Rubisco activase diversity and heat tolerance in native Australian plants

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Keywords:
Photosynthesis, Rubisco activase, Heat tolerance, Rca

Abstract:

To anticipate how future thermal regimes will affect plant photosynthetic capacity, it is first necessary to characterize how elevated temperatures impact fundamental processes underlying photosynthesis. Among photosynthetic components, Photosystem II (PSII) is well recognized as heat sensitive (1,2). In contrast, the carbon-fixing enzyme Rubisco exhibits substantial thermostability, and its thermal inactivation is linked to the heat-lability of its regulatory chaperone, Rubisco activase (Rca) (3). Research to date suggests that Rca has lower thermal tolerance than either PSII or Rubisco, however, thus far investigations into the thermal sensitivity of Rca have largely focused on crops and are often restricted to narrow temperature assays (4). To address this limitation, we optimized a protocol for extraction and visualization of Rca from native – neither crop nor model – plant species spanning diverse families and leaf morphologies. This approach works across phylogenetic breadth and leaf mass per area (LMA) variation, overcoming the need to design species-specific methodologies. As a proof of concept, we subsequently applied this protocol to a set of phylogenetically and morphologically diverse native Australian plants to assess changes in leaf Rca content in response to progressive increases in temperature and exposure duration. Through our study, we illustrate how this straightforward experimental setup not only captures Rca content across diverse species but also serves as a cost-effective tool for explorations of Rca heat stress responses. Integrating such data with existing measures of PSII thermal tolerance will be critical for advancing our mechanistic understanding of photosynthetic performance under future climatic conditions.

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Illuminating GABA signalling in plants at single-cell resolution

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Keywords:
GABA, fluorescence imaging, biosensor, single cells

Abstract:

γ-Aminobutyric acid (GABA), a non-proteinogenic amino acid, is a conserved messenger for cellular communication across kingdoms. In plants, GABA's traditional roles were thought to be confined to pH regulation, cellular energy production, and carbon–nitrogen balance as a metabolite. GABA has recently been shown to function as a cytosolic guard-cell signalling molecule with spatiotemporal dynamics[1]. However, conventional bulk-tissue GABA quantification lacks the resolution to assess cytosolic levels at the single-cell scale[2]. A genetically encoded fluorescent sensor for GABA (iGABASnFR1, K_d=~9 μM, ΔF/F =2.5 *in vitro*) has been developed for extracellular GABA imaging in mammalian neurons[3]. Here, we adapted this biosensor to monitor cytosolic GABA in live plant cells. In *Arabidopsis thaliana* root protoplasts, iGABASnFR1 was adapted to show cytosolic localisation, with changed sensitivity of K_d=~270 μM and ΔF/F=1.6, indicating reduced affinity *in planta*. However, it is noted that protoplast fluorescence may not reflect native cytosolic GABA, as cell wall removal mimics wounding and influences GABA metabolism. In intact roots under non-stress conditions, iGABASnFR1 fluorescence exhibited low spatial resolution, possibly because most GABA is sequestered in vacuoles, making cytosolic levels hard to detect. To overcome this, we employed and adapted iGABASnFR2 with improved signal-to-noise ratio[4]. Together, our findings highlight the importance of calibration and comparison of different GABA biosensors with distinct dynamic ranges for optimal *in vivo* imaging. This work establishes a platform to investigate cytosolic GABA dynamics at single-cell resolution in plants, paving the way to decode its roles under varying environmental conditions.

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Distinct fructan accumulation patterns in leaves and crowns under diurnal temperature fluctuations in wheat: possible relevance to freezing tolerance

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Keywords:
Freezing tolerance, Fructan, Diurnal temperature, Cold acclimation

Abstract:

Freezing severely damages plants and threatens their survival. Many plant species develop cold acclimation mechanisms that enhance freezing tolerance in response to low non-freezing temperatures. Most studies of these mechanisms have been conducted under constant temperature conditions, highlighting the roles of soluble sugars and membrane lipids[1,2]. Through these studies, it has become clear that in wheat, fructans, which are soluble oligosaccharides, play a particularly critical role in freezing tolerance, as they accumulate at high levels in the crown containing the meristem and enhance freezing tolerance by increasing cellular water potential[3,4]. In natural environments, however, plants experience diurnal temperature fluctuations, creating conditions distinct from the constant regimes. To clarify how wheat responds, we simulated autumn temperature patterns in Japan using a controlled chamber and investigated cold acclimation mechanisms with a particular focus on fructans. Because soluble sugars are primarily synthesized in leaves and then transported to the crown, we hypothesized that fructan dynamics would differ between leaves and crown, and therefore analyzed these organs separately. Our results showed that fructan levels increased in both organs under diurnal fluctuations. While fructan content remained stable under constant temperature, under fluctuating conditions it displayed diurnal patterns that closely tracked changes in temperature. Gene expression analysis further revealed that transcripts of fructan branching enzymes were more than 20-fold higher in crowns than in leaves, suggesting structural differences in fructans between the two organs. Therefore, Wheat may modify fructan structures in leaves and the crown under diurnal temperature fluctuations, which may be associated with freezing tolerance.

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Cellular aspects of Na^+ homeostasis in plants: Quantitative approaches.

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Keywords: Sodium homeostasis, sodium transport, membrane transport models, salinity

Abstract:

To survive saline conditions, plants maintain cytosolic Na^+ concentrations around 10 mM while sequestering Na^+ to the vacuole. Using steady-state "homeostat" modelling (1.2), we show that at the cellular level, cytosolic Na^+ homeostasis is achieved by two different types of transport system at both the plasma membrane and tonoplast: inward Na^+ permeable channels and outward Na^+:H^+ antiporters, energised by proton pumps. Coupling these systems with K^+ transport adds further complexity and strict transport selectivity is crucial for avoiding conflicting ion fluxes. Chloroplasts, which occupy a large volume fraction of the cytoplasm, might also contribute to cytosolic Na^+ homeostasis. Our models predict that at the plasma membrane, Na^+:H^+ antiport regulation has a greater effect on cytosolic Na^+ concentrations than channel regulation, but both channel and antiport regulation are equally important at the tonoplast. The energetic consequences of homeostatic regulation are investigated and discussed in the context of evolutionary selection for particular ion channel characteristics.

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Transcriptomic profiling of wheat grain filling under elevated CO_2 and temperature: Discovery of key stress-responsive genes and pathways for climate resilient breeding.

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Keywords:
Elevated CO_2, heat stress, DEGs, transcription factors, RT-qPCR.

Abstract:

Wheat (*Triticum aestivum* L.), one of the world's most vital cereal crops, faces significant challenges from global climate change, particularly elevated CO_2 (eCO_2) and temperature conditions. Understanding the complex molecular and physiological responses of wheat to these combined stresses is essential for securing sustainable wheat production. In view of this, we performed RNA sequencing analysis of wheat during grain filling to assess gene expression responses to the combined effects of elevated CO_2 (eCO_2) and heat stress. A total of 7,051 significant differentially expressed genes (DEGs) were identified, comprising 2542 up-regulated and 4,509 down-regulated genes. Gene Ontology (GO) analysis classified 9,115 DEGs into various functional categories, while KEGG pathway analysis revealed the enrichment of DEGs across various abiotic stress pathways." Transcription factors such as HSP101, SOD1, WRKY33, and DREB2A, along with key regulatory genes including RCA, rbcS, rbcL, GS, PHO, and IRL, which play crucial roles in photosynthesis, nitrogen metabolism, micronutrient assimilation, and yield, were found to be highly abundant among the differentially expressed genes (DEGs). Validation of selected DEGs by RT-qPCR showed expression patterns consistent with the transcriptome data, confirming its reliability. The results of the present study could facilitate further functional validation and exploration of the mechanisms underlying elevated CO_2 (eCO_2) and heat stress responses in wheat during grain filling. These findings provide a basis for breeding climate-smart wheat.

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Genome engineering barley root systems for future climates.

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Keywords:
Root system architecture, barley, CEPR1, peptide hormone, drought

Abstract:

Optimising barley root systems to attain water and nutrients during adverse growing conditions common to Australia has the potential to significantly improve yield stability. Due to the challenges in visualising and phenotyping root systems, breeding programs have traditionally focused on above-ground traits and yield maximisation. As a result, much of the genetic variation controlling root systems has been inadvertently fixed as a by-product of selection. This is likely particularly true in elite germplasm due to intensive selection over the past century.

C-TERMINALLY ENCODED PEPTIDE RECEPTOR 1 (CEPR1) has been shown to influence root system architecture in the model species *Arabidopsis* and *Medicago*[1] and we have previously reported[2] that editing *CEPR1* to generate a loss-of-function *cepr1* barley line produced a novel root system architecture with similar traits as seen in the model species. Briefly, a steep and compact root system of similar depth to the WT. *cepr1* barley plants also display a shoot-penalty compared to WT and produce less and lighter grain per plant.

Our current work includes identifying targets downstream of *CEPR1* for editing in an attempt to 'fine tune' the *cepr1* phenotype and produce a steep and deep root system. We are also currently assessing the performance of our barley *cepr1* line under conditions of imposed abiotic stress as well as in the field and plan to present key findings of these experiments at the conference.

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Development of biomarkers for thermotolerance in wheat

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Keywords:
Heat shock protein, Circadian Clock, GABA, heat tolerance, RT-qPCR

Abstract:

Global warming represents a significant threat to global agricultural production, particularly for staple crops such as wheat. Despite its importance, the genetic mechanisms underlying thermotolerance in wheat remain insufficiently investigated. In this study, we are trying to develop molecular biomarkers capable of distinguishing wheat genotypes with differential thermotolerance. Candidate biomarkers were derived from transcriptional profiles associated with three major pathways: heat shock proteins (HSP), circadian clock (CC), and γ-aminobutyric acid (GABA) metabolism.

A total of 543 wheat genotypes were analysed under control conditions (17 °C) and following a 2-hour heat treatment at 27 °C. Expression profiling revealed consistent upregulation of HSP genes (HSF23 and HSP90-1) across all genotypes, with fold-changes ranging from <10 to several hundred. Conversely, CC genes (LHY and PRR59) were generally downregulated, although a subset of genotypes exhibited upregulation in response to heat. GABA pathway genes showed more variable responses: GABAP was upregulated in most genotypes, while SSADH expression remained largely unchanged.

Based on these transcriptional patterns, ~100 genotypes have been selected for controlled heat-stress experiments in glasshouse conditions. Subsequent field validation will be undertaken at two sites, Roseworthy (SA) and Wagga Wagga (NSW), to assess the predictive value of these biomarkers under agronomic conditions.

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High-throughput screening and structure-guided design of small molecules enable on-demand modulation of chloroplast stress signalling

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Keywords:
retrograde stress signalling, high-throughput chemical screening, enzyme inhibition, structure-activity relationship (SAR), chemical genomics

Abstract:
Chloroplasts serve as environmental stress sensors, triggering acclimation responses through chloroplast-to-nucleus retrograde signalling. A key pathway in this process involves the SAL1 phosphatase and its catabolic substrate, 3'-phosphoadenosine 5'-phosphate (PAP). The SAL1-PAP pathway is evolutionarily conserved across land plants, making it a promising target for enhancing crop resilience. However, complete genetic deletion of SAL1 results in complex pleiotropic effects, highlighting the need for precise modulation of its activity. Here we performed a high-throughput in vitro screen of 13,000 small molecules to identify novel SAL1 inhibitors and uncovered a lead compound—V20. Accelerated molecular dynamics (MD) simulations, biochemical assays, SAL1 mutagenesis, and chemical modifications to V20 revealed that V20 binds to two regions on the SAL1 protein previously not known to affect SAL1 biochemical activity. Exogenous application of V20 to the model plant Arabidopsis led to increased PAP accumulation, activation of PAP-responsive transcriptomic changes, and enhanced

plant stress tolerance. Our results establish a proof-of-concept for targeted modulation of SAL1 activity via small molecule inhibitors, demonstrating the potential to selectively manipulate chloroplast-to-nucleus retrograde signalling pathways to enhance plant stress resilience.

Foliar cuticular wax chemistry influences host-recognition processes in pathogenic fungi

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Keywords:
Cuticular wax, mass spectrometry, fungal germination, surface chemistry

Abstract:

Fungal disease costs the Australian grains industry $930 million annually. Current mitigation strategies for these diseases often include widespread application of fungicides, which can be costly, environmentally harmful and promote fungicide resistance. Many fungal diseases in grains begin at the initial contact between a fungal spore and the surface of a leaf. With leaf surfaces coated in a waxy cuticular layer which is chemically dynamic across plant species and cultivars, there is increasing research to suggest that fungal spores use this cuticular layer for host-recognition. In this project, cuticular waxes of economically important grain crops and their cultivars are profiled by GCMS and SEM to understand their chemical and morphological diversity. Fungal germination assays are then performed with powdery mildew (*Blumeria graminis*) conidia to investigate the effect of wax chemistry on fungal pre-penetration process such as germination. This work demonstrates that small changes in wax composition can have distinct and important implications for early fungal disease progression.

Unravelling root growth responses to salt stress in barley

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Keywords:
Salinity, root growth, barley, crops

Abstract:

Salinity is a major cause of crop yield losses in agriculture, significantly impacting on sustainability. Soil salinization is increasing due to the impacts of climate change, reducing the area of arable land for crop production. The ability of crops to alter root system architecture in saline soils is an adaptive mechanism to maintain water and nutrient uptake, whilst ensuring seedling establishment. Saline soils result in a reduction in primary root growth impacting significantly on crop growth and yield. Barley (*Hordeum vulgare*) cv. Clipper (malting barley) and Sahara (North African landrace 3771) have been shown to have contrasting root growth responses to salinity stress [1, 2]. We aimed to identify the genes involved in root elongation maintenance and Na^+ exclusion in response to salt stress. To elucidate the genetic basis for these mechanisms, a Clipper x Sahara 3771 double haploid (DH) mapping population was screened for root phenotypic traits in response to salt stress. Quantitative Trait Loci (QTL) and bulk segregant RNAseq analyses were employed to identify candidate genes. We have identified six significant QTLs for root traits, with two co-located QTLs for seminal root length and average root diameter on chromosome 2HS. Within these loci we have identified several differentially expressed genes with Single Nucleotide Polymorphisms (SNPs), that are potential candidates for root adaptation in saline conditions. In the future, combining root phenotyping with advances in crop genetics will allow us to uncover root traits that can be incorporated into crop breeding programs for yield stability in saline soils.

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Developing Novel Herbicide-Tolerant Wheat Cultivars Through Genome Editing and EMS Mutagenesis

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Keywords:
Herbicide tolerance, Wheat, EMS, CRISPR/Cas9, Resistant weeds.

Abstract:

Global crop production is threatened by weeds, which have caused a 34% reduction in yield worldwide. To solve this problem, growers use herbicides to control weeds. The continued use of herbicides with similar modes of action has caused the rapid evolution of herbicide-resistant weeds. Research has revealed the molecular basis of these effective resistance strategies.

Wheat is a staple crop grown globally as a source of calories. Due to the complexity of the wheat genome, it has been challenging to develop herbicide-resistant varieties in wheat through conventional breeding. Our project aims to equip wheat with genetic resilience by translating herbicide resistance mechanisms from resistant weeds to wheat.

We hypothesize that mutations found in resistance weed species can be used to develop useful herbicide tolerance traits in wheat. To test this hypothesis, CRISPR/Cas9 gene editing technology will be used to induce precise mutations in wheat genes (*GS2*, *PPO* & *ACCase*), similar to the resistance mechanisms seen in resistant weeds. Simultaneously, we will characterize mutant wheat population generated through chemical mutagenesis (Ethyl methane sulfonate) for tolerance to three groups of herbicides (HRAC Group 1, 10 & 14). We will then combine these approaches to introduce mutations across sub-genomes, resulting in more effective herbicide tolerance. The results of this project will help diversify herbicide options, improve integrated weed management strategies, and maintain wheat yields, which are essential for sustainable agriculture amid increasing global food demands.

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Finding the missing links in salt and water transport in plants

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Keywords:
Tonoplast Intrinsic Proteins, Ion transport, Two-electrode voltage clamp, Salinity

Abstract:

Tonoplast intrinsic proteins (TIPs) are vacuolar membrane channels that facilitate the movement of water and small solutes, contributing to plant growth and stress adaptation [1]. Some have now been reported to be associated with ion transport [2-4] and AtTIP2;1 transports water and ammonia [5]. In this study, we examined the transport properties of selected TIP isoforms from barley (*Hordeum vulgare L.* cv. Sloop and Sloop SA), and *Arabidopsis thaliana*. *HvTIP2;2* Sloop and Sloop SA isoforms differ in a single amino acid (L117F) and were evaluated to determine if they differ in function. Functional characterization was performed in *Xenopus laevis* oocytes; swelling assays were used to determine water permeability (P_{os}) and two-electrode voltage clamp (TEVC) electrophysiology to assess ion conductance. Results showed that water transport by HvTIP2;2 (Sloop and Sloop SA) was inhibited by $MeNH_4Cl$ and TEA. In Arabidopsis, AtTIP2;1 and AtTIP2;3 water transport was inhibited by TEA, while AtTIP2;2 was more strongly inhibited by $MeNH_4Cl$. Furthermore, AtTIP2;1, AtTIP2;2, and AtTIP2;3 appeared unlikely to mediate Na^+ or NH_4^+ transport. To evaluate tissue specific expression of *HvTIP2;2* under salt stress, we used paper roll-supported hydroponics [6], and analysed the expression of *HvTIP2;2* in Sloop and Sloop SA following treatment with 150 mM NaCl. *HvTIP2;2* was predominantly expressed in the root tips and lateral roots, with increased expression under salt stress suggesting a role in root stress adaptation. The results highlight the importance of TIPs in regulating water flux under stress conditions, pointing to their broader contribution to plant adaptation and resilience. This work provides a basis for enhancing crop resilience to salinity and drought through targeted modification of vacuolar transport mechanisms.

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Title: Understanding salt uptake and accumulation in grapevines

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Keywords:
Salinity, ion distribution, grapevine, abiotic stress, rootstock

Abstract:

Salinity is a global problem and arable land area is decreasing due to human induced salinisation. Grapevine is considered moderately sensitive to salt, especially chloride (Cl^-). To gain a better understanding of the fundamental mechanisms of ion and salt homeostasis in plants, we aim to generate new information on the spatial and temporal accumulation of sodium (Na^+), Cl^- and nutrient ions e.g. potassium (K^+) in grapevine organs and tissues when subject to salt stress. To do this, we are comparing *Vitis vinifera* L. cv. Cabernet Sauvignon (good Na^+ exclusion and moderate Cl^- exclusion capacity) with a salt-tolerant (140 Ruggeri, excellent Cl^- excluder) and salt-sensitive (K51-40, poor Cl^- excluder) rootstock cultivar, using grapevine rooted leaves as a simple root-to-shoot model. The lamina K^+, Na^+ and Cl^- have varietal dependent distribution patterns, and the spatial distribution of K^+ has weak correlations with Na^+ and Cl^-. When exposed to 50 mM or 100 mM NaCl, root [K^+] was reduced however the petiole and lamina [K^+] were generally not reduced. Leaf hydraulic conductivity dropped in K51-40 under salt stress. We propose that salt stress induced leaf necrosis is caused by preferential deposition of ions in the outer rim when the salt accumulation rate is relatively slow, however ion accumulation is not the main reason for necrosis when lamina has a fast ion buildup. We will employ transcriptomics to uncover the transporters and regulatory mechanisms underlying the movement and spatial accumulation of salt and nutrient ions.

Temporal regulation of mitochondrial and peroxisomal stress-responsive genes in wheat

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Keywords:
Alternative oxidase, Alternative respiration, Abiotic stress, Triticum aestivum

Abstract:

Heat and drought are environmental conditions that severely reduce the yield of bread wheat (*Triticum aestivum*). One key mechanism behind yield loss is the excessive production of reactive oxygen species (ROS) during stress events, which can damage lipids, proteins and DNA, ultimately impairing photosynthetic and respiratory performance. To maintain cellular homeostasis during stress, many plants have been shown to activate antioxidant pathways and/or alternative respiratory pathways (Hickey et al., 2022), although studies in wheat are limited. Recently, peroxisome abundance has also been demonstrated as a key response to oxidative stress in wheat (Hinojosa et al, 2019). The peroxisomal antioxidant system functions to scavenge ROS and involves catalase (CAT1) activity and peroxisome biogenesis via the activity of peroxins (PEX), while the mitochondrial alternative oxidase (AOX) acts to prevent ROS formation through bypassing energy-conserving complexes of the respiratory electron transport chain (Li et al., 2024). This study investigates genotypic variation in transcriptional responses of mitochondrial and peroxisomal stress-related genes during severe water limitation, as well as early responses to temperature elevation under the same water-limiting conditions. RT-qPCR analysis revealed significant differences in both the timing and magnitude of gene transcript responses between genotypes, indicating that rapid induction of these pathways may contribute to early stress responses. Interestingly, a higher-yielding genotype maintained higher stomatal conductance under terminal drought (<10% soil moisture) compared to a lower-yielding genotypeand showed a unique transcriptional pattern during the first hour of heat application for *TaAOX1a*, *TaAOX1c*, *TaAOX1d*, *TaPEX11-7A*, and *TaCAT1*, suggesting a distinct regulatory balance between mitochondrial and peroxisomal ROS mitigation mechanisms. Overall, these preliminary findings suggest genotype-specific strategies in the timing of mitochondrial and peroxisomal responses that may underpin differences in resilience and yield stability under combined drought and heat stress.

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Interactive Effects of Temperature and Water Potential on Whole-Plant Hydraulic Conductance

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Keywords:
stomata, temperature, VPD, hydraulic conductance, DRST

Abstract:

Little is known about effects of temperature (*T*) on whole-plant hydraulic conductance (K_{plant}) in intact plants, due to the conflating influence of changes in water potential that often occur when temperature is changed, because of shifts in evaporative demand and/or stomatal conductance. This study aimed to isolate the effect of temperature on K_{plant} from that of water potential. Whole plant transpiration was measured by mass loss, water potential was measured in transpiring leaves, source water potential was estimated using both bagged leaves and bagged plants. K_{plant} was then calculated from these measurements using Darcy's Law. In experiment #1, these measurements were conducted at different temperatures, causing changes in both *T* and water potential; in experiment #2, they were conducted at different VPDs but constant temperature, causing changes only in water potential. Experiment #2 was used to determine the vulnerability curve for K_{plant}, which was then used to correct for shifts in water potential in experiment #1. Preliminary results indicate small changes in K_{plant} with temperature. These results have implications for effects of T on K_{plant}, including as a possible mechanism driving stomatal opening in response to temperature.

Testing a hydraulic mechanism to the total stomatal temperature response

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Keywords:
Temperature, stomata, VPD, hydraulic conductance

Abstract:

Stomata are known to respond to many environmental variables, including CO_2[1], light[2], and VPD[3]. The response to temperature with VPD held constant, however, has rarely been examined, however, with sparse data from the literature and little replication[4]. The mechanisms behind this response have received even less attention. Here we test a hydraulic mechanism for the stomatal temperature response. Temperature should increase hydraulic conductance via viscosity, increasing leaf water potential and thus stomatal conductance. This predicts a more positive % response to temperature at high VPD than at low VPD. We measured the stomatal response to temperature at two VPDs and compared the results with predictions based on published effects of temperature on hydraulic conductance. Our results suggest that hydraulics do contribute to the stomatal response to temperature, but the response exceeds our predictions, suggesting other mechanisms are at work. Future experiments should directly measure the effect of temperature on whole-plant hydraulic conductance.

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Genome-wide association study reveals novel loci regulating amylose content in wheat (*Triticum aestivum* L.) under high temperature stress

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Keywords:
Grain quality, GWAS, starch, high temperature, sink

Abstract:

About 11-13% of the global population is affected by diabetes, obesity, and high blood pressure. This metabolic syndrome can be corrected by dietary changes and improved food quality. Wheat is a cereal that nourishes 35-40% of the population. Wheat usually contains a low amount (approximately 20-30%) of amylose, a resistant starch, and has the capacity to provide health benefits against diabetes, obesity, and high blood pressure. Thus, breeding for high amylose wheat is crucial to tackle these health issues. To achieve this, we must identify the genetic mechanisms involved in amylose content. In this study, we phenotyped 260 wheat genotypes for apparent amylose content over two seasons and employed genome-wide association studies to elucidate the link and genetic causes of the significant differences in amylose content between the genotypes. We identified two serine threonine protein kinase genes (TraesCS7D02G530500 and TraesCS1D02G026200) associated with amylose variation. Further, we studied the effects of high temperature on the amylose biosynthesis pathway after the anthesis stage in four contrasting genotypes. The results showed that granule-bound starch synthase (GBSS) and soluble starch synthase (SSS) were affected by high temperature, whereas ADP-glucose pyrophosphorylase (AGPase) was not. Gene expression studies revealed that starch-binding enzyme IIa (SBEIIa) shifted from a low correlation to a positive correlation with other starch synthase enzymes under high-temperature stress.

Oxygen-Sensitive ACO1 Regulates Ethylene Production under Hypoxia in Arabidopsis

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Keywords:
Ethylene Biosynthesis, Hypoxia Stress, Stress Adaptation, Oxygen Sensing

Abstract:

Ethylene is a central phytohormone that enables plants to adapt to diverse stresses. Its biosynthesis is catalysed by 1-aminocyclopropane-1-carboxylate oxidase (ACO), which is encoded by a multigene family in plants. However, the isoform-specific contributions of ACOs to ethylene biosynthesis remain poorly understood. Here, we reveal that in *Arabidopsis thaliana*, ACO1 plays a critical role in linking oxygen availability to ethylene production during hypoxia stress. Although Arabidopsis contains five ACO isoforms, only ACO1 is transcriptionally upregulated during hypoxia. Through phenotyping and biochemical assays, we demonstrate that ACO1 is functionally distinct from other isoforms and is essential for maintaining ethylene-mediated hypoxia adaptation. Kinetic analysis further shows that ACO1 possesses a higher oxygen affinity, enabling ethylene biosynthesis under low-oxygen conditions. Together, our findings establish ACO1 as an oxygen-sensitive enzyme and uncover a novel mechanism by which plants fine-tune ethylene biosynthesis to adapt to hypoxia.

Interrogating plasmodesmatal regulation in compartmentalizing chloroplast signals in C_4 photosynthesis

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Keywords:
Chloroplast signalling, Plasmodesmata, C_4 photosynthesis

Abstract:

C_4 photosynthesis involves the partitioning of photosynthetic machinery between mesophyll (M) and bundle sheath (BS) cells to facilitate the C_4 cycle and concentrate CO_2 around Rubisco[1]. As such, M and BS are intimately connected by plasmodesmata (PD) to facilitate the exchange of metabolites and C_4 photo-assimilates[2,3]. We have previously demonstrated that M and BS cells in the model C_4 monocot *Setaria viridis* accumulate specific chloroplast stress signals and perform cell-specialized chloroplast signalling under combined high light and heat stress (HL+H). However, given their high degree of interconnectivity it is unclear how this compartmentalization of chloroplast signals between M and BS cells is achieved under HL+H. Here I test the hypothesis that regulation of PD aperture and thus permeability may contribute to cell-type specific chloroplast signalling. I found that callose, which is associated with PD closure, is deposited at the M-BS interface within 60 minutes of HL+H stress, resulting in decreased intercellular transport as demonstrated using 5(6)-carboxyfluorescein diacetate transport assays. Interestingly, this callose deposition required the activity of both NADPH-oxidases and photosynthetic electron transport, suggesting the involvement of both plasma membrane- and chloroplast-derived reactive oxygen species (ROS). To test if callose deposition is the mechanism enabling cell-specific chloroplast signal compartmentalization, I will utilise a system for the inducible overexpression of the native callose degrading enzyme β-1,3-glucanase, or the callose biosynthesis enzyme callose synthase[5]. This system will confer selective control of PD permeability by regulating callose deposition, providing a platform to directly test the role of PD permeability in compartmentalizing chloroplast signals.

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Exploring wheat aquaporin substrate permeability

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Keywords:

Aquaporins, membrane proteins, Phosphorylation, abiotic stress, wheat

Abstract:
Aquaporins (AQPs) are integral membrane proteins that facilitate transport of water and other solutes across cellular membranes[1]. Subsets of AQPs play crucial roles in regulating various physiological and pathological processes in plants[1,2]. Regulation of crop plant AQP substrate specificity, such as permeability to water, salts, nutrients and signalling molecules like hydrogen peroxide, is not yet fully understood. Post-translational modifications, such as differential phosphorylation, ubiquitination, and methylation across regions of structural importance (termini or loops), have been shown to impact AQP solute transport [1,4].

Here, substrate permeability profiles for two wheat (*Triticum aestivum* L.) AQPs (TaAQPs) were tested using yeast heterologous AQP expression assays[3]. Regulatory control of the TaAQP permeability was tested by mutating serine residues present in different loops to mimic phosphorylated and dephosphorylated states. Both native TaAQPs were identified as novel candidates for water, hydrogen peroxide, ammonium and sodium transport.

Mutant versions of the TaAQPs that mimicked different phosphorylation states showed varied solute transport phenotypes, indicating that differential regulation of the phosphorylation status of the sites tested could influence the function of the TaAQPs. The observations are consistent with previous studies exploring the influence of post-translational modification in the dynamic regulation of AQP function[4].

Regulation of TaAQP function by differential phosphorylation is likely to be relevant to redox signalling, nutrient use efficiency and resilience to environmental stress[1,4,5]. Future work targeting the engineering of wheat TaAQP function is expected to contribute to understanding the roles of TaAQPs in responses to osmotic and oxidative stress.

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Investigating Pectin RG-II Glycosyltransferases in Plant Cell Wall Biosynthesis

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Keywords:
Plant, cell wall, pectin, Rhamnogalacturonan-II

Abstract:

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 id 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 implemented a bioinformatics pipeline to identify candidate pectin biosynthesis genes (glycosyltransferases, GTs). Having shortlisted candidates for further characterisation, we are using gene-editing to produce null mutants. To overcome issues of lethality, we are editing during callus formation, and maintaining the lines in tissue culture, thereby avoiding the reproductive phase. Having characterised the cell wall monosaccharide composition, we are now developing a workflow for RG-II structural analysis, which we are benchmarking against RG-II isolated from red wine.
We have also successfully expressed a selection of these candidates in human embryonic kidney cells (HEK293), with the generation of at least one crystal structure to 2.6A. We will screen the enzymes for affinity binding against a panel of NDP substrates to confirm predicted function.

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PMC11288761.

Barley ovule development is regulated by B-sister MADS-box genes

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Keywords:
Female reproduction; Ovule development; Nucellus; B-sister MADS-box; Gene function

Abstract:

Barley (*Hordeum Vulgare*) is a critical economic crop in Australia, and its seeds develop from ovules. Recent study has shown that *MADS31*, a B-sister class MADS-box gene, regulates ovule development by controlling the nucellus patterning, further affecting seed-set. Another B-sister gene, *MADS29*, is also likely to play a role in ovule development, potentially in coordination with *MADS31*.

This study investigates whether *MADS29* has similar regulatory function to *MADS31* and explores relationship between these two B-sister-class MADS-box genes. To address this, we generated double mutant and performed genetic rescue crosses between *MADS29* and *MADS31* to assess potential functional redundancy. Ovary structures were observed by making and staining of semi-thin sections. Transcriptomic data from *mads31* and recent *mads29* were compared to identify shared downstream targets, and molecular assays were conducted to test the interaction between two B-sister genes.

The genetic rescue experiment partially restored nucellus patterning in the ovule but did not rescue seed-set. Molecular and transcriptomic analysis suggest that *MADS29* and *MADS31* have the potential to cooperate in regulating nucellus patterning.

This is the first time report of both B-sister class genes' function in barley ovule development. Understanding how these genes regulate nucellus patterning provides new insight into ovule development in cereals and highlights its importance for successful seed set.

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Enhanced Plant Growth and Stress Tolerance via miR156a Overexpression from chickpea: Insights into miR156-SPL Regulatory Network in Arabidopsis

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Keywords:
miRNA, transcription factors, Arabidopsis, Chickpea

Abstract:

The interaction between miR156 and the SPL gene family is pivotal in plant biology, particularly in developmental processes and growth regulation. miR156, a microRNA in plants, targets mRNA transcripts of the SPL gene family, which includes transcription factors governing critical aspects of plant development like phase transitions, leaf initiation, flowering, and fruit ripening. miR156 binds to SPL gene transcripts, reducing their expression either through degradation or translational inhibition. In this work, we generated overexpressed transgenic lines of chickpea mir156 (CamiR156a) in Arabidopsis. CamiR156a at higher levels and we observed the huge differences in transgenic and wild plants changes at various stages. As we know mir156 is also well known for its role in different stresses, hence we also studied these transgenic lines under drought, salinity. Under these stress conditions transgenic plants were showing tolerance in comparison to wild type Arabidopsis thaliana (Col0). Under drought and salinity stress, the transgenic Arabidopsis seedlings developed longer main roots than the natural type. Furthermore, under these stresses, the Arabidopsis lines overexpressing CamiR156a showed superior growth compared to the wild type plants confirmed by biochemical and molecular analysis. They also showed unique developmental traits such dwarfism, prolonged vegetative growth period, increased shoot branching, and shorter siliques, in addition to producing more seeds. The miR156-SPL module is evolutionarily conserved and has significant agricultural implications, offering potential for crop improvement by manipulating flowering time, yield, and stress tolerance in chickpea. Understanding this regulatory network is key to advancing plant biology and agricultural practices

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Carboxylate exudation and reproductive efforts are associated with leaf phosphorus-resorption efficiency in chickpea

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Keywords:
Canopy senescence, P-use efficiency, phosphorus resorption, phosphorus acquisition, reproductive investment.

Abstract:

Efficient leaf phosphorus (P) resorption is a key process that enhances internal P-use efficiency in plants. However, the interactions among leaf P resorption, senescence dynamics, and seed P accumulation remain poorly understood. We evaluated 266 chickpea accessions with diverse genetic backgrounds in a temperature-controlled glasshouse experiment. Measurements included leaf P-resorption efficiency (PRE), reproductive allocation, phenology, canopy senescence traits, and biomass distribution, along with rhizosheath carboxylates, which is an indicator of root P-acquisition strategy. Accessions with higher leaf P concentrations exhibited greater PRE, initiated canopy senescence earlier, and maintained a faster canopy P translocation rate. These combined traits significantly increased aboveground productivity and enhanced P-use efficiency. We also identified a non-linear relationship between carboxylate exudation and PRE: resorption efficiency rose with increased exudation before reaching a plateau. Structural equation modelling revealed that higher carboxylate exudation and greater reproductive allocation both contributed to enhanced P resorption. Substantial variation in PRE was observed across accessions, ranging from 70% to 89%. This study is the first to systematically integrate leaf P resorption, root P acquisition, reproductive investment, biomass partitioning, and senescence dynamics. Our findings provide new insights into P-use strategies in chickpea and highlight potential pathways for improving crop P efficiency.

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Organ-specific phosphorus remobilization strategies in wheat under contrasting phosphorus supply

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Keywords:
Phosphorus use efficiency, remobilization efficiency, root–shoot dynamics, Organ-specific responses.

Abstract:

Efficient phosphorus (P) utilization is essential for crop productivity and seed nutrition. We assessed P concentrations and phosphorus remobilization efficiency (PRE) in wheat glumes, leaves, stems, and roots under high (HP) and low P (LP) supply. P concentrations differed markedly among organs and generally declined from anthesis to maturity, with the strongest reductions in glumes and leaves, while roots maintained relatively stable levels. HP plants consistently accumulated more P than LP plants at both stages. PRE showed contrasting organ-specific responses: aboveground organs displayed higher PRE under LP than HP, whereas roots showed the opposite trend. Correlation analysis revealed that PRE in glumes and stems was strongly associated under both P regimes, suggesting coordinated remobilization within aboveground tissues. PRE was positively related to P concentration at anthesis, but negatively related to P concentration at maturity, highlighting that large initial reserves promote effective remobilization, while high residual P reflects limited efficiency. These findings demonstrate that aboveground organs rely on efficient remobilization under P limitation, whereas roots contribute more under sufficient supply. This organ-specific insight provides a physiological basis for improving P use efficiency in wheat.

Identification, functional analysis and validation of circRNA role in seed protein accumulation by regulation of corresponding miRNA in chickpea

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Keywords: CircRNA, miRNA, Chickpea, Seed Protein Content

Abstract

Circular RNAs (circRNAs) are stable RNA molecules formed by back-splicing, known to regulate various plant developmental stages, including flowering, fruit formation, and seed maturation. circRNA function primarily by acting as molecular sponges for microRNAs (miRNAs), thereby modulating miRNA-mediated gene regulation. In this study, we analyzed RNA-seq data from two chickpea genotypes differing in seed protein content (SPC): ICC8397 (high SPC) and FG212 (low SPC). Using two circRNA prediction tools, CIRCExplorer3 and CIRIquant, we identified 103 and 1228 putative circRNAs, respectively, with 67 being common to both. Among these, 7 differentially expressed circRNAs were validated through qRT-PCR. Notably, 3 circRNAs (circRNA1, circRNA2 and circRNA3) exhibited stage specific expression pattern at 30 and 40 days after anthesis (DAA), indicating developmental regulation. circRNA3, derived from genes encoding Legumin and Legumin A-like seed proteins, displayed consistently higher expression in high protein ICC8397 genotype. Interactome analysis revealed inverse expression correlations between several circRNAs and miRNAs, including miR156 and miR21. Importantly, miR156, which targets the SPL gene family critical for the vegetative-to-reproductive transition, was differentially expressed between genotypes. This transition influences photosynthate allocation, a key determinant of SPC. Moreover, mi156 also regulates negative SPC modulators such as ABC transporters involved in lipid transport, indirectly affecting protein accumulation in seeds.

The present findings highlight the regulatory roles of circRNA and miRNA data offers promising strategies for chickpea improvement, particularly for enhancing seed development and nutrition quality.

Ammonium (NH_4^+) regulates carbon metabolism and spatial–diurnal assimilate partitioning to improve growth and nitrogen use efficiency in maize

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Keywords: *Zea mays*, assimilate allocation, energetic efficiency, nitrogen use efficiency, root morphology

Abstract

Nitrogen (N) is primarily taken up by most plant species in the form of nitrate (NO_3^-) and ammonium (NH_4^+) to support growth and metabolic functions. However, the regulatory mechanisms modulating carbon (C) assimilate allocation under varying N forms remain unclear. This study investigated C metabolism and its spatial distribution in maize seedlings subjected to five N treatments (T1–T5): T1, nitrogen-free (control N); T2, 1 mM NO_3^- (sole NO_3^-); T3, 1 mM NH_4^+ (sole NH_4^+); T4, 0.5 mM NH_4NO_3 (mixed N supply); and T5, substitution of 1 mM NH_4^+ with 1 mM NO_3^- ($NH_4^+ \rightarrow NO_3^-$) at 10 days after seedling transfer (DAT). NH_4^+ treatment triggered significant physiological and molecular adaptations, such as enhanced growth, improved photosynthetic performance, and increased sucrose and starch accumulation. These elevated carbohydrate levels were closely associated with increased activity of sucrose-metabolizing enzymes (SuSy, SPS, and INVs) and starch-metabolizing enzymes (AGPase, SS, AMY, and BAM), alongside the upregulation of key genes involved in sucrose metabolism (ZmSPS1, ZmSuSy1, and *ZmINVs*), sucrose transport (*ZmSWEET14*, *ZmSUT2*, and *ZmSTP2*), and starch metabolism (*ZmSS1*, *ZmAGPase1*, *ZmAMY1*, and *ZmBAM1*). Spatial and diurnal analyses revealed dynamic patterns of C partitioning across the leaves, roots, and leaf sheaths. These findings advance our understanding of how different N forms, particularly NH_4^+, regulate C metabolism and shoot–root allocation to facilitate carbon utilization in sink tissues to improve plant resilience to N fluctuations. Future research will focus on exploring these adaptive mechanisms across diverse maize genotypes under field conditions, with the goal of improving nitrogen use efficiency (NUE) and productivity in variable N environments.

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Comparative Analysis of *KNATM* Function in *Arabidopsis thaliana* and *Cardamine hirsuta*

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Keywords:
TALE
KNATM
Leaf shape

Abstract:

Leaves are morphologically diverse, specialized plant organs involved in energy capture and gas exchange. Leaf complexity is a key morphological characteristic describing natural variation in the dissection of laminar tissues across the leaves of different species. Specifically, this feature describes a morphological spectrum where leaves range from simple, with undissected lamina, to compound, where lamina are dissected into distinct units known as leaflets. Leaves and leaflets can be further modified by marginal outgrowths such as lobes, or serrations, increasing complexity. Leaf complexity is established early in development, and in many species depends upon the activities of Three Amino Acid Loop Extension (TALE) homeodomain transcription factors.[1] The plant TALE family is an ancient family of genes comprised of two sub-families known as *BEL1-like-homeodomain (BLH)* and *Knotted1-like-homeobox (KNOX),* and also a related gene, *KNATM*, which lacks the conserved homeodomain.[2] Interactions between the proteins encoded in these genes are thought to be critical to their developmental functions.[3] Further, differences in the expression domains of TALE genes between species with simple leaves, such as *Arabidopsis thaliana*, and species with compound leaves, such as *Cardamine hirsuta*, might modify the effect of TALE proteins on leaf complexity.[4] Understanding how TALE genes modify leaves could provide insight into how evolutionary forces may have progressively shaped and diversified leaf forms, as well as how those forms arise in the plants of today. Here we present results investigating the function of several TALE genes in *Arabidopsis thaliana* and *Cardamine hirsuta*.

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Intercropping enhances wheat performance under low phosphorus by rhizosheath exudates and microbiomes in a genotype-specific manner

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Keywords:
Intercropping, root exudates, low phosphorus, rhizosheath microbiome, genotypes

Abstract:

Intercropping enhances crop productivity under nutrient limitation, yet the belowground mechanisms—especially root exudation, mycorrhizal associations, and microbial communities—remain poorly defined, particularly regarding intraspecific genotypic variation.

This study conducted a split-barrier pot experiment using two chickpea lines contrasting in root traits and exudation pattern: ICC456 (LC: low carboxylate exudation, extensive root system) and ICC2884 (HC: high carboxylate exudation, small roots), intercropped with wheat to disentangle plant–exudates–microbiome interactions under phosphorus (P) limitation.

Wheat growth and P uptake significantly increased only when roots were allowed to interact physically or chemically with chickpea roots, highlighting the critical role of belowground interactions. Root exudates rather than arbuscular mycorrhizal fungal (AMF) networks primarily facilitated wheat performance. Metagenomic analyses further revealed that chickpea line identity significantly shaped wheat rhizosheath microbiomes, with distinct microbial communities strongly linked to carboxylate exudation and acid phosphatase activity.

These findings highlight genotype-specific biochemical facilitation mechanisms in intercropping and provide a trait-based framework for designing intercropping systems to enhance resource use in low-input agroecosystems.

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Growth Regulation Mechanism of EMF modulating *Rhododendron moulmainense* to high-temperature stress: Integrated microbiome, transcriptome, and metabolome insights

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Keywords: High-temperature stress, photosynthesis, chlorophyll fluorescence, starch-sucrose metabolism, ABC transporters,

Abstract:

Rhododendrons domestication in urban habitats is limited due to their vulnerability to higher air temperatures, and their adaptive mechanisms are poorly understood. In this study, the isolated strains of ericoid mycorrhizal fungi (EMF) from *Rhododendron moulmainense*, such as HJ21 and HJ144, with a similarity rate of 98.43% and 98.06% to Om19, and HJ106 99.05% to *Pezicola ericae* were tested to examine their role and potential in ameliorating the adverse effects of high-temperature stress on *R. moulmainense*. The findings demonstrated that high-temperature stress decreased chlorophyll fluorescence, chlorophyll contents, and photosynthetic rates, which were validated by the damaged chloroplast structure in transmission electron microscopy and reduced leaf stomatal traits in scanning electron microscopy. The results showed that the EMF treatments promoted soil physiochemical properties, enzymatic activities and enhanced fungal and bacterial community abundance, composition, and functional pathways. Transcriptome and metabolome analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment upregulated differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) of the starch-sucrose metabolism and ABC transporters metabolism pathways under high-temperature stress of 40ºC to reveal the synergistic effects of EMF regulating these pathways. The findings revealed that DEGs and DAMs in starch-sucrose metabolism, encoding SUS, TPS1, BAM1, sucrose, D-ribose, and D-fructose, and ABC transporters such as ABCC1, ABCC2, ABCG2, Thiamine, and Betaine were significantly higher in EMF treatment under T42 to regulate the plant growth compared to HT treatment without EMF. These results may help us better understand the growth regulation and domestication processes of Rhododendron under high-temperature stress scenarios.

Decoding SPX4 Proteins and the proteostatic regulation of plant phosphate homeostasis

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Keywords: Phosphate homeostasis, SPX4 protein degradation, Proximity labelling (PUP-IT), Nutrient signalling, Ubiquitin-proteasome system

Abstract:

In response to changes in soil phosphate (Pi) availability, plants have evolved strategies to regulate Pi acquisition. Pi signalling and uptake hinges on SYG1/Pho81/XPR1 (SPX) domain proteins, which act as sensors of cellular phosphorus status and posttranslational co-repressors of PHOSPHATE STARVATION RESPONSE REGULATOR1 (PHR1) —a master transcriptional regulator of the Pi starvation response (PSR) [1, 2]. Co-repressor-PHR1 interaction and PSR repression enable plants from N-limited habitats to fine-tune Pi uptake and prevent overaccumulation. Here, we focus on SPX4, which acts primarily in shoots [2]. Unlike the well-studied isoforms SPX1 and SPX2 that act in the nucleus, SPX4 is subject to proteasomal degradation in P-limited Arabidopsis. In *Oryza sativa*, E3 ligases SPX4 DEGRADATION E3 LIGASE1 (OsSDEL1, Os12g35320), and its homolog OsSDEL2 were identified as regulators of OsSPX4 ubiquitination [3]. However, the functional conservation of SDEL homologs in Arabidopsis has not been confirmed. Considering the challenge of detecting short-lived protein interactions regulating protein turnover, we utilized a novel pupylation-based proximity labelling technique (PUP-IT) to detect transient interactions between target proteins *in vivo* [4]. We identified two members of the ubiquitin-specific protease (UBP) family, associated with protein turnover regulation, as potential interactors of SPX4. While low Pi availability targets SPX4 for proteasomal degradation, this process must be reversed upon Pi resupply to prevent toxicity and nutrient imbalance. The UBP deubiquitinases identified here may modulate SPX4 protein degradation. In depth characterization of SPX4 turnover will enhance our understanding of how plants regulate Pi uptake, utilization, and reallocation to maintain nutrient homeostasis, with implications for sustainable agricultural practices.

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Integrated transcriptional profiling of nodules reveals novel processes regulating symbiotic nitrogen fixation in legumes

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Keywords:
Legumes, symbiotic nitrogen fixation, nodule senescence, RNA-seq, DAP-seq

Abstract:

Leguminous plants form specialized root nodules with rhizobia that enable the conversion of atmospheric nitrogen into plant-available forms. Because nodules are the primary sites of symbiotic nitrogen fixation, manipulating the pathways that regulate their development and function offers a powerful strategy to reduce dependence on chemical fertilisers and promote more sustainable cropping systems. A key master regulator of this process is Fixation Under Nitrate (FUN), a TGA-family bZIP transcription factor, which in the presence of high nitrogen limits symbiotic nitrogen fixation by targeting nitrogen signalling and nodule senescence processes[1]. To further identify how FUN regulates nodule function in cowpea (*Vigna unguiculata*), we have integrated RNA-seq data from cowpea nodules treated with high nitrate together with DAP-seq datasets of FUN from cowpea, *Lotus japonicus*, and soybean (*Glycine max*). RNA-seq analysis identified 8731 genes that are differentially expressed in cowpea nodules in response to high nitrate, while the DAP-seq analysis identified 4899, 12036 and 11147 possible direct DNA binding sites for FUN and therefore regulation across the cowpea, lotus, and soybean genomes respectively. By integrating datasets, we can more confidently identify regulatory and biologically meaningful binding events, distinguish direct from indirect pathways, and pinpoint interactions conserved across species. These findings will then be further validated and expanded in upcoming RNA-seq analysis of lotus *ljfun* and CRISPR/Cas9 edited cowpea *vufun* knockout mutants, providing more causal evidence of the role of FUN in regulating nitrogen fixation, deepening our understanding of the genetic networks critical for symbiosis.

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Dissecting *sol35*: One Line, Multiple Mutations, and a Path to Mucilage Suppression

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Keywords: Suppressor, LEUNIG-HOMOLOG, Mucilage, Phenylpropanoid pathway, Floral defects

Abstract:

Upon hydration, myxospermous plant seeds release mucilage, a polysaccharide-rich gel that aids germination and protects against desiccation. In *Arabidopsis thaliana*, mucilage release is easily visualised using ruthenium red staining, and the availability of mutants makes it a powerful model for studying cell wall regulation. The transcriptional regulator LEUNIG_HOMOLOG (LUH) is essential for mucilage release through activation of MUCILAGE MODIFIED 2 (MUM2), and the *luh-5* mutant fails to secrete mucilage. To identify new regulators, we performed an EMS suppressor screen in the *luh-5* background and isolated suppressor of *luh-5 (sol35)*, which partially restored mucilage release. In addition to this suppression, *sol35* seeds displayed a yellow phenotype, and plants exhibited floral defects. Segregation analysis suggested that these phenotypes were caused by independent mutations. Whole-genome resequencing confirmed that the yellow seeds resulted from a mutation in TRANSPARENT TESTA4 (TT4), while the floral phenotype was due to a mutation in TOUSLED (TSL). Neither mutation accounted for the restored mucilage release, indicating that the causal *sol35* suppressor allele is distinct from TT4 and TSL. Ongoing fine mapping and chromosome walking are being used to identify this novel locus. These results establish *sol35* as a genetic entry point to uncover new components regulating mucilage release in *Arabidopsis*.

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Identification of a novel plant height QTL on chromosome 6H and its agronomic effects in field-grown spring barley (*Hordeum vulgare*)

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Keywords:
Semi-dwarf, field, stem, barley, genetics, QTL

Abstract:
Barley (*Hordeum vulgare L.)* is well adapted to Australia's climate but can be prone to mechanical constraints such as lodging, head loss and straw strength issues, which negatively impact yield and crop profitability. Since the Green Revolution, many semi-dwarf genes such as *semidwarf 1* and *ari-e.GP* have been discovered and used in Australian barley breeding, but they can be associated with negative pleiotropic effects. A breeding line showing an ideal phenotype with intermediate height and erect plant architecture was used as a donor parent to develop two biparental mapping populations. From phenotyping the RILs (recombinant inbred lines) across 6 environments, a novel quantitative trait locus (QTL) on chromosome 6H was identified that is stable across multiple environments. This QTL is associated with reduced height, resistance to lodging and tolerance to head loss compared to wild-type, and it does not negatively impact yield nor have any maturity effects. *QTL6H.1* has similar height to *sdw1.d* and an additive effect in reducing height, internode length and spike count. Genes underlying the *QTL6H.1* are being investigated using a candidate approach. This height QTL will be a valuable addition to the breeders' semi-dwarf gene toolkit.

WUSCHEL-D1 upregulation induces the formation of supernumerary floral organs in wheat

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Keywords:
Shoot apex meristem, bread wheat, inflorescence architecture, floral organs, grain yield

Abstract:

Wheat inflorescence architectures that deviate from the conventional unbranched spike, highly favoured during domestication, have the potential to increase grain yield. In contrast to the single grain-producing florets found in wild-type plants, multi-ovary (MOV) plants are characterised by the development of three fertile pistils per floret, all capable of forming grains, along with high frequency of fertile secondary spikelets. Here, we show that the dominant trait is caused by upregulation of a WUSCHEL homolog, *TaWUS-D1*, in the *Mov-1* locus on chromosome 2D. Genomic sequencing and RNA-seq studies revealed that the gene is flanked by a complex chromosomal rearrangement unique to MOV plants, which enables widespread upregulation of surrounding genes. Gene expression and *in situ* hybridisation data show that *WUS-D1* is suppressed in wild-type plants, but it is activated and overexpressed in spikelet primordia during early inflorescence development in MOV plants. Over-proliferation of stem cells was observed at these stages, inducing the development of larger meristems with competency to produce supernumerary organs. Mutagenised MOV lines that lost WUS-D1 function reverted to a single-ovary phenotype, demonstrating that *WUS-D1* is essential for the MOV trait. The insights gained into the genetic control of the additional inflorescence organs provide a foundation for the development of new alleles for breeding that may improve yield-related traits in bread wheat.

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From model species to crops: targeting root system architecture and reproductive development with a peptide hormone signalling pathway

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Keywords:
Root system architecture, peptide hormone receptor, cereal crop, reproductive development, barley

Abstract:

Crops with improved nutrient capture and utilisation are required to sustainably intensify crop productivity to meet future food demands[1]. Work primarily in dicots has revealed important roles for peptide hormones from the C-TERMINALLY ENCODED PEPTIDE (CEP) family and their receptor, CEP RECEPTOR 1 (CEPR1), in integrating root system architecture, overall plant growth, and reproductive success with nutrient cues[2-4]. It is unknown, however, if CEPR1 functions similarly in agronomically important cereal crops. To investigate cereal CEPR1 function, we used genetic approaches combined with root and shoot phenotyping[5]. Barley, rice, and maize CEPR1 orthologs were introduced into an Arabidopsis *cepr1* mutant to determine if root, vegetative, and reproductive phenotypes could be rescued. Consistent with an ability to perceive endogenous Arabidopsis CEPs, the cereal CEPR1s restored wild-type-like phenotypes to *Atcepr1*. To validate cereal *CEPR1* function, we knocked out *CEPR1* in barley using CRISPR-Cas9 which recapitulated *Atcepr1* root, shoot, and fecundity phenotypes. To gain insight into AtCEPR1 and HvCEPR1 ligand preference, we used a root growth inhibition assay to screen CEP peptides from either species on *Atcepr1* expressing barley or Arabidopsis CEPR1. AtCEPR1 favoured AtCEP3 due to divergence at the HvCEP *N*-terminus, whereas HvCEPR1 had a broader ligand preference, interacting with both Arabidopsis and barley CEPs. The results demonstrate CEP receptor function for cereal CEPR1s, roles for barley CEPR1 in root and reproductive development, and highlight the utility of Arabidopsis to identify functionally equivalent genes in cereal crops. Modulating CEPR1 signalling

components could provide new avenues for improving crop nutrient capture, utilisation and yield.

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Exogenous Gamma-Aminobutyric Acid Application Delays Onset Of Ripening in Grenache, Chardonnay and Shiraz Grape Berries

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Keywords:
antioxidants, berries, delay, γ-aminobutyric acid (GABA), ripening, *Vitis vinifera*

Abstract:

Physiological and biochemical changes in *Vitis vinifera* L. (grape) berries during development have been extensively studied (Coetzee and du Toit 2012; Robinson and Davies 2000), yet the role of γ-aminobutyric acid (GABA), a non-protein amino acid (Shelp et al., 1999), is poorly understood but believed to play an important role in the plant's abiotic stress response. In this study, changes in GABA concentrations during berry development and the effect of exogenous GABA application on ripening was studied in three cultivars of grape berries. GABA accumulated concomitantly with sugar accumulation commencing at veraison in Chardonay, Shiraz and Grenache grape berries sampled from the Coombe vineyard over two seasons (2018–2020), but cultivar dependent variability was noted in hydrogen peroxide (H_2O_2) and antioxidant capacity. During berry development, levels of Reactive Oxygen Species (ROS) increase at the onset of veraison and gradually decrease as berries develop. However, under abiotic stresses such as drought and heat, excess accumulation of ROS results in poor quality berries (Pilati et al. 2014). Thus, treatments which can reduce ROS could delay ripening. In this study, veraison was delayed in berry bunches treated with 5 mM GABA weekly from the pea stage until veraison. Decrease in berry mass, total soluble solids, cell death, and increased antioxidant capacity was observed in berries treated with exogenous GABA. The findings from two seasons suggest that GABA may modulate ripening by interacting with antioxidant systems to detoxify ROS and this treatment could be useful in modulating berry response to climatic changes.

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Mutation in the strigolactone *D53* gene paralogues improve adaptation to low N conditions in barley

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Keywords:
CRISPR, hormone signalling, plant architecture, nitrogen, yield

Abstract:

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 cultivars which can grow with less fertiliser inputs. Strigolactone (SL) is an important hormone in plants that controls plant architecture, by inhibiting branching, and enable more efficient resource use in sub optimal conditions. We created barley plants with mutated strigolactone *d53* signalling mutants (Hv*d53a-153* and *Hvd53b+1*) using site directed nucelogensis-1 gene editing at the University of Adelaide, Waite campus. The performance of these lines was assessed under low and high nitrogen conditions in a replicated greenhouse experiment to determine their nitrogen use efficiency (NUE). The results revealed major differences between these mutants and the wildtype in respect to tillering, flowering, biomass accumulation, yield and seed morphology. The results revealed that the mutants can maintain tillering (a factor of yield) and accumulate more biomass under low N condition compared to the wildtype barley. Further assessment through transcriptome analyses shows that the mutants are highly enriched in pathways that are associated with adaptation to low N. Therefore, these findings suggest that barley plants with a mutated *d53* signalling pathway can have more efficient use of resources under sub optimal condition. This research is aimed at using gene editing to introduce nitrogen use efficiency (NUE) into barley

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From the sea to the pond: Lipid profiling and biosynthesis of VLC-PUFAs in duckweeds

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Keywords:
Duckweeds, Turions, Omega-3 fatty acids

Abstract:

Duckweeds (Lemnaceae) have been proposed as a promising candidate for future plant-based nutrition and a bioreactor for biomolecule synthesis[1]. While they are rich in protein, fibre and micronutrients, some key dietary components are still missing. Lipids are an important source of calories, and specifically omega-3 very long-chain polyunsaturated fatty acids (VLC-PUFAs) play an important role in inflammatory reactions, carcinogenesis, and thrombosis[2]. Omega-3 can be divided into marine sources that provide eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and plant sources that provide alpha-linolenic acid (ALA), with EPA/DHA more bioavailable in a human diet. Here, I profiled the fatty acids present in different duckweed species (*Lemna minuta, Wolffia australiana* and *Spirodela polyrhiza*) and developmental stages (fronds, turions and seeds) as a foundation for further modification. Palmitic acid (C16:0) and oleic acid (C18:1) levels increased significantly in phospholipids of fronds and turions compared to total fatty acids. These differences indicate acyltransferase preference in phospholipid metabolism. Furthermore, fatty acids unsaturation level in phospholipids of turions increased compared to the fronds, potentially as an overwintering mechanism to maintain membrane fluidity.

Subsequently, we transiently expressed genes which will boost both VLC-PUFA biosynthesis, and funnel conversion into bioavailable forms in *Nicotiana benthamiana, W. australiana* and *L. minuta*. The presence of novel LC-PUFAs including EPA and its precursors were detected via gas chromatography flame

ionization detector (GC-FID). Future work will include iterative engineering to enhance the conversion of EHA to DHA, and profiling of other lipid species such as galactolipids and glycosyl inositol phosphoryl ceramides (GIPCs).

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The Impact of Subcellular Localisation on AmA1 Protein Accumulation in *N. benthamiana* and *L. sativa* leaves

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Keywords:
Recombinant protein expression, Plant-based protein production, Subcellular targeting, Amaranth Albumin 1 (AmA1), Space nutrition

Abstract:

The rising demand for high-quality protein in the human diet has emerged not only from issues of famine and malnutrition across the globe, but also with recent developments relating to interplanetary space travel. Protein sources such as animal meat come with various environmental limitations in its production, while being impractical for consumption in space. As an alternative, plants can be harnessed as hosts for the recombinant expression of nutritional proteins, offering a highly scalable and convenient source of protein. Here, subcellular targeting is explored as an approach to improve the accumulation of a nutritionally balanced seed storage protein, Amaranth Albumin 1 (AmA1), in both *Nicotiana benthamiana* and *Lactuca sativa* leaves, alongside the co-expression of LEAFY COTYLEDON 2 (LEC2), a transcription factor found to increase recombinant protein accumulation when ectopically expressed. Using tGFP as a proxy to quantify AmA1 protein expression, an AmA1-tGFP fusion protein was targeted to the cytosol, apoplast, ER, lytic, and protein storage vacuoles of *N. benthamiana* leaves, with the protein appearing to favour cytosolic localisation by almost 1-fold. Interestingly, confocal microscopy revealed that LEC2 co-expression also induced the formation of protein storage vacuole-like structures, usually found exclusively in seeds. The transformation of AmA1-tGFP constructs into *L. sativa* protoplasts indicates increased expression over time, but can be improved with co-expression of ratiometric controls. In future work, the integration of AmA1-tGFP into viral vectors will significantly improve its accumulation, eventually allowing for the development of high-expressing nutritional protein in plants that can be consumed on Earth or in space.

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Differential Regulation of Chloride and Nitrate Transport under Water Deficit: Role of At ALMT10 in Root-to-Shoot Ion Partitioning

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Keywords: AtALMT10, Nitrate, Stress and Ion Transport

Abstract:

Reprogramming ion and water homeostasis is a key determinant of plant and crop acclimatisation to water deficit (WD). Several fundamental processes orchestrated by the stress phytohormone abscisic acid (ABA) are involved, including stomatal closure and a reduction in xylem-mediated root-to-shoot translocation of ions implicated in root osmoregulation. For example, in the presence of ABA, the expression of the *Stelar K^+ Outward Rectifier* (*SKOR*) channel[1] and that of the slow-type anion channel *SLAH1*[2] are strongly inhibited, leading to increased accumulation of Cl^- in the root[2] and possibly of K^+, thereby hypothetically facilitating the rapid recovery of osmotic potential and root growth in a dry soil. In contrast to Cl^-, NO_3^- translocation is either unaffected[2] or even increased after WD or exogenous ABA application. Previous *in silico* analyses of microarray and RNA-seq data identified *AtALMT10* as an anion channel highly co-expressed with *AtSLAH3*, *AtSLAH1* and *SKOR* in the xylem-pole pericycle cells of *Arabidopsis thaliana*. Unlike the genes encoding the stelar K^+ and Cl^- channels, *AtALMT10* expression is induced by WD, leading to the hypothesis that *ALMT10* may function as a NO_3^- channel that facilitates its xylem translocation under drought conditions. We propose that under WD, Cl^- is preferentially used for root osmoregulation, whereas NO_3^- is preferentially transported to aerial organs to maintain nitrogen (N) availability for the biosynthesis of N-containing protective compounds such as amino acids (e.g.: GABA), glycine betaine and polyamines.

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Uncovering nitrogen-responsive regulation of dhurrin biosynthesis in Sorghum bicolor

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Keywords:
Dhurrin, cyanogenic glucoside, specialised metabolism, nitrogen, *Sorghum bicolor*

Abstract:

Dhurrin, a cyanogenic glucoside, plays key roles in *Sorghum bicolor* physiology and defence, yet the molecular basis of its regulation remains poorly understood. Dhurrin accumulation is highly responsive to nitrogen status[1] and biochemical evidence supports a role for dhurrin in nitrogen storage[2,3], suggesting coordination between nutrient signalling and specialised metabolism. This study investigated the transcriptional regulation of dhurrin biosynthesis during nitrogen resupply following nitrogen limitation. A time-course RNAseq experiment was conducted across eight time points (0 - 60 hours) alongside measurements of dhurrin concentration, growth, and nitrate content. Nitrogen resupply led to significant increases in dhurrin levels in leaves and sheaths, with expression of the three dhurrin biosynthesis pathway genes (*SbCYP79A1*, *SbCYP71E1*, and *SbUGT85B1*) upregulated in leaves from 24 hours onward. Gene regulatory network (GRN) analysis identified candidate transcription factors (TFs). Two of these candidate genes were further characterised using transient expression assays in *Nicotiana benthamiana*. Results will be presented that clarify the timing and tissue specificity of nitrogen-responsive dhurrin regulation and the identification of a promising candidate linking nitrogen signalling with specialised metabolism in sorghum. This work provides new insight into the transcriptional control of cyanogenic glucoside biosynthesis and establishes a foundation for functional studies of regulatory integration between nutrient availability and defence metabolism.

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A unique PGRL1 paralog evolved in grasses offers faster photoprotection

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Keywords:
PGRL1 paralogs, Grass Evolution, C_4 Photosynthesis, Rapid PSI Photoprotection

Abstract:

The expansion of grasses into open habitats in the early Miocene transformed terrestrial ecosystems but required the development of photoprotection mechanisms better protecting plants under high and fluctuating irradiance[1,2]. The PGR5/PGRL1 pathway is critical for the adaptation of angiosperms to fluctuating light[3], but functional roles of the two PGRL1 paralogs found in grasses remained unknown[4]. Here we show that a grass-exclusive PGRL1 paralog, PGRL1β, is enriched in bundle sheath cells of NADP-ME C_4 grasses. Contrastingly, an ancestral PGRL1 paralog, PGRL1α, conserved across land plants, is enriched in mesophyll cells of NADP-ME C_4 grasses. By generating CRISPR/Cas9 gene-edited *Setaria viridis* (a model NADP-ME C_4 grass) lacking either PGRL1 paralog, we demonstrate that PGRL1β enables 5 s faster protection of Photosystem I compared to PGRL1α in response to increase in irradiance. Using protein structural modelling, we suggest that these diverged functions are underlined by the distinct lumenal regions of each paralog, suggesting lumen pH–dependent regulation of PGRL1α, but not PGRL1β. This difference in regulation can confer distinct speed of triggering photoprotection because of the known delay in establishing low lumenal pH during increases in irradiance due to the dynamic partitioning of proton motive force between proton and electrical gradients. A dual PGRL1 system is grasses enables PGRL1β rapidly respond when maximum photoprotection is required, complementing the slower-acting PGRL1α balancing photoprotection and productivity. This innovation likely

enhanced grass resilience to dynamic light environments and contributed to the ecological dominance of grasslands globally.

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Molecular cloning and characterisation of a new restorer gene for hybrid breeding in wheat

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Keywords:
Hybrid breeding, *Triticum aestivum*, Cytoplasmic male sterility (CMS), *Restorer*-of-*fertility (Rf)* gene, mitochondrial gene expression.

Abstract:

Hybrid breeding is a core strategy to increase crop yield and resistance. For example, maize hybridization traditionally relies on manual detasseling to prevent self-pollination. However, this approach does not work in wheat because its numerous florets are densely packed within the spikelets. Cytoplasmic male sterility (CMS) refers to a mutation in the mitochondrial genome, leading to plants producing nonfunctional pollen and causing male sterility. CMS can be used as an alternative tool in hybrid breeding. To restore fertility, nuclear-encoded Rf proteins suppress the expression of CMS-causing mitochondrial genes. CMS/*Rf* systems are widely used in rice and maize. However, the establishment of a CMS/*Rf* system in common wheat (*Triticum aestivum*) remains challenging. In wheat, the main CMS type is T-CMS derived from *Triticum timopheevii*, and eleven *Rf* genes have been found to be able to restore fertility of T-CMS plants. Among them, *Rf1* and *Rf3* were recently cloned and shown to restore fertility by cleaving transcripts of the CMS-associated gene *orf279* in the mitochondria, but the restoration is often incomplete in elite lines. To better understand the molecular basis of fertility restoration in wheat, this study will focus on the molecular cloning and characterisation of another *Rf* gene, called

Rf4. In contrast to *Rf1* and *Rf3* restorer genes, which are mapped on chromosomes 1A and 1B, respectively, the *Rf4* locus has been mapped on chromosome 6B, and it originates from *Aegilops speltoides.* Further understanding of *Rf* genes will advance the development of more effective CMS/*Rf* systems for wheat hybrid breeding.

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Uncovering the molecular function of Mitochondrial Transcription Termination Factors in *Arabidopsis thaliana*

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Keywords:
Mitochondrial transcription termination factors; mTERFs, *Arabidopsis thaliana*; mitochondrial RNA metabolism.

Abstract:

Mitochondrial transcription termination factors (mTERFs) form a highly conserved protein family that regulates multiple aspects of organellar gene expression. In higher plants, several mTERFs have been shown to localise to chloroplasts or mitochondria, where they regulate gene expression, and contribute to plant development and responses to environmental stress. Comparative genomic studies in cereals such as wheat and rye recently revealed a novel sub-group of mTERFs referred to as Restorer-of-Fertility like mTERFs (RFL-mTERFs), whose homologues are evolutionarily linked to restoration of fertility in plants carrying cytoplasmic male sterility (CMS) genes. CMS is a mitochondrially inherited trait that causes pollen defects and male sterility, and RFL genes in crops can suppress or modify expression of CMS-associated open reading frames to restore fertility. In contrast, *Arabidopsis thaliana* doesn't possess a naturally occurring CMS system yet contains homologous RFL-mTERFs predicted to localise to mitochondria and function primarily in RNA metabolism.

Three Arabidopsis RFL-mTERFs with strong predictions for mitochondrial targeting and high floral expression were selected for investigation. Subcellular localisation, mutant characterisation and expression profiling collectively confirmed mitochondrial targeting, high transcript levels in reproductive tissues and no obvious growth or fertility phenotypes in knockout lines. Although no phenotypic differences were observed, ongoing RNA analyses will help to determine whether subtle molecular effects occur.

By integrating these approaches, this study will help to understand the molecular function of RFL-mTERFs in Arabidopsis and it will provide a model for future research on this clade in crops where CMS and RFL-mTERF genes are applied in hybrid breeding to control plant self-pollination. High-yielding hybrid crops will help to meet future food demands of the growing human population.

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PLANT TIMEKEEPERS:
How the Circadian Clock Shapes Stomatal Movements in Non-Flowering Plants

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Keywords:
Circadian clock, stomata, nonflowering plants, transpiration, stomatal conductance.

Abstract:

The plant circadian clock is a biological timekeeper, synchronising physiology with daily environmental cycles. While circadian regulation of stomatal conductance is well studied in angiosperms, its role in other land plants is still being elucidated[1,2,3]. We investigated circadian regulation of stomatal opening in the lycophyte *Selaginella moellendorffii*, and ferns *Adiantum aethiopicum* and *Marsilea mutica*, in comparison with the angiosperm *Arabidopsis thaliana*. Plants were entrained with 12:12h day/night cycles before transfer to continuous darkness to test for circadian-driven stomatal opening responses. Whole plant transpiration was measured gravimetrically[4] for two day/night cycles, followed by 48h in the dark. Stomatal opening speed was further assessed at subjective dawn and dusk using the LI-COR Li-6400, recording stomatal conductance in response to light until steady maximum conductance. Unlike Arabidopsis, which maintained a clear circadian rhythm, with increasing transpiration at subjective dawn, *S. moellendorffii* and *A. aethiopicum* did not exhibit circadian-driven stomatal opening under continuous darkness. *M. mutica* exhibited transpiration that initiated later in the subjective day compared to Arabidopsis, a delay which persisted under 8h or 16h day lengths. Furthermore, unlike Arabidopsis, where the speed of stomatal opening was significantly higher at subjective dawn compared to dusk, opening speed in the ferns and lycophyte remained consistent regardless of time. These results indicate that strong circadian regulation of stomatal opening and some aspects, including stomatal priming, are

unique to flowering plants, broadening the understanding of the evolution of mechanisms controlling stomatal movement.

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Targeted genetic manipulation and yeast-like evolutionary genomics in the green alga *Auxenochlorella,* a new model for plant science and bioengineering

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Keywords:
green algae, site-specific transformation, chlorophyll biosynthesis, evolutionary genomics, hybridisation

Abstract:

Green algae present excellent opportunities for fundamental research in plant science and are highly amenable to bioengineering. *Chlamydomonas* has served as the primary algal model for a century, although site-specific genetic manipulation and consistent transgene expression have long been challenging. Here, we present *Auxenochlorella*, a genus of oleaginous and mixotrophic green algae whose streamlined genomes can be readily manipulated by homologous recombination, enabling the targeted editing, addition or removal of genes[1,2]. We produce a telomere-to-telomere phased diploid genome assembly of *Auxenochlorella* UTEX 250 (haploid length 22 Mb, ~7,500 genes) and introduce a genetic toolkit for site-specific manipulation of the nuclear genome in multiple strains, featuring several selectable markers, inducible promoters, and fluorescent reporters for protein

localization. We demonstrate the utility of *Auxenochlorella* for fundamental research by knockout of a chlorophyll biosynthesis enzyme. Furthermore, we demonstrate that *Auxenochlorella* is subject to several evolutionary phenomena that are associated with vegetatively diploid organisms such as yeast: allodiploid hybridisation, mitotic recombination, loss-of-heterozygosity and aneuploidy. UTEX 250 is an allodiploid hybrid of *Auxenochlorella protothecoides* and *Auxenochlorella symbiontica*, two closely related species differentiated by extensive chromosomal rearrangements. UTEX 250 haplotypes are a mosaic of each parental species following mitotic recombination, and loss-of-heterozygosity events are pervasive across *Auxenochlorella* and can evolve rapidly in the laboratory. Two UTEX-250 chromosomes are trisomic, which we confirm by allele-specific transformation. These results demonstrate the generality of several evolutionary forces associated with vegetative diploidy and provide a foundation for use of *Auxenochlorella* as a reference organism in plant science and bioengineering.

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Jumping into action: discovery of novel promoters from plant transposable elements

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Keywords:
Promoters, synthetic biology, plants, recombinant genes

Abstract:

Plants are extraordinary platforms for developing synthetic biology tools and as a chassis for manufacturing valuable bioproducts. Reconstructing pathways to synthesize bioproducts require effective and robust expression of multiple recombinant genes. Gene expression is controlled by promoters and the utilization of different promoters in a genetic construct prevents early silencing from the plant cell. However, plant biotechnology has mostly relied on the use of legacy viral-based 35S and CmYLCV promoters and promoters from endogenous genes such as *UBIQUITIN* and *ACTIN*, lacking a comprehensive collection of other efficient promoters. Transposable elements, mobile DNA that integrates into genomes, contain intrinsic promoters called Long terminal repeats (LTRs). LTRs are used by transposable elements to drive the expression of their own genes but are often silenced by the host as a defence mechanism against transposition. However, past studies have found that exogenous transposons escape identity-based silencing when introduced into a different host. In this study, we showed that a heavily methylated LTR promoter in the duckweed, *Wolffia australiana*, is an active promoter in *Nicotiana benthamiana* leaves and drove transient expression of a *GFP* reporter gene. LTR retrotransposons are diverse and widespread in plant genomes and, hence, these results led to further exploration of LTR promoters from multiple different plant species and engineering thereof for improved activity *in planta*. Through this work, we hope to expand the repertoire of promoter elements in the plant synthetic biology toolkit.

GABA Supplement Influences Basal ROS Production in Guard Cells of *Arabidopsis thaliana*

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Keywords:
GABA, ROS, Guard cell, Redox state, Signalling

Abstract:

Reactive oxygen species (ROS) are central regulators of guard cell signalling (1,2), and their dynamics are tightly modulated by metabolic pathways such as the γ-aminobutyric acid (GABA) shunt (3). While GABA is known to influence ROS production, its dose-dependent effects in wild-type guard cells remain unclear. We quantified ROS accumulation in guard cells of Arabidopsis wild type plants exposed to exogenous GABA at different concentrations (2, 5, and 10 mM) for 10 min and compared this to basal ROS at 0- and 10-min. Basal ROS levels showed a modest increase within 10 min. Comparing to ROS content at 10 min mark, 2 mM GABA further elevated ROS fluorescence intensity by more than 35% higher, 5 mM GABA, retained 27% higher ROS. However, 10 mM GABA decreased ROS content 18% lower compared to 10min mark. Therefore, guard cell ROS production was reduced by 10 mM GABA supplement, lower than the content under 2- and 5-mM supplement at 0 min.

These results demonstrate that apoplastic GABA accumulation influences basal ROS accumulation in guard cells in a **dose-dependent and non-linear manner**: moderate GABA concentrations enhance ROS generation, while more than 5mM extracellular GABA brings down ROS production. This work provides new insight into how GABA contributes to redox homeostasis in Arabidopsis guard cells and establishes a foundation for future studies on the integration of GABA–ROS signalling in stomatal regulation.

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RGG/RG-rich RNA-binding Proteins: What is their Role in in Salt Tolerance?

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Keywords: protein translation, salt stress, RNA-binding proteins, halophytes, salt tolerance

Abstract:

Soil salinization is an increasing threat to global agriculture, reducing arable land and limiting crop yields (1). High concentrations of sodium chloride (NaCl) in the soil lead to osmotic stress, ion toxicity, and nutrient imbalances in most glycophytic (salt-sensitive) plants, ultimately stalling protein synthesis and growth (2, 3). A major consequence of salinity stress is the destabilisation of the translation machinery, resulting in reduced ribosome engagement and lower translational efficiency (4). In contrast, halophytic species such as *Salicornia bigelovii* maintain robust translation even under high salt conditions. Recent studies have implicated RNA-binding proteins (RBPs) - particularly those rich in RGG/RG motifs - in mediating this resilience by stabilising ribosomes, binding RNA, and facilitating phase separation during stress responses.

Our team's prior research demonstrated that SbiSALTY2, an RGG/RG-rich RBP from *S. bigelovii*, enhances salt tolerance in a salt-sensitive yeast mutant AXT3, suggesting its involvement in protecting translational machinery during salinity stress (5). Comparative analysis of RGG/RG-rich RBPs across taxa revealed four hydrophobic amino acids within the STM1 domain, as well as three RGG/RG-rich regions that were highly conserved between *SbiSALTY* and the Arabidopsis *AtRGGA* ortholog. When expressed in a heterologous system, we discovered that mutations in the second RGG/RG-rich region of *AtRGGA*, and within all three RGG/RG-rich regions in *SbiSALTY* significantly impaired salt-rescue of yeast AXT3 strain.

By elucidating how SbiSALTY2 supports translation under salt stress, this project will advance our understanding of stress-adaptive RBPs and provide new molecular tools for engineering salt-tolerant crops - an essential goal for food security under climate change and soil degradation.

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Advancing CRISPR based gene editing to create tomato male sterile lines for F1-hybrid seed production in Australia

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Keywords:
Tomato, breeding, male sterile line, CRISPR, F1 hybrid seed

Abstract:

Plant breeding has played a foundational role in the advance of human societies[1]. F1 hybrid seeds remain central to commercial annual crop production because of their many advantages, including hybrid vigour and uniformly high yield potential[2]. The fundamental principle of F1 hybrid seed production is controlled pollen transfer from one inbred parent to the stigma of another inbred parent[2]. This process poses significant challenges in crop breeding, however, as self-fertile species will predominantly self-pollinate. Preventing self-pollination is essential in these species in order to enable hybrid seed production. One labour intensive mechanism for ensuring outcrossing in the breeding process is to manually emasculate the female parent[3]. This method is labour intensive and not always effective. The use of male sterile lines, where flowers produce no or unviable pollen, presents a more efficient solution for large-scale hybrid seed production[4]. This project aims to develop a male sterile tomato line as a step towards enabling domestic F1 hybrid seed production in Australia. Based on the critical role of male sterility in hybrid breeding system, this project is generating a male sterile tomato line through biotechnology and tissue culture techniques. To ensure the resulting male sterile line has the potential to be integrated into F1 hybrid breeding programs in Australia, the system was designed with two criteria: (1) the male sterile seeds must be easily identified and suitable for storage, and (2) the male sterile line must be free of foreign DNA.

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Identification and functional characterization of *VsDET1* in Common vetch

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Abstract:

Global protein demand is expected to double by 2050, yet current livestock-based systems are environmentally unsustainable. Plant proteins offer a viable alternative, but cereal crops such as wheat are increasingly vulnerable to drought under climate change. Legumes provide greater resilience, and *Vicia sativa* (common vetch) is a promising candidate, combining drought tolerance, high seed protein content (24–32%), a balanced amino acid profile, and ecological benefits via nitrogen fixation. However, vetch adoption is constrained by the accumulation of two non-proteinogenic amino acids—β-cyano-L-alanine (BCA) and γ-glutamyl-β-cyano-L-alanine (GBCA)—which limit its use in food and animal feed.

Using whole-genome sequencing, phylotranscriptomics and gene expression term analysis we identified *VsDET1* as a candidate gene regulating BCA and GBCA biosynthesis in vetch seeds. In transgenic vetch roots expressing *VsDET1*, BCA or GBCA were not detectable using sensitive mass spectrometry. Furthermore, we demonstrated in two vetch varieties, Languedoc and Blanchefleur, that accumulate high levels of BCA and GBCA *VsDET1* mRNA was not detectable using qRT-PCR. In Languedoc, we identified a miniature inverted-repeat transposable element (MITE) insertion in an exon of *VsDET1*. We named the MITE, ALEXA. Bioinoformatic analysis showed that there are only 7-8 copies of ALEXA in vetch genomes. Consensus analysis showed that ALEXA has 14 nt terminal inverted repeats and causes target site duplications of 8 bp. Using ALEXA TIR sequences, we identified ALEX relatives in other legume genomes like soybean. These findings uncover a genetic mechanism underlying anti-nutritional compound accumulation in vetch and provide a foundation for breeding protein-rich, low-toxin legume crops suited to climate-resilient agriculture.

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Uncovering the Role of GABA Metabolism in Regulating Organic Acid Profiles and Stomatal Dynamics in Duckweed

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Keywords:
Duckweed, GABA shunt, stomatal movement, malic acid

Abstract:

Duckweeds (*Lemnoideae spp.*) are rapid growing and highly nutritious aquatic plants, holding a great potential for sustainable food production for indoor farming. Recent research has demonstrated that γ-aminobutyric acid (GABA) metabolism contributes to stomatal regulation and organic acid (e.g., malic acid) storage in plants via negative regulation of tonoplast-localised Aluminium-activated Malate Transporters (ALMTs), fine-tuning water-use-efficiency (WUE). However, it remains unknown whether GABA exerts similar effect in duckweeds. Here, we employed duckweed genomic and single cell data to identify GABA synthesis (e.g., glutamate decarboxylases, GADs) and catabolism (e.g., GABA transaminase, GABA-T) homologs within the GABA shunt –the primary GABA metabolic pathway in plants– in two duckweeds genera *Wolffia australiana* and *Lemna minuta*. Meanwhile, we optimized the method for measuring stomatal pores and found that the stomata on daughter fonds of *Lemna minuta* responded more rapidly, than those on mother fonds, to closing signals, such as abscisic acid (ABA) and darkness, highlighting the frond age may influence sensitivity to environmental stimuli. This work is expected to leverage on modified GABA metabolism to fine tune stomatal behaviour. Furthermore, as GABA, glutamate and organic acids contribute flavour we will investigate how GABA modification could influence taste.

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Investigating the role of mucilage in rhizosheath using exudate-releasing cotton root analogues

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Keywords:
Rhizosheath, rhizosphere, mucilage, roots

Abstract
Rhizosheaths are coatings of soil physically bound to the roots of plants, typically angiosperms (York et al. 2016). These adaptations are known to regulate water uptake into the root system and protect the plant from drought stress. However, many knowledge gaps exist in the scientific literature, including the role of exuded mucilage in both rhizosheath formation and controlling water movement in the soil. To investigate the contribution of mucilage in rhizosheath formation, I ran experiments based on the method of Rahim et al. (2023), involving macrame strands buried in soil to act as root analogues wicking water or mucilage. The macrame strands wicked water or mucilage at field-relevant concentrations, with the resulting rhizosheath-like structures compared. To test for the presence of mucilage bridges, which are thought to assist in rhizosheath soil adhesion and water uptake, I used scanning electron microscopy and light microscopy of soil samples dosed with water or field-relevant mucilage concentrations. Root analogues (i.e. macrame strands) wicking mucilage had greater rhizosheath cover in soil and artificial soil than root analogues wicking water. The greater rhizosheath cover of root analogues wicking mucilage likely reflects the ability of mucilage to form bridges between particles, as observed by microscopy. The relationship between mucilage, rhizosheath cover and drought tolerance can inform the field of plant physiology and assist in plant breeding initiatives, especially in the development of drought tolerant crops.

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Decoding Natural Genetic Diversity for Flowering Time and Maturity in Australian Soybean Varieties via Whole Genome Sequencing

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Keywords:
Genetic variation, Flowering time, Days into maturity, E1-E4 genes, Australian Soybean

Abstract:

Soybeans are cultivated across a wide range of latitudes in Australia, with production largely determined by days to flowering (DTF) and days to maturity (DTM), which vary according to regional conditions. At present, benchmark soybean varieties are classified into only three maturity groups: early, mid, and late; however no genetic information is available on the control of flowering time, maturity, or other agronomic traits in Australian commercial cultivars. In this study, we performed whole-genome sequencing (WGS) of nine commercial soybean varieties and germplasm representing three maturity groups including early (Burrinjuck, New-Bunya), mid (Gwydir, Richmond, SCH-634411Y, A6785, Hutcheson), and late (Hayman, Kuranda). WGS identified candidate variant annotations in major loci associated with earliness, the long juvenile phase, and other agronomic traits. Analysis of the *E1–E11* maturity genes revealed functional and non-functional alleles, including the *e1-as* allele in Burrinjuck and New-Bunya and the *E2^I220V* missense mutation in Gwydir, Hutcheson, and Kuranda. Notably, intronic SNPs were detected in *E8* (New-Bunya, Hutcheson), while polymorphisms in *E9 (FT2a)* included the *E23D* substitution in Gwydir and the *S451fs* frameshift in Burrinjuck and Hayman. Variants in long-juvenile regulators (*Tof16-1a, Tof16-2a*) further highlighted their role in delaying flowering under short-day conditions. We also identified allelic variation in seed-quality genes, including those related to gelling capacity and hilum colour. Additionally, ANOVA revealed that SNPs in 25 flowering-related genes were significantly associated with flowering time and maturity in the sequenced lines. KASP markers were successfully developed to distinguish

between parental lines with contrasting phenotypes for maturity, hilum colour, and gelling capacity. These markers can be used in the future to validate SNP mutations in segregating populations.

Root carboxylate exudation enhances seed silicon accumulation and grain yield in barley under low phosphorus

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Keywords:
phosphorus-use efficiency; *Hordeum spontaneum*; *Hordeum vulgare*; Silicon; low-input agriculture

Abstract:

Phosphorus (P) deficiency is one of the most severe constraints limiting global crop productivity, especially in low-input agroecosystems. Silicon (Si) has emerged as a beneficial element enhancing plant stress tolerance and nutrient use efficiency, yet the mechanisms linking root exudation, Si accumulation, and crop yield under low P remain poorly understood. Here, we investigated the relationships between root carboxylate exudation, seed Si concentration, and grain yield in cultivated and wild barley (*Hordeum vulgare*) grown under P-deficient soils. Our results demonstrated that carboxylate release strongly promoted seed Si accumulation, particularly in wild barley genotypes, and this enhanced Si concentration was positively correlated with grain yield under low P. By contrast, cultivated barley exhibited weaker associations between root exudation, Si uptake, and yield. These findings reveal a novel interaction between root exudates and Si nutrition under P deficiency, highlighting

genotypic variation in the efficiency of this mechanism. Collectively, our study provides new insights into how root exudation contributes to nutrient interactions and yield stability, and underscores the potential of exploiting wild germplasm to improve P use efficiency and crop resilience in sustainable agriculture.

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Quantifying Nitrogen Fixation in Legumes using CRISPR-Derived Non-Nodulating mutants

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Keywords:
Biological Nitrogen Fixation, CRISPR/Cas9

Abstract:

Legume crops form symbiotic relationships with compatible rhizobia bacteria, reducing the need for additional synthetic fertilisers in agricultural systems. Quantifying the contribution of biological nitrogen fixation (BNF) remains challenging, as plants draw nitrogen from both soil and symbiotic sources. Generating non-nodulating mutants provides a useful baseline to quantify BNF in varieties that can nodulate, providing a useful tool for breeding programs.

Nodulation requires perception of signal molecules, called Nod Factors, by transmembrane LysM receptor kinases, called Nod Factor Receptors, located on host plant roots. Compatible Nod Factor signals initiate rhizobia recognition and subsequent nodule development, with knock-out mutations resulting in non-nodulating phenotypes.

We have identified NFR5 encoding gene orthologs in mung bean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and common bean (*Phaseolus vulgaris*). Using CRISPR/Cas9, novel vectors have been designed to target these regions. We are now generating knockout mutants in these species that can be used in future trials as a baseline for quantifying to what extent nitrogen is derived from biological nitrogen-fixation in these species. Updates on current progress will be presented.

AO1 Regulates Extrorse Stamen Morphology via Subcellular Localization in *Brassica rapa*

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Keywords:
Outcrossing, Filament, AO1, Localisation, *B.rapa*

Abstract:
To enhance genetic diversity and fitness, flowering plants have evolved a range of adaptive mechanisms that promote outcrossing, including self-incompatibility, herkogamy, heterostyly, and variation in stamen orientation. In the Brassicaceae family, stamen orientation occurs in two distinct morphs: extrorse and introrse. Extrorse orientation is considered the ancestral state**,** while introrse orientation represents a derived condition that reduces pollen dispersal and limit genetic diversity.

Here, we show that the extrorse stamen morph arises from filament twisting during cellular elongation immediately prior to bud opening in Brassica rapa. Prior to filament elongation, extrorse morphs exhibit an increased cellulose microfibril angle, suggesting that secondary cell wall architecture influences filament biomechanics and twisting. Interestingly, filament twisting is reduced at lower temperatures during filament elongation, indicating a potential environmental modulation of this trait.

In *B. rapa*, our unpublished data support a genetic model in which the extrorse stamen trait is governed by a single, dominantly inherited gene, designated Anther Orientation 1 (AO1)**.** To identify AO1, we employed a multi-faceted approach combining bulked segregant analysis (BSA) from plants of a F_2 mapping population, RNA-seq to detect filament-expressed genes, whole-genome sequencing of extrorse and introrse *Brassica* species, and biochemical prediction of gene function. This led to the identification of a single candidate gene, and genetic complementation experiments in *B. rapa* are currently underway to validate its function.

To investigate subcellular localization, we generated translational fusion constructs of the candidate AO1 proteins (extrorse and introrse variants) tagged with cerulean

fluorescent protein and transiently expressed them in *Nicotiana benthamiana* leaves. Interestingly, the extrorse AO1 fusion protein localized to both the cell periphery and nucleus, whereas the introrse variant was restricted to the cell periphery. Future experiments will aim to resolve the precise subcellular localization at the cell periphery and its functional implications for filament biomechanics and stamen orientation.

Protecting crops from lepidopteran insect pests using RNAi

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Keywords:
Crop protection, lepidopteran, dsRNA, RNAi

Abstract:

Spodoptera frugiperda, the fall armyworm (FAW), is a lepidopteran pest that causes devastating economic losses worldwide on a wide range of crops. FAW was first discovered in Australia in 2020 after spreading from the Americas to Africa in 2016. Several approaches to control crop infestations by lepidopteran pests, including FAW, are commercially available with conventional chemical insecticides the most widely used. Unfortunately, the long-term reliance on these insecticides has led to the development of resistant insect populations and negative environmental impacts. Effective crop protection strategies with new modes of action, safer for the environment and for human health, must be developed to mitigate against insect resistance. RNA interference (RNAi) can be considered as a viable alternative to chemical insecticides. RNAi represents an environmentally friendly pest management strategy due to its RNA sequence-specific mechanism of action. Recently, the development of transgenic crops expressing dsRNA specifically targeting essential genes of insect pests, known as trans-kingdom RNAi (TK-RNAi), has emerged as a promising technology for insect control. While RNAi

is effective with insect pests such as Coleopterans, Lepidopteran insects are notoriously difficult to control with RNAi initiated by ingested dsRNA, requiring effective delivery of intact dsRNA at relatively high doses. In our work, we describe new ways of plant-based dsRNA delivery to lepidopteran insect pests including FAW and the cotton bollworm, *Helicoverpa armigera*.

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Utilising RNA silencing to modulate cannabinoid biosynthesis in *Cannabis sativa*

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Keywords:
Cannabis sativa, Cannabinoid biosynthesis, MicroRNA (miRNA), Artificial microRNA (amiRNA), Spray-Induced Gene Silencing (SIGS)

Abstract:

Cannabis sativa (*Cannabis*) produces cannabinoids, unique bioactive molecules with therapeutic potential for use in the treatment of neurological disorders, cancer, epilepsy, diabetes, and bacterial infections (1). Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), the major cannabinoids, are synthesised from the precursor, cannabigerolic acid (CBGA), a precursor which also gives rise to minor cannabinoids such as cannabichromene (CBC) and cannabigerol (CBG). Increasing evidence supports the medicinal value of such minor cannabinoids, however, compared to THCA and CBDA, these compounds are produced in trace amounts.

MicroRNAs (miRNAs) are small molecules of regulatory RNA essential for the control of gene expression. Tightly conserved and highly abundant across plant species, miRNAs control critical developmental processes and adaptive responses to stress. Artificial miRNAs (amiRNAs) are engineered miRNA molecules designed to structurally mimic native miRNAs for the highly specific silencing of select target genes. To date, amiRNA technology has been successfully applied in plants to enhance resistance to pathogens and to drive adaptive responses to abiotic stress (2, 3). Delivery of amiRNAs has been achieved through the traditional genetic modification (GM) approach or via the novel avenue of Spray Induced Gene Silencing (SIGS) (4).

This project aims to: (i) profile the temporal changes in the miRNA landscape during female floral development of a high-CBD *Cannabis* variety, (ii) design and optimise a *Cannabis*-specific amiRNA to manipulate key steps in the cannabinoid

biosynthesis pathway, and (iii) experimentally validate the efficacy of our amiRNA technology in *Cannabis*. Collectively, this project will develop amiRNA technology for gene expression manipulation in *Cannabis* via a non-GM approach.

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Differential Roles of PGR5 and PsbS in Photoprotection of C4 Photosynthesis

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Keywords:
Photoprotection, C_4 Photosynthesis, Light Stress, PGR5, PsbS

Abstract:

In C_3 species, the PGR5 and PsbS proteins both contribute to photoprotection under light stress by regulating energy-dependent non-photochemical quenching (qE)[1,2]. In C_4 plants, a carbon concentrating mechanism which operates across mesophyll and bundle sheath cells makes the regulation of photosynthesis more complex[3]. To elucidate the role of these proteins in C_4 photosynthesis, we have generated gene-edited *Setaria viridis* lacking PGR5 or PsbS. PGR5 is less critical under constant growth irradiances but is essential for maintaining efficient photosynthesis and growth under fluctuating light. In the absence of PGR5, plants cannot build up the proton motive force (*pmf*) required to trigger qE and have an overreduction at the donor side of Photosystem I, resulting in increased ROS production in both cell types and causing photodamage. To overcome this deficit, PGR5-deficient plants instead engage slow-relaxing, likely zeaxanthin-dependent, non-photochemical quenching. In contrast, PsbS-deficient plants exhibit less severe photodamage and minimal growth impairments under either constant or fluctuating growth irradiances. In the absence of PsbS, plants are deficient in qE, but still build up *pmf* and maintain oxidisation at the donor side of PSI. Therefore, PGR5 is more important for maintaining photoprotection in C_4 photosynthesis as it is required for both qE and photosynthetic control, while PsbS is more dispensable as photosynthetic control can still protect plants in its absence. Our results are informative of the various mechanisms of photoprotection employed by C_4 plants, and provide insight into the role these proteins may have played in the evolution of C_4 photosynthesis.

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Contrasting Sodium Management Strategies Underpin Salinity Tolerance in Rice (*Oryza sativa* L.)

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Keywords:
Salinity, rice, leaf tissue tolerance, ion exclusion, osmo-protection.

Abstract:

Rice is a staple cereal that ensures global food security, nourishing more than half of the world's population. However, it is highly salt-sensitive, suffering up to 50% yield reduction when exposed to soil electrical conductivity of 6 dS m^{-1}. Therefore, improving salt tolerance in rice is essential to sustain productivity and secure food supplies under increasing soil salinity stress. Identifying salt-tolerant rice genotypes is crucial for developing resilient varieties. We assessed the salt tolerance of seven contrasting rice genotypes under three levels of NaCl stress. We measured changes in photosynthetic parameters, chlorophyll content, inorganic (Na^+,K^+) and organic (proline, sugar) osmolyte accumulation, membrane stability, growth and yield. The results showed that Vyttila 10 performed well under salinity, maintaining higher chlorophyll content, osmolyte accumulation, photosynthetic efficiency, cell membrane stability, and yield compared with the other genotypes. In contrast, Pournami experienced the greatest reduction in growth, physiological performance, and yield, and accumulated excessive sodium in its leaves. Further analysis of leaf tissue tolerance revealed that Kamini, a landrace from mangrove regions, was able to accumulate high sodium in its leaves without compromising chlorophyll content, whereas the otherwise tolerant Vyttila 10 exhibited a sharp decline in chlorophyll under high sodium. These findings suggest that Vyttila 10 functions as a sodium excluder, limiting sodium entry into leaf tissue, while Kamini operates as a sodium includer with strong leaf tissue tolerance, making it a valuable genetic resource for developing leaf tissue tolerant rice varieties.

Title: Lessons from the wild: heat and drought tolerance in rice relatives.

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Keywords:
Climate change, abiotic stress, climate resilience, T_{opt}

Abstract:

Climate change poses a serious threat to the sustainability of global rice production, a staple food for over half of the world's population[1]. Raising temperatures and erratic rainfall leading to drought are the most potent abiotic stressors that severely impact productivity[2]. Exploring the natural variation within wild rice relatives offers a promising avenue for enhancing the climate resilience of rice.

The study aimed to characterize the heat and drought resilience of four wild rice species (*Oryza australiensis, O. meridionalis, O. rufipogon* and *O. nivara*). Rice variety *Kitaake,* was used as a commercial control. The overarching aim of our project is to: (1) determine their thermal optima (T_{opt}), (2) assess their photosynthetic responses to a heatwave, and (3) evaluate growth and recovery under severe drought.

Three separate experiments were conducted in controlled glasshouse environments (1) a temperature response experiment, to determine T_{opt} for each species; (2) a 5-day heatwave (45/35°C day/night) experiment; and a drought experiment, where plants were subjected to severe soil water deficit (60-80 % SWD) for 5 weeks followed by a 2-weeks recovery period. Photosynthetic gas exchange was measured from each species in each experiment.

The wild species displayed variation in thermal tolerance, with *O. nivara* exhibiting the highest T_{opt}. Under heatwave conditions, *O. australiensis* and *O. nivara* maintained stable photosynthetic rates. Interestingly, *Kitaake* also performed well under heat stress. However, *Kitaake* showed high drought susceptibility, exhibiting a 77% loss in total aboveground biomass. In contrast, both *O. australiensis* and *O. nivara* maintained superior physiological function under severe drought conditions.

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Development of cell-specific optogenetic tools for studying guard-cell signalling

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Keywords:
optogenetics, guard cells, stomatal movements.

Abstract:

Controlled indoor growth facilities are being developed for growing plants in space stations as well for sustainable agriculture on earth. Australian agriculture uses more than 70% of the water resources, with the majority of this being transpired through stomatal pores, surrounded by guard-cell pairs[1,2]. Transmembrane ion fluxes across guard cells regulate stomatal opening and closure[3]. Recent advances in optogenetics, particularly the use of channelrhodopsins (CHRs)—light-gated ion channels– have enabled the discovery of novel guard-cell signalling elements during stomatal movement[4]. However, current plant optogenetic approaches typically involve constitutive *CHR* overexpression throughout the whole plant, which may introduce artefacts when studying cell-specific signalling, especially when activating light illuminates large area of leaves, likely activating signalling events from both mesophyll and guard cells[4]. To address this, we designed and built a guard cell-specific promoter to drive *CHR* expression in guard cells, thereby facilitating stomatal signalling studies without perturbing signalling regulation in other cell types[5]. Unlike in stable transgenic lines which had guard cell specific expression, *Agrobacterium tumefaciens*-mediated transient expression failed to restrict CHR expression specifically to guard cells in *Nicotiana benthamiana* leaves. Instead, expression was strong in the epidermal layer but absent from guard cells. These results suggest that transient expression is not an ideal system for studying guard-cell-specific signalling, and processes have to be studied following selection of homozygous stable lines being isolated.

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

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Keywords:

1. Common vetch (*Vicia sativa*)
2. Plant-based protein
3. Anti-nutritional compounds (β-cyano-alanine, γ-glutamyl-β-cyano-alanine)
4. Genome assembly and transformation
5. CRISPR genome editing

Abstract

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 of 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, Riley 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 market.

To accelerate common vetch improvement we built a (1) high-quality chromosome-level reference genome (1.65 Gbp, n=6), assembled using Oxford Nanopore, Illumina, and Hi-C data, with 98% completeness and annotated 53,218 protein-coding genes (Xi, Nguyen 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 and Searle 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.

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Developing a model system for Functional Characterisation of PGRL1 Paralogs

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Keywords:
Photosynthesis, Photoprotection, CRISPR-Cas9, gene-editing

Abstract:

Photosynthesis is the basis of plants development and productivity but it is often limited by the process of photoprotection which dissipates some of the light energy absorbed by leaf as heat [3,4]. The PGRL1 and PGR5 proteins are the main regulators of photoprotection. While all land plants apart from grasses have one PGRL1 paralog encoded by the *PGRL1Alpha* gene, grasses have evolved the second paralog encoded by *PGRL1Beta.* Previous research suggests that PGRL1Beta might enable faster photoprotection and therefore it could have facilitated inhabiting of open, high-stress environments (grasslands) [4].

To investigate the roles of PGRL1Alpha and PGRL1Beta in photoprotection, we are developing *Arabidopsis thaliana* model system allowing fast swapping between PGRL1 paralogs. We have targeted Cas9 to *PGRL1A* and *PGRL1B* (the two copies of *PGRL1Alpha* in *A. thaliana*) to generate knockout alleles and selected plants lacking native PGRL1s. Alternatively, we have generated *A. thaliana* plants overexpressing *PGRL1Alpha* or *PGRL1Beta* sequences from grass *Setaria viridis* from the same promoter. Crossing of the gene-edited and overexpression lines will generate *A. thaliana* plants expressing *PGRL1Alpha* or *PGRL1Beta*. These plants will enable functional comparison of PGRL1Alpha and PGRL1Beta in regulation of photoprotection and impact on plant productivity and stress resilience. We will identify whether PGRL1Beta could provide faster photoprotection than PGRL1Alpha has potential increase stress resilience in non-grass crops.

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Hyperspectral imaging of transformed plants to estimate valuable products to facilitate space exploration.

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Keywords:
Hyperspectral, biomanufacturing, transgenic, plants, caffeine

Abstract:

As we move towards the goal of producing valuable compounds in plants to facilitate deep Space travel, the question of compound analysis and extraction arises. With limited resources and the requirement of a near zero waste economy, standard on-Earth methods are less applicable. The utilisation of plant hyperspectral imaging, coupled with machine learning, can allow us to detect unique spectral signals relating to the presence and concentration of target molecules within a plant. This reduces the requirement for onboard equipment and reagents and increases automation. Strong modelling can allow for the detection of problem lines and optimal harvest time without the need for human intervention. In this study we have utilised synthetic biology techniques to introduce the genes (*CaXMT1*, *CaMXMT1*, *CaDXMT1*) responsible for caffeine production in *Coffea arabica*, into *Nicotiana benthamiana*. Caffeine concentration was measured using HPLC-MS/MS across two time points (two and four days post-agroinfiltration). Day 4 had a maximum production of 75.92 µg/g FW (μ = 29.92, SD = ±9.78, n = 52). Whereas day 2 had a maximum production of 21.28 µg/g (μ = 9.56, SD = ±4.64, n = 20). Hyperspectral radiometric measurements were taken on the adaxial and abaxial side of the leaves at 350-2500 nm across 2151 bands of the EMS. Current LOOCV-PLSR models show a R^2 of 0.62 (RMSE = 8.23, MAE = 5.92). For pharmaceutical use cases like this and with model improvements, this technology could allow plants to be used as direct medicinal vectors, further reducing the need for downstream processing.

Developing a method for accurately genotyping genome edited plants.

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Keywords:
Biotechnology, bioinformatics, gene-editing, regulatory compliance

Abstract:

Gene-editing offers the potential for addressing many of the pressing issues facing modern agriculture, such as improving drought tolerance, disease resistance, and yield. However, in Australia, the commercialisation of gene-edited crops is limited by strict regulation which are often time-consuming and costly to meet. A key regulatory step is demonstrating the absence of foreign DNA that is typically introduced during the gene editing process in the form of a transformation plasmid into the edited plants.

This project focuses on developing a streamlined, cost-effective method for genotyping gene-edited crops, aiming to make these technologies more accessible to breeders. Our approach uses a computational pipeline to detect sequence similarity between transformation plasmids and crop genomes. Unlike whole-genome assembly or limited PCR screening, this method is more efficient and scalable, especially for large genomes like wheat. Before we can detect what has been added to a gene edited plant, it is important to understand what innate similarity is already present between transformation plasmids and crop genomes. This research has shown that there is a substantial amount of sequence similarity between these two DNA sequences with stretches of similarity greater than 100 bases. These results have highlighted the difficulty of demonstrating that a non-gene edited plant is free from foreign DNA and the challenges that arise when moving to work with gene edited plants.

By simplifying and improving the genotyping process, this work will help reduce regulatory bottlenecks, lower development costs, and facilitate the broader adoption of gene-edited crops in Australian agriculture.

Morpho-physiological traits and proteomic signatures in QTL-hotspot- introgressed chickpea under drought stress: Implications for yield improvement

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Keywords:
Chickpea, drought stress, QTL-hotspot, recombinant inbred lines, differential abundant proteins

Abstract:

Chickpea is a key source of protein and fibre globally, but drought stress significantly reduces its yield and nutritional quality. This study investigated aboveground morpho-physiological and root proteomic responses to progressive drought in four QTL-hotspot-introgressed recombinant inbred lines (RILs) and their recurrent parents grown in a controlled environment. Drought was imposed at early podding. Plant transpiration was monitored continuously using automated weighing and watering stations. While phenology remained consistent across RIL pairs during sowing, flowering, and podding, significant differences were observed at maturity. Drought notably reduced aboveground dry weight, seed yield, harvest index, leaf gas exchange, and water use. Proteomic analysis of root tissues revealed both constitutive and drought-induced differentially abundant proteins (DAPs) under DS. Several constitutive DAPs were located on chromosome 4 and were associated with drought-responsive pathways such as phenylpropanoid biosynthesis and lignin polymerisation. Advanced microscopy and omics techniques further support the role of QTL-hotspot-linked genes in enhancing lignification under DS. These findings enhance our understanding of drought adaptation in chickpea and highlight genetic targets for improving productivity in water-limited environments.

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How will future climate change affect wheat yield and nutritional quality?

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Keywords:
Triticum aestivum, elevated CO_2, elevated temperature, high-throughput phenotyping, crop ecophysiology

Abstract:

In the coming decades, climate change will bring higher atmospheric CO_2 concentrations, along with warmer temperatures. Despite their joint presence under future climate conditions, the combined effects of elevated CO_2 (eCO_2) and elevated temperature (eT) on wheat performance remain understudied[1]. When tested separately, eCO_2 increases wheat yield while diminishing grain nutritional values[2,3], whereas eT decreases yield[4] with undetermined effects on grain nutrition. Whether there are wheat varieties that can take advantage of the extra CO_2 and/or temperature to achieve better performance in both yield and/or quality is unclear.

To better understand the combined effects of eCO_2 and eT on Australian wheat, we will present early insights into one of the largest experiments in this area — 90 diverse Australian wheat lines grown under realistic current and future climate conditions for the year 2100 (800 ppm CO_2 and +5°C). In the first year of the experiment, we found yield declined by 17% on average in the future climate treatment. Grain quality showed little response to the future climate treatment, except for zinc levels, which decreased by 2%. High-throughput phenotyping showed that decreases in yield were correlated with predicted changes in photosynthetic capacity ($V_{cmax;\ 25}$). Our work will support a better understanding of the underlying mechanisms contributing to variation in wheat yield and nutrition under future climate conditions, thus helping identify breeding targets to ensure food security.

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Pushing Boundaries: Overcoming technical barriers in spatial RNA-seq of plant tissues

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Keywords:
Cryosectioning, Fresh-frozen tissue preparation, Spatial gene expression, Visium HD 3'

Abstract:
Spatial RNA sequencing (spatial RNA-seq) enables comprehensive transcriptome analysis at cellular resolution, while preserving the spatial context of gene expression within the intact tissue architecture. However, its application to plant tissues presents unique challenges due to high cellular heterogeneity and complex tissue structure. The Visium HD 3' platform was employed to analyse spatial RNA-seq in developing wheat grains. The available protocol (10x Genomics, 2025a, 2025b), optimised for mammalian tissues, was not inherently compatible with the sections of developing wheat grains, as complete tissue detachment from slides occurred during early processing stages. To address this issue, the protocol was extensively refined through the introduction of 30% sucrose infiltration containing RNase inhibitor, which notably enhanced tissue sectioning and mounting of developing grains. Further optimisation included initial fixation with cold methanol and isopropanol (10x Genomics, 2025c), reduction of PBS concentration during post-fixation washes, post-enhancer washes, and destaining processes, as well as lowering the temperature during preparation of the enhancer mix. These modifications substantially improved tissue adherence throughout the preparation of fresh-frozen tissue and the capture of Poly(A) RNA via CytAssist. Consequently, these adjustments dramatically improved the quality of tissue cross-sections, producing adequate and high-quality cDNA suitable for library preparation, allowing spatial RNA-seq in wheat immature grains. These findings are anticipated to facilitate the broader application of spatial RNA-seq technology in plant research.

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INVESTIGATING CONTRADICTORY FINDINGS RELATED TO AUXIN AND *THOUSAND-GRAIN WEIGHT 6* (*TGW6*) GENE AND THEIR RELATIONSHIP WITH GRAIN WEIGHT IN WHEAT

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Keywords:
Auxin, grain development, grain size, *TGW6*, *Triticum aestivum*

Abstract:

The effect of auxin on wheat (*Triticum aestivum* L.) grain size is contentious, with uncertainty over whether indole-3-acetic acid (IAA) accumulation arises from *de novo* synthesis or hydrolysis of IAA-glucose. We described the first comprehensive study of tryptophan aminotransferase (*TAR*) and indole-3-pyruvate mono-oxygenase (*YUCCA*) expression from 5 to 20 days after anthesis. Expression data combined with liquid chromatography–tandem mass spectrometry using internal standards indicated *TaTAR2-B3*, *TaYUC9-A1*, *TaYUC9-B*, *TaYUC9-D1*, *TaYUC10-A* and *TaYUC10-D* are responsible for IAA production in developing grains. These genes are also grain-specific, as in rice and maize. Although IAA synthesis occurs primarily in the endosperm, the *TaYUC9-1* group is expressed in the embryo, especially in aleurone and transfer cells, suggesting a role in tissue differentiation. Our results also cast doubt on the proposed role of *THOUSAND-GRAIN WEIGHT* gene, *TaTGW6*, in increasing grain size by reducing IAA, as previous work overlooked the contribution of IAA biosynthesis from tryptophan. Wheat has nine and rice three TGW6-like genes encoding proteins with over 80% identity, making it unlikely that one inactive allele significantly affects IAA levels in grains. Neither *TaTGW6* nor *OsTGW6* is expressed in grains; instead, both are expressed in pre-emergent inflorescences; *TaTGW6* is expressed particularly in microspores prior to mitosis. This evidence, combined with undetectable esterified IAA and lack of IAA–glucose synthase expression in grains, suggests that TaTGW6 and OsTGW6 do not regulate grain size via the hydrolysis of IAA–glucose. Instead, their similarity to rice strictosidine synthase-like (OsSTRL2) suggests they play a key role in pollen development.

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Using optogenetic tools to explore rapid switching of plant water transport and energetics of ion transport

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Keywords:
optogenetics, aquaporins, root membrane transport, ion transport energetics

Abstract:

Green-light-activated ion channels from algae (channel rhodopsins, CHRs) are powerful, non-invasive tools for probing physiological processes in plants (1). Their effectiveness is enhanced because plants utilise green light poorly. This research uses CHRs to address two outstanding questions in plant physiology.

First, we investigate the energetic cost of ion homeostasis (2). By expressing the anion-conducting CHR GtACR1 in *Arabidopsis* roots, we can use light to induce a controlled anion leak that leads to membrane depolarisation. Our aim is to measure the resulting increase in root respiratory demand as the plant returns to ion homeostasis. This will quantify the energy required to maintain this crucial ion balance.

Second, we explore the rapid gating of aquaporins, which are vital for plant water use efficiency. Some aquaporins (e.g. AtPIP2;1) can switch from conducting water to conducting cations (3), but the signals that trigger this switch are unknown. Two candidates are cytosolic calcium (Ca^{2+}) and pH. By co-expressing a Ca^{2+}-permeable CHR (ChR2-XXM) and a H^+ permeable (HcKCR2) with AtPIP2;1 in *Xenopus* oocytes, we can use light to generate precise signals and observe their real-time effect on the aquaporin's function.

This work will present preliminary data illustrating how CHRs can be used to dissect these complex physiological mechanisms.

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17	Saha	Saurabh	Saurabh Saha
New technologies and AI in plant science	Saric	Rijad	Rijad Saric
35	Schaefer	Mara	Mara Schaefer
13	Schleyer	Kathryn	Kathryn Schleyer
44	Seward	Alex	Alex Seward

18	Sharma	Kritika	Kritika Sharma
67	Shelden	Megan	Megan Shelden
24	Siazon	Stephen Eunice	Stephen Eunice Siazon
123	Singh	Anshuman	Anshuman Singh
64	Situmorang	Apriadi	Apriadi Situmorang
14	Sohrabi	Sab	Sab Sohrabi
Beneficial plant-microbe interactions	Sun	Jiacan	Jiacan Sun
93	Taleski	Michael	Michael Taleski
113	Tao	Yunpeng	Yunpeng Tao
New technologies and AI in plant science	Tongson	Eden	Eden Tongson
61	Tyerman	Stephen	Stephen Tyerman
130	Tyerman	Stephen	Stephen Tyerman
Plant biotic and abioitc stress signalling	Tyerman	Steve	Steve Tyerman
91	Vu	Hien Minh	Hien Minh Vu
122	WANG	JUNYI	JUNYI WANG
Plant biotic and abioitc stress signalling	Walker	Louise	Louise Walker
85	Wallis	Daniel	Daniel Wallis
89	Wee	Benjamin	Benjamin Wee
Plant biotic and abioitc stress signalling	Wellawatta	Ayesha	Ayesha Wellawatta
8	Wen	Yan	Yan Wen
Synthetic Biology/Green biomanufacturing	Wen	Allen	Allen Wen
114	Weston-Olliver	Grace	Grace Weston-Olliver
118	Woodford	Russell	Russell Woodford
31	Wu	Xingyu	Xingyu Wu
70	Wu	Yue	Yue Wu
59	Xu	Bo	Bo Xu
79	Xu	Ling	Ling Xu
Frontiers in Genetics and Molecular Biology	Yang	Qinqin	Qinqin Yang
19	Yee	Suyan	Suyan Yee
92	Yoshikawa	Guilherme Ventura	Guilherme Ventura Yoshikawa
90	ZAREAN	MARYAM	MARYAM ZAREAN
58	Zen	Sabrina De	Sabrina De Zen
5	Zhang	Shunlin	Shunlin Zhang
16	Zhang	Rose	Rose Zhang

Beneficial plant-microbe interactions	Zhang	Zhe	Zhe Zhang
34	Zheng	Fei	Fei Zheng
78	Zhou	Xingyi	Xingyi Zhou
127	Zhuang	Zixiong	Zixiong Zhuang