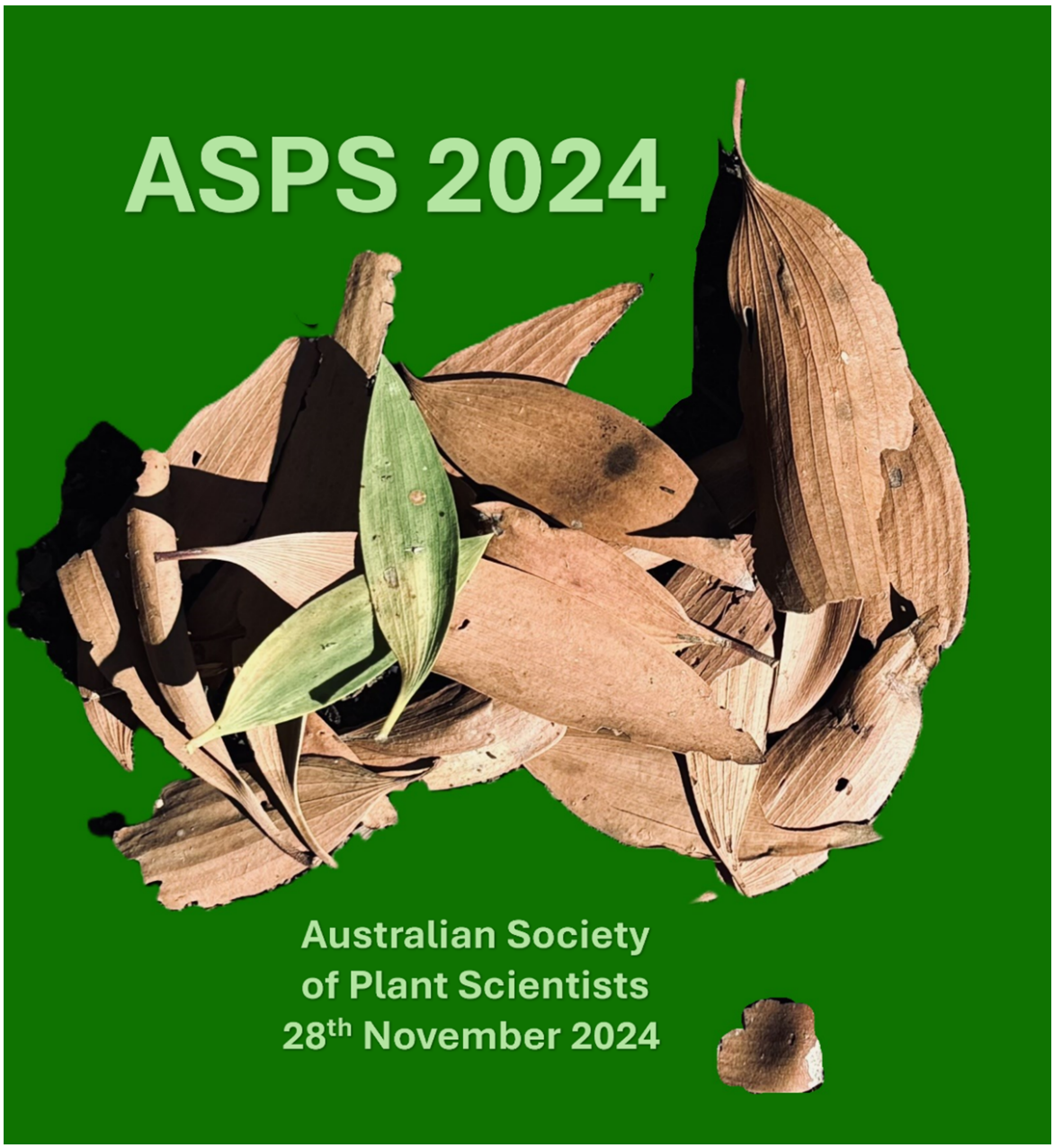
ASPS 2024 Abstract Book

Brisbane, QLD



Acknowledgement of Country

We are meeting today on Meanjin (Brisbane) Aboriginal land. The ASPS acknowledges, with deep respect, the Turrbal and Yugara as the traditional owners of the land on which we meet. We pay respect to their Elders, lores, customs and creation spirits. We recognise the important role Aboriginal and Torres Strait Islander people play within the community and their valuable contributions to Australian and global society.

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Contents

Australian Society of Plant Scientists (ASPS) Queensland Node Conference 2024

📍 **Location**: Abel Smith Lecture Theatre, UQ St Lucia Campus (Room 23-101)
📅 **Date**: 28th November 2024

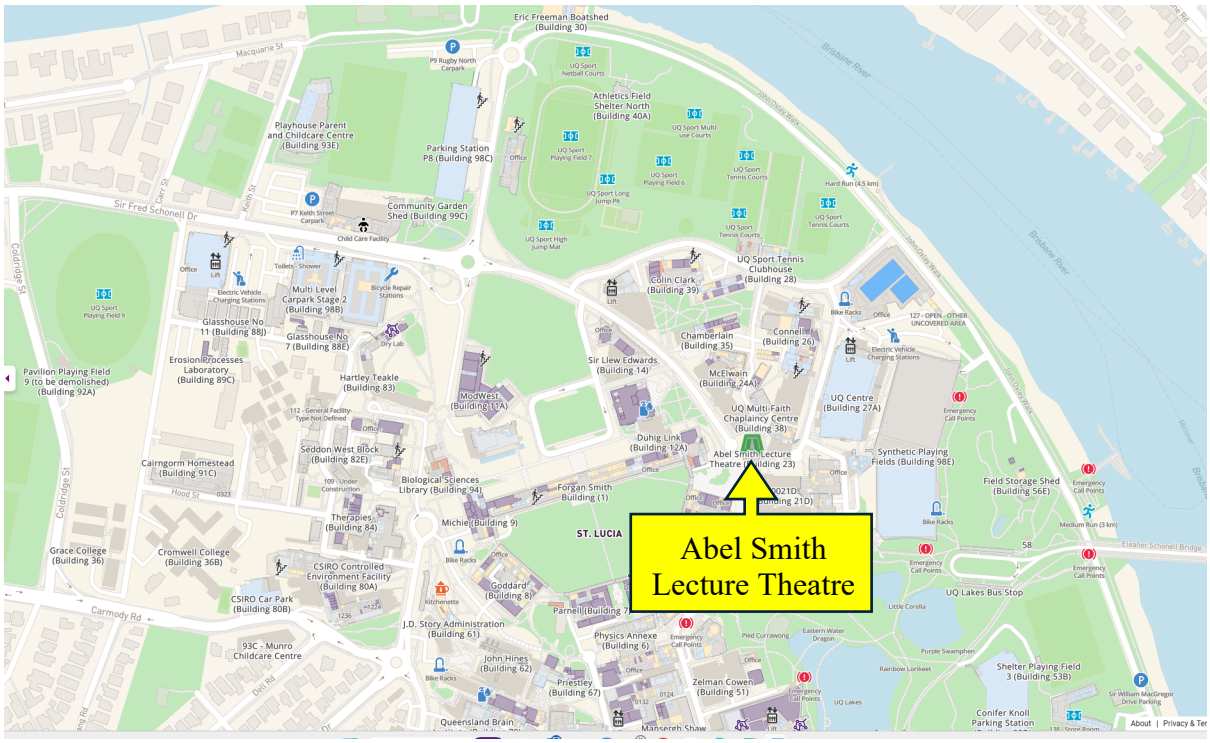
🌟 Session	⏰ Time
🔷 **Registration**	8.00-8.30
🔷 **Welcome and Opening** *Nipuni Thanthrige*	8:30 – 8:45
🔶 **Keynote Seminar** *Chair: Peter Prentis* **Dr Edward Gilding** - Department of Agriculture and Fisheries Atypical characteristics of albumin-1 genes in a basal Phaseoleae are linked to defence peptide cyclisation	8:50 – 9:20
🔷 **Session 1: Discovery Research** *Chairs: Peter Crisp, Stephanie Kerr* • **Buddhini Ranawaka** Factors affecting genome editing in plants • **Alexandria Mattinson** Regulation of P. vulgaris CLAVATA3/EMBRYO SURROUNDING-related (CLE) Peptides During Pathogenic Interactions • **Anuradha De Silva** Mimicry of stress tolerant proteins from related wild species to improve crops tolerance • **Caitlin Dudley** Time to flowering and flowering duration in mungbean are unrelated physiological traits with independent genetic controls	9:25 – 10:25

☕ **Morning Tea & Networking**	10:25 – 10:55
🔶 **Session 2: Translational Research** *Chairs: Brett Williams, Pauline Okemo* • **Stephanie Kerr** Using Functional Genomics To Characterise The Genetic Pathways Regulating Flowering In Horticultural Tree Crops • **Juel Datta** Genomic approaches to investigate the molecular mechanisms of phase transition period in Citrus species • **Stephanie Brunner** A haplotype-based approach to exploring genotype by environment interactions for barley yield • **Shanice Van Haeften** Tapping into genebanks to reveal geographic patterns of temperature adaptation in chickpea	10:55 – 11:55
Lunch	11:55 – 12:30
🏆 **ASPS Award Seminars – Virtual** *Chairs: Martha Ludwig and Lynette Brownfield* • NZSPB Roger Slack Award - David Chagné • ASPB Goldacre Award – Peter Crisp • ASPB Education Award - Ashley Jones & Benjamin Schwessinger • Break • NZSPB elected fellow to the NZ Royal Society - Andy Allan and Kevin Davies • ASPB Jan Anderson – Jenny Mortimer • ASPB JG Wood – Sergey Shabala	12:30 – 3:15
☕ **Afternoon Tea**	3:15 – 3:45
⚡ **Session 3: Flash Talks** *Chairs: Yasmine Lam, Grace Weston-Olliver, Juel Datta, Chamilka Ratnayake* • **Ming Cheng** Maximising pineapple production by controlling flowering time using CRISPR/Cas9 • **Andrea Putri Subroto** Establishment of Protoplast Isolation and PEG-Mediated Transformation for a Pilot Study of Genome Editing in Avocado by Targeting the PDS gene	3:45 – 4:35

• **Dominic Previtera** Heritable transgene-free genome editing using mobile CRISPR elements in plants • **Jordan Gemmell** What Doesn't Kill You Makes You Stronger: Photoinhibitory Priming as a Technique to Enhance Yield and Abiotic Stress Tolerance in Crops • **Nicholas Lester** Hyper-recombinant faba bean for accelerated breeding • **Dylan Bowman** Oral delivery of plant produced compounds • **Julia Solonenka** Anti-freeze proteins as novel fusion partners for the non-chromatographic purification of recombinant plant-produced proteins • **Katherine Pinto-Irish** De novo genome assembly and comparative transcriptome analysis reveal divergence in Neptunia species and insights of Selenium hyperaccumulation and tolerance in Neptunia amplexicaulis from Australia • **Chamilka Ratnayake** Anthocyanin – An alternative to conventional reporter systems: Towards a combinatorial strategy for novel regulatory networks and modified metabolic pathways in *Nicotiana benthamiana* • **Zuba Ahmed** Forward genetic screening in *Nicotiana benthamiana* to unravel genes responsible for successful anthocyanin production	
🏅 **Closing and Award Ceremony** *Chairs: Brett Williams, Nipuni Thanthrige*	4:35 – 4:45
🍽 **Conference Dinner** The Plough Inn - South Bank Parklands	6:30 until late

Maps

23-101 - Abel Smith Lecture Theatre



Session One: Discovery research

Chairs: Peter Crisp, Stephanie Kerr

Factors affecting genome editing in plants

Budhhini Ranawaka[1,2], Satomi Hayashi[1,2], Sally Roden[1,2] and Peter Waterhouse[1,2]

[1] Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, QLD, Australia
[2] Australian Research Council Centre of Excellence for Plant Success in Nature and Agriculture, Queensland University of Technology, Brisbane, QLD, Australia

Abstract:

Successful CRISPR/Cas-mediated genome editing depends on the induction of double-strand breaks (DSBs) at a specific DNA sequence and the subsequent initiation of error-prone repair mechanisms. However, the factors affecting the efficiency of CRISPR/Cas-mediated DSB and repair fidelity remain largely unexplored in plants. This study uses Nicotiana benthamiana to explore the impact of the DSB repair mechanism on the editing efficiency of Cas9 and Cas12a enzymes. Multiple target sites in intergenic (Bur2 promoter) and exonic (RDR1) regions were tested for susceptibility to cleavage in vitro and to in vivo editing. The in vivo editing and in vitro cleavage efficiencies of Cas9 and Cas12a differed considerably among the target sites. Moreover, in vitro cleavage efficiency was not reflected in the in vivo editing efficiency. These results imply that perfect re-ligation by DNA repair mechanism compromises the apparent editing efficiency, suggesting that indel accumulation may not accurately reflect CRISPR/Cas-mediated genome editing efficiency. A plant system that can quantify and reduce the perfect re-ligation during DSB repair is successfully designed. The ongoing testing on this system shows that different Cas enzymes have different levels of perfect re-ligation during DSB repair, offering insights for further optimising editing strategies in plants.

Regulation of *P. vulgaris* CLAVATA3/EMBRYO SURROUNDING-related (CLE) Peptides During Pathogenic Interactions

Alexandria Mattinson[1], April Hastwell[1], Elizabeth Aitken[1] and Brett Ferguson[1]

[1] University of Queensland, Brisbane, Australia

Abstract:

Plants contain a multitude of intricate and tightly regulated molecular signalling pathways to control growth, development, and response to biotic and abiotic stimuli. CLAVATA3/EMBRYO SURROUNDING-related (CLE) peptides are a family of small signalling molecules involved in diverse pathways that regulate and optimise plant development. CLE peptides, such as RIC1, RIC2, and NIC1, are known for their ability to control legume nodulation as part of the Autoregulation of Nodulation mechanism. However, the role of CLE peptides in the context of pathogen interactions has not yet been thoroughly investigated. We used legume fungal pathogens *Macrophomina phaseolina* and *Sclerotium rolfsii* to investigate the differential expression of the complete family of CLE peptide encoding genes in common bean (*P. vulgaris*). Several differentially expressed candidates have now been identified that respond to infection with these pathogens. We are now functionally analysing these candidates to establish their role in symbiotic and pathogenic interactions. Findings could help in the development of synthetic peptides, or the identification of genetic targets, that help enhance crop resistance to harmful pathogens in agriculture.

Mimicry of stress tolerant proteins from related wild species to improve crops tolerance

Anuradha De silva[1], Julia Bally[1], Satomi Hayashi[1], Thi Thuy Trang Li[1], Muthurajan Raveendran[3], Peter Prentis[1], Sagadevan Mundree[2] and Brett Williams[1]

[1] Queensland University of Technology, Brisbane, Australia
[2] University of Queensland, Brisbane, Australia
[3] Tamil Nadu Agricultural University, Tamil Nadu, India

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 survive 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 variations in stress tolerance proteins significantly enhance their function. Our models predict 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 demonstrate that rice expressing the *Tripogon* and APO I 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.

Time to flowering and flowering duration in mungbean are unrelated physiological traits with independent genetic controls

Caitlin Dudley[1], Shanice Van Haeften[1], Eric Dinglasan[1], Lee Hickey[1], Hannah Robinson[1], Christine Beveridge[2,3], Michael Udvardi[1] , Karen Massel[1], Elizabeth Dun[2,3] and Millicent Smith[1,2]

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[2] School of Agriculture and Food Sustainability, Faculty of Science, The University of Queensland, QLD, Australia
[3] ARC Centre of Excellence for Plant Success in Nature and Agriculture, The University of Queensland, QLD, Australia

Abstract:

Mungbean (*Vigna radiata L.*) is a valuable grain legume crop grown in sub-tropical regions. Mungbean typically has a long, asynchronous flowering window which can increase vulnerability of grain production to abiotic stress and results in challenges at harvest due to staggered pod maturity. While previous research has documented genetic variation in days to flowering (DTF) and flowering duration (FD), there remains a gap in our understanding of how these flowering traits interact with environmental factors. Specifically, the dynamics of how DTF and FD respond to varying environmental conditions and their implications for plant performance are still underexplored. This includes a lack of knowledge of the genetic controls underpinning key flowering traits. Utilising a diverse mungbean nested association mapping (NAM) population, DTF and FD were evaluated across four field experiments conducted in Queensland, Australia in 2022 and 2023. Extensive phenotypic variation was observed for DTF (35 - 70 days after sowing) and FD (20 – 60 days after sowing). Both flowering traits displayed scale Genotype x Environment interactions, with FD more interactive with environment compared to DTF. No relationship was evident between DTF and FD across all environments. Genome wide association studies identified eight quantitative trait loci (QTL) for DTF and one for FD, where no QTL were in common. The phenotypic effect, presenting as variations in flowering time, associated with the accumulation of early or late alleles at DTF QTL highlights the potential to develop mungbean varieties with distinct flowering time. These findings provide a foundational understanding of flowering behaviour in mungbean, paving the way for targeted crop improvement strategies to enhance adaptation and performance in diverse agricultural environments.

Session Two: Translational research

Chairs: Brett Williams, Pauline Okemo

Using Functional Genomics To Characterise The Genetic Pathways Regulating Flowering In Horticultural Tree Crops

Stephanie Kerr[1], Zachary Stewart[1], Amanda Johnson[1], Juel Datta[1], Malcolm Smith[2], Peter Prentis[1]

[1] Centre for Agriculture and the Bioeconomy, School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Australia
[2] Department of Agriculture and Fisheries, Bundaberg Research Station, Bundaberg, Australia

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. As part of the National Tree Genomics program, we performed phylogenetic analyses to identify homologs of key flowering genes in the nationally important tree crops: almond, avocado, citrus, mango, and macadamia. RNA-seq analyses from almond, macadamia, and mango leaf and bud samples collected over time-courses have highlighted genes likely to be involved in floral induction in these species, while association studies in citrus have identified QTLs and candidate genes controlling early flowering. Furthermore, we are using transformation techniques to functionally characterise these key flowering homologs in model species, as well as developing novel techniques to functionally characterise these genes within the trees themselves. Here, I will present our latest research and update our understanding of the genetic pathways regulating flowering in these important horticultural tree species.

Genomic approaches to investigate the molecular mechanisms of phase transition period in Citrus species

Juel Datta[1,2], Zachary Stewart[1,2], Brett Williams[1,2], Sudipta Das Bhowmik[1,2], Malcolm Smith[3], Peter Prentis[1,2], Stephanie Kerr[1,2]

[1] School of Biology and Environmental Science, Queensland University of Technology (QUT), Brisbane, Australia
[2] Centre for Agriculture and the Bioeconomy, Queensland University of Technology (QUT), Brisbane, Australia
[3] Department of Agriculture and Fisheries (DAF), Queensland, Australia

Abstract:

The phase transition period from vegetative juvenile to reproductive stage in *Citrus* is a lengthy process, during which flowering is suppressed. TERMINAL FLOWER1 (TFL1) likely plays a role in this process by repressing flowering promoting genes such as *APETALA1* and *LEAFY*. The overexpression of *Citrus sinensis TFL1* (*CsTFL1*) in *Arabidopsis*, can suppress flowering, however, the function of TFL1 in *Citrus* itself is yet to be characterized. Although, in other tree crops species such as pears, poplars, and apples *TFL1* loss-of-function mutations result in earlier flowering. In this study, genomic approaches such as quantitative trait loci (QTL) analysis, and CRISPR-Cas9 were used to understand the molecular mechanisms of early and late flowering in both wild and commercial citrus species. QTL sequencing of precocious wild citrus species identified a single genomic region linked to early flowering, and further analysis will be done to predict candidate flowering genes. Furthermore, CRISPR-Cas9 was used in citrus epicotyls to edit *CsTFL1* via *Agrobacterium*-mediated transformation methods. Regenerated transgenic citrus shoots are being screened for gene editing events. Overall, the results of this project aim to accelerate the citrus breeding process by identifying genetic markers and candidate genes for early flowering and creating early flowering gene edited citrus lines.

A haplotype-based approach to exploring genotype by environment interactions for barley yield

Stephanie Brunner[1], Zachary Aldiss[1], Samir Alahmad[1], Silvina Baraibar[2], David Moody[2], Lee Hickey[1], Kai Voss-Fels[3] and Hannah Robinson[3]

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia
[2] InterGrain Pty Ltd, Perth, WA, 6163, Australia
[3] Department of Plant Breeding, Hochschule Geisenheim University, Geisenheim, Germany

Abstract:

Barley is grown in every Australian state across a diverse range of growing environments, thus genotype by environment interactions (GEIs) are of great interest to barley breeders. With climate change threatening production, it is increasingly important to understand the GEIs impacting barley yield. A robust data set 668 genotypes grown across 21 sites was used to explore the questions of the importance of fitting GEIs into models for yield, to detect haplotypes with varied effects across environments and to co-locate these haplotypes with key physiology genes to better understand the drivers of GEIs.

Two Genomic Best Linear Unbiased Prediction (GBLUP) models for barley yield were fitted to the data, identical except one model contained a GEI term with a factor analytic 4 (FA4) variance structure. Comparison of model statistics illustrated that the GEI GBLUP model accounted for a greater amount of genetic variance indicating the important role of GEI in barley yield. Using the first three factor loadings from the FA4 model the 21 sites were clustered into 4 environmental groups.

The barley SNPs were partitioned into 1758 haplo-blocks based on linkage disequilibrium and the local genomic estimated breeding value (LGEBV) (Voss-Fels et al. 2019) was calculated for each haplotype in each environmental cluster. The GEIs of each haplo-block across the environmental clusters was explored and blocks under large GEIs were identified. These blocks were co-located with several key phenology genes to understand and interpret the complex relationship between genotype and environment. This study provides a novel framework for teasing apart GEIs at a haplotype level which can be applied to other traits and crops in the future.

Tapping into genebanks to reveal geographic patterns of temperature adaptation in chickpea

Shanice Van Haeften[1], Lee Hickey[1] and Millicent Smith[1,2]

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland
[2] School of Agriculture and Food Sustainability, The University of Queensland

Abstract:

The increasing frequency of extreme temperature events due to climate change poses significant challenges to global chickpea production, necessitating the development of more resilient varieties across diverse growing regions. One potential strategy to tackle this challenge is to explore and utilise diverse chickpea accessions available in genebanks to identify novel alleles that can confer adaptation to high temperature. This study employed two complementary approaches to understand environmental adaptation in 1,330 diverse chickpea accessions collected from the Americas, Asia, Africa, the Middle East, and the Mediterranean. The first approach uses passport data and regional environmental data to investigate the genomic regions underlying QTL for potential extreme temperature adaptation. The second approach uses eigen values from population structure to detect changes in larger scale structural variants in chickpea genomes across regions. Using these passport, environmental and eigen variables, genome-wide association studies were undertaken to identify potential adaptive genes. These analyses revealed several significant marker-trait associations linked to temperature adaptation. Notably adaptive alleles showed clear geographical distribution patterns, suggesting local adaptation to specific environmental conditions. The comprehensive mapping of adaptive alleles across diverse geographical and climatic zones provides valuable insights into the environmental adaptation mechanisms in chickpea and provide promising targets worthy of further exploration in the development of climate-resilient chickpea varieties.

Session Three: Hybrid Session

Chairs: Martha Ludwig and Lynette Brownfield

NZSPB Award Roger Slack

Dr David Chagné

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

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

[1] Victoria University of Wellington, Wellington
[2] Plant & Food Research, Papaioea
[3] Rangitāne o Manawatu, Papaioea
[4] Genomics Aotearoa

Abstract:

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

ASPS Peter Goldacre Award

Dr Peter Crisp is a Group Leader and Senior Lecturer in the School of Agriculture and Food Sustainability at The University of Queensland. Peter's research program is focused on crop functional genomics, epigenetics and biotechnology, and has significantly advanced our understanding of the contribution of epigenetics to heritable phenotypic variation in plants. His group has invented groundbreaking technologies for harnessing (epi)genetic variation and their discoveries have led to exciting new avenues for decoding genomes and for the rational engineering of gene regulation for trait improvement in plants. Peter is a former recipient of an ARC DECRA Fellowship and a UQ Amplify Fellowship. Having benefited immensely from brilliant mentors, Peter is passionate about training. He leads a budding group of talented students and researchers and is a Chief Investigator in the ARC Training Centre in Predictive Breeding and the International Research Training Group for Accelerating Crop Genetic Gain. Peter is also an affiliate of the Queensland Alliance for Agriculture and Food Innovation and the ARC Centre of Excellence for Plant Success in Nature and Agriculture.

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

Peter Crisp[1]

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

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

Abstract:

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

References:

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4. Ganguly, D.R., Hickey, L.T., and Crisp, P.A. (2021). Harnessing genetic variation at regulatory regions to fine-tune traits for climate-resilient crops. *Molecular Plant*.

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ASPS Teaching and Outreach Award

Dr Ashley Jones & A/Prof Benjamin Schwessinger

Over the past seven years, we have continuously innovated our teaching of the latest genomic analysis approaches in plant sciences, spearheading the establishment of Oxford Nanopore sequencing across Australia and in the class room. Our impact in plant science teaching and beyond has been significant, influencing multiple sectors including universities, industries, government, and communities. Key to these achievements, is that we pursue a highly integrative approach to teaching from wet lab protocol development to dry lab analysis pipeline sharing. In addition, we focus on development of wider scientific communities, as we share teaching materials and protocols online via Protocols.io.

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

Ashley Jones[1] and Benjamin Schwessinger[1]

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

Keywords: Genomics, Education, Community, Outreach

Abstract:

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

NZSPB Elected Fellow to the NZ Royal Society

Professor Andy Allan and Dr Kevin Davies

Fast flowering as a tool for gene discovery in woody perennials

Allan, A.C.[1,2]

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

Abstract:

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

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

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

The Evolution Of Flavonoid Biosynthesis

Kevin M. Davies[1], Nick W. Albert[1], Yanfei Zhou[1], Samarth Kulshrestha[1], Rubina Jibran[2], John W. van Klink[3], David Chagné[1], Marco Landi[4], Peter Schafran[5], Fay-Wei Li[5], Stefan J. Hill[6], John L. Bowman[6]

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Abstract:

The flavonoid pathway is characteristic of land plants and a central biosynthetic component enabling life in a terrestrial environment. It is one of the most studied plant characters, and the subject of >15,000 journal articles each year. However, it is only with the recent advent of model systems for non-seed plants, that we have started to understand how and why the pathway may have evolved. The phylogenetic and functional data on non-seed plants challenge the idea of a canonical flavonoid pathway inherited from the Last Common Ancestor (LCA) of all land plants. Rather, it suggests extensive gene losses and gains within each lineage.

Some flavonoid pathway branches are well conserved and may have been present in the LCA. In particular, the UVR8/HY5-mediated induction of colourless flavonoids for tolerance of UVB-light is strongly conserved between Arabidopsis and the liverwort *Marchantia polymorpha*. In contrast, the stress-related red pigments have striking biosynthetic and functional diversity. Notably, the red pigments of liverworts are a previously unreported flavonoid type 'auronidins' that are cell-wall located polymers that provide protection against abiotic and biotic stresses. One lineage, the hornworts, has lost flavonoid biosynthesis entirely. Yet genome sequencing for eight hornwort genera found a single 'canonical' flavonoid biosynthetic gene in the phylogenetic outlier hornwort species. Thus, the hornwort ancestor may have inherited the flavonoid pathway but the biosynthetic and regulatory genes were lost during lineage-specific evolution. The results illustrate the importance of extending studies out from the usual suspects of plant models and across the embryophyte diversity.

ASPS Jan Anderson Award

Jenny Mortimer is Associate Professor of Plant Synthetic Biology in the School of Agriculture, Food and Wine at the University of Adelaide (UoA), Australia, where she is also Deputy Director (Interim) of the Waite Research Institute. She is Chief Investigator (CI) and UoA node leader of the ARC Centre of Excellence Plants for Space (P4S). She is also an Affiliate Staff Scientist at Lawrence Berkeley National Laboratory, USA, and a Director of Plant Systems Biology at the Joint BioEnergy Institute, USA. After completing her PhD at Cambridge University, UK, she began exploring how engineering the plant cell wall could deliver sustainable and economically viable biofuels: first as a postdoc in Cambridge, then as a research fellow at RIKEN Japan, before joining Berkeley Lab in 2014, and Adelaide in 2021. Her team's research focuses on understanding and manipulating plant cell metabolism, with a focus on complex glycosylation. The goal is to develop knowledge and crops which contribute to a sustainable and renewable bioeconomy.

At Adelaide, her group is using synthetic biology to develop new crops for food and materials production in controlled growth environments – including for Space settlement (P4S), applying new agricultural biotechnologies to develop resilient field crops as a CI in the ARC Training Centre for Future Crops Development, and developing Australian feedstocks for sustainable jet fuel as a CI in the ARC Research Hub for Engineering Plants to Replace Fossil Carbon. She collaborates extensively internationally, and projects include a UK Space Agency funded project to develop a plant growth facility for Axiom Station, and a NASA funded project to develop a payload for Artemis III, the mission that will return humans to the surface of the moon. She was selected as a World Economic Forum Young Scientist (2016/17), where she contributed to the WEF Code of Ethics for Researchers (widgets.weforum.org/coe), and she is an editor for the society journals Plant Cell Physiology and Plant Journal. You can find out more information on the lab here: mortimerlab.org/

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

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

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

Abstract:

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

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

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ASPS J. G. Wood Award

Prof Sergey Shabala

I was trained as an electrical engineer (B Eng Hon 1984) but then became fascinated by living systems and moved into biology, receiving a PhD in Plant Physiology in 1989 from the Institute of Experimental Botany in Minsk (former USSR). In 1995 I came to University of Tasmania as a post-doc in biophysics. In 1998 I got my tenure in the School of Agricultural Science where I have been working until 2023. During this time, I have built a highly productive laboratory focusing on stress physiology and membrane transport in plants, exploring mechanisms of plant sensing and adaptation to harsh environmental conditions such as drought, salinity, waterlogging, oxidative stress, and nutritional disorders. After working at UTAS for 28 years, I have moved to Western Australia to become a UWA Chair in Plant Physiology in June 2023. Over my research career, I have published over 460 peer-reviewed papers and supervised to completion 58 PhD students.

Cell-based phenotyping for breeding crops for future climates

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

Abstract:

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

Session Four: Flash talks

Chairs: Yasmine Iam, Grace Weston-Olliver, Juel Datta, Chamilka Ratnayake

Maximising pineapple production by controlling flowering time using CRISPR/Cas9

Ming Cheng[1], Yuri Trusov[1], Guoquan Liu[2], Yanfei Mao[3] and Jimmy Botella[1]

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[2] Centre for Crop Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia.
[3] Shanghai Institutes for Biological Sciences, Shanghai, China

Abstract:

The pineapple *(Ananas comosus (L.) Merr.)* holds a significant position as a highly prized tropical fruit, ranking second in terms of production and third in terms of global consumption and exportation. However, the field of pineapple breeding has long been associated with challenges. In the history of pineapple breeding, a pivotal shift occurred in the 2000s when the previously dominant Smooth Cayenne cultivar was supplanted by the MD-2 cultivar. This breakthrough, originating from conventional pineapple breeding efforts dating back to 1973, has gained widespread popularity worldwide. The limited success achieved in enhancing pineapple traits is primarily attributed to the extended juvenile period and self-incompatibility of the pineapple plant. These factors render traditional breeding methods exceedingly time-consuming and labor-intensive, with limited success in achieving desired trait improvements.

Furthermore, the new dominant pineapple cultivar MD-2 is known to exhibit partial sensitivity to natural flowering, a phenomenon referred to as precocious flowering. This persistent issue in pineapple farming disrupts harvesting schedules, affects year-round supply, and escalates market prices, ultimately rendering years of agricultural toil by farmers futile. However, understanding the mechanisms behind early flowering induction has proven challenging, primarily due to the complexities associated with transforming pineapple plants.

Studies have established that various environmental stimulus, including cold temperatures, shorter daylight hours, and mechanical disturbance, can trigger flowering in pineapples. Notably, the stress-related hormone ethylene has emerged as a key regulator of pineapple flowering induction. According to Yang's cycle, the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) plays a crucial role in ethylene biosynthesis, controlling the rate-limiting step. Silencing the *AcACS2* gene has been demonstrated to delay pineapple flowering.

In a bid to gain deeper insights into the roles of *AcACS2* genes in pineapple flowering, we have employed the CRISPR/Cas9 system to modify MD-2 pineapple plants. We have optimized the pineapple transformation protocol, encompassing tissue culture and

antibiotic selection. Currently, we have generated pineapple transformants containing Cas9, and ongoing analyses using molecular techniques are underway. We anticipate achieving a more comprehensive understanding of the roles of *AcACS2* genes by the project's conclusion. Additionally, we aim to develop an improved pineapple line with controlled flowering as a valuable outcome of this endeavor.

Establishment of Protoplast Isolation and PEG-Mediated Transformation for a Pilot Study of Genome Editing in Avocado by Targeting the *PDS* gene

Andrea Putri Subroto[1], Yixiong Wu[1], Thi Thao Ninh[1], Alice Hayward[1] and Christopher O'Brien[1]

[1] Centre for Horticulture Science, QAAFI, The University of Queensland

Abstract:

The fruit avocado has increased in popularity due to its beneficial health benefits. Breeding new avocado cultivars with superior traits better suited for growers and consumers is severely hampered by its long juvenility period and heterozygosity. In this study, a system for avocado genome editing using protoplasts as an explant is being developed. Protoplasts are plant cells that have had their cell walls removed chemically. Protoplasts have the capability to uptake foreign genetic material, therefore they become a powerful tool for genome editing to create non-chimeric edited plants. New genome-editing techniques, including the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein (Cas), allows breeders to target specific locations in the genome, holding great potential to speed up crop improvement. In the CRISPR/Cas system, protoplasts are useful not only for rapidly validating the mutagenesis efficiency of sgRNA designs or Cas proteins but also as a platform for DNA-free gene editing. The pilot study targeted *phytoene desaturase* (*PDS*) gene as a CRISPR/Cas9 editing proof of concept in avocado. The knockout of the *PDS* gene will result in albino plants. We successfully isolated protoplasts from avocado leaves with a yield of approximately 7.9×10^6 g^{-1} fresh weight and a viability of ±80-100%, and transformed protoplasts with green fluorescent protein (GFP) plasmid. Two sgRNAs for the avocado *PDS* gene were designed and cloned into the CRISPR/Cas9 vectors. The activities of CRISPR/Cas9 vectors will be validated using PEG-mediated avocado protoplast transformation system.

Heritable transgene-free genome editing using mobile CRISPR elements in plants

Dominic Previtera[1], Zhiwei Ye[1], Zheng Gong[1] and Jimmy Botella[1]

[1] Plant Genetic Engineering Laboratory - UQ

Abstract:

Current regulatory concerns and time consuming transformation methods have led to rapid advances in the field of viral-induced genome editing (VIGE). However, VIGE is still limited by several factors, primarily the size constraints of most viral vectors and the subsequent requirement of constituent overexpression of the highly efficient Cas9 endonuclease. The production of transgene-free genome edited first generation progeny has proven difficult, but it has now been achieved through the use of transgenic Arabidopsis rootstocks expressing Cas9 and gRNA's augmented with tRNA-like-structures (TLS). This research will aim to improve the efficiency of this process using the model species *N. benthamiana,* testing both intra and cross-species grafts with agronomically relevant species. A range of TLS configurations as well as the mobile flowering signal *Flowering Locus T (*FT) will be tested to maximise the mobility of Cas9 mRNA from the transgenic rootstock into the Wildtype scion. gRNAs fused to FT to improve germline access will be supplied from a range of viral vectors following grafting, rather than included in T-DNA during transformation, an approach that will increase editing efficiency in germline cells through greater expression of gRNA as well as remove the need to create new transgenic *N. benthamiana* for each desired genome edit. Currently, transgenic lines have been validated and viral vectors with mobility sequences have been tested to determine their efficiencies. Some grafted plants have been supplied with viral vectors expressing gRNA and results should be obtained soon. Ultimately, this research will produce a VIGE system that can quickly and efficiently produce desirable gene edits in a range of model and agriculturally relevant species.

What Doesn't Kill You Makes You Stronger: Photoinhibitory Priming as a Technique to Enhance Yield and Abiotic Stress Tolerance in Crops

Jordan Gemmell[1], Julia Bally[1] and Brett Williams[1]

[1] Queensland University of Technology

Abstract:

To combat the threat of food insecurity, techniques are required to increase food production. Controlled environment agriculture (CEA) offers the opportunity to address these challenges, by facilitating a greater degree of control over plant growth, enhancing yield and nutritive value. However, optimisation is hindered by knowledge deficits, regarding how plants respond to artificial growth environments. Therefore, by increasing understanding of how plants respond to differences in artificial environmental conditions, optimisation of CEA systems can be achieved.

Exposure to stressful conditions causes organisms to adapt. Recent research has indicated that transient applications of stressful environmental parameters (e.g. excessive light availability) result in enhancement of plant performance. Specifically, it appears that by exposing plants to stressful conditions temporarily, that they can be forcibly adapted into higher rates of photosynthetic activity, by upregulating the activity of photochemical and electron transport apparatus. Our project aimed to optimise both the intensity and frequency of transient high-light stress applications, by comparing crops grown under optimal conditions with those that have been exposed to excessive light at different timings, frequencies, and intensities. The leaf optical response suggests that not only can transient photoinhibition enhance the photosynthetic performance of crops, but also tolerance to future high-light events.

Hyper-recombinant faba bean for accelerated breeding

Nicholas Lester[1], Brett Ferguson[1], Sven Weber[2] and Karen Massel[1]

[1] The University of Queensland, St Lucia, Australia
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Abstract:

Meiotic recombination is a fundamental biological process in plant reproduction where paternal and maternal chromosomes are intermixed, making it essential process for evolution and adaptation. It's harnessed in plant breeding to create novel genetic combinations, purging deleterious loci, as well as used in applications for rapid introgression and improved fine mapping of traits. Since natural low recombination rates are such a limiting factor in crop improvement, research has long focussed on increasing recombination by using biotechnology in model species. This research seeks to translate this work into the largely neglected legumes species, such as faba bean (*Vicia faba*). Faba bean is an important temperate legume, where Australia is the single largest global exporter. Improvement of this crop is more important than ever given growing use cases for soil remediation and alternative proteins; however, yield improvement has stagnated at 0.4% per year. Recent genomic advancements, such as the generation of the first faba genome as well as pangenome, are continuing to make genetic research a more viable option. This study seeks to utilise cutting-edge biotechnological approaches to investigate candidate genes for hyperrecombination in faba bean. These approaches include CRISPR-mediated genome editing of anti-crossover factors (such as RECQ4 and FIGL1), as well as overexpression of crossover promoting factors (such as HEI10). The value of these changes in plant breeding will be assessed by quantifying recombination by marker genotyping and cytology. Hyper recombinant elite lines could be used for high-resolution mapping in a NAM scheme, or for rapid introgression of landrace loci, while non-GM CRISPR mutants could be directly included in breeding efforts. This research will reveal the usefulness of this potential technology and will deepen our fundamental understanding of recombination in legumes, an area which is sorely neglected.

Oral delivery of plant produced compounds

Dylan Bowman[1], Julia Bally[1], Zacharie Le Blanc[1], Melody de Laat[1] and Peter Waterhouse[1]

[1] Queensland University of Technology, Brisbane, Australia

Abstract:

The economic burden of intestinal parasitic worms on the Australian sheep industry reaches $430 million per year. The financial toll on the industry is set to increase as worms have developed resistance to traditional drugs. This presents a need from the agriculture industry for a novel, effective, and cheap treatment for intestinal parasitic worms. Using plants, we aim to produce an already well characterised anti-parasitic protein (APP) at an industrial scale to answer the need of the industry. Traditional large scale protein production in plants is performed transiently using agrobacterium after which the leaves are harvested and then purified. Biopharming aims to remove the expensive and limiting steps involved with plant protein production by utilising stable expression in plants. Stable expression in biopharming is often performed with transgenics as they are simple to develop and can have good levels of yield. There is another option for stable expression though, transplastomics. Transplastomics utilise the unique biology of the plastome to have over 10,000 copies of a transgene per cell. This facilitates much higher levels of expression in theory compared to transgenics. A further step in biopharming is to use edible crops to produce foreign protein. Edible crops can bioencapsulate foreign protein in the plant material, removing the need for expensive purification steps. Since beginning this project, I have developed a transgenic *Nicotiana benthamiana* plant but I'm aiming to modulate the expression in the genome and develop more transgenics in the future using inducible promoter systems. I am currently developing transplastomic lines in *Nicotiana benthamiana* but also trying to modulate expression in the chloroplast. In order to bioencapsulate the APP I am developing transgenic lines in rice and transplastomic lines in lettuce, chosen for their non-toxicity in sheep. Through the success of this project I hope to show that plants can be used as platforms for drug production and delivery across industries.

Anti-freeze proteins as novel fusion partners for the non-chromatographic purification of recombinant plant-produced proteins

Julia Solonenka[1], Zacharie LeBlanc[1] and Peter Waterhouse[1]

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Abstract:

The recombinant expression of proteins in plants (i.e., “biopharming”) is a rapidly growing production method for biologics. Compared to industry standard platforms, scaling up is easier, cheaper, and safer (Eidenberger et al., 2023). Despite advantages, few plant-based biopharmaceuticals have made it to market, partly due to challenges and expenses associated with downstream processing— the plant matrix contains more contaminants than other fermentation systems (Buyel et al., 2015). There is a trade-off between the scalability of plants and the production efficiency; therefore, new purification strategies could drive the mainstream adoption of biopharming. Recently, non-chromatographic methods involving fusion proteins have gained popularity for their ease of use and mild conditions. Antifreeze proteins (AFPs), which are expressed by numerous organisms to protect against freeze damage (Białkowska et al., 2020), are underexplored candidates for this purpose. AFPs have been expressed in transgenic crops to improve frost resistance, including *Nicotiana benthamiana* (Huang et al., 2021) and recent work has shown the purification of these proteins by using their affinity to ice (Marshall et al., 2016). As ice grows, other solutes are excluded, thereby enriching the AFPs. Thus, I hypothesized that AFPs could be applied as fusion partners to enrich recombinant proteins produced in *N. benthamiana* via ice affinity purification. I fused dimeric Antifreeze Protein III isoform RD3 from *Lycodichthys dearborni* to mCherry and an Anti-Parasitic Protein (APP) currently under investigation at the Waterhouse laboratory. The fusions were transiently expressed in *N. benthamiana*, and the clarified plant extract was subjected to ice shell purification. My preliminary findings demonstrated the successful enrichment of both mCherry and APP via this strategy. Based on the ratio of incorporation of AFPs and contaminants in the ice shell, an optimized strategy for purification is proposed for future experiments.

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De novo genome assembly and comparative transcriptome analysis reveal divergence in Neptunia species and insights of Selenium hyperaccumulation and tolerance in *Neptunia amplexicaulis* from Australia

Katherine Pinto-Irish[1], Yibi Chen[2], Jeroen van der Woude[3], Mark G. M. Aarts[3], Subash Rai[4], Valentine Murigneux[4], Maggie-Anne Harvey[3], Peter D. Erskine[1], Antony van der Ent[1,3] and Cheong Xin Chan[2]

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Abstract:

Selenium (Se) hyperaccumulators are plant species characterized by their ability to accumulate >1000 µg Se g^{-1} in their tissues. As the chemical similarity between Se and sulphur allows it to use sulphur pathways in plants, the mechanisms of Se accumulation in hyperaccumulator species have been related to enhanced pathways of sulphur acquisition, transport, and assimilation. *Neptunia amplexicaulis* is a Se hyperaccumulator whose molecular mechanisms of Se tolerance and accumulation at genomic and transcriptomic levels have not been described so far. Here, we conducted a genomic and transcriptome analysis of *N. amplexicaulis*, and its close relative but non-accumulator *Neptunia heliophila*. Genomes were sequenced using a combination of Illumina and Oxford Nanopore Technologies from leaf tissue, and RNA sequencing was performed in a combination of Illumina and PacBio sequencing from root and leaf tissue from plants grown in hydroponic conditions exposed to low, medium, and high Se in the form of Na_2SeO_4 and Na_2SeO_3. Our results suggest that although the two genomes exhibit high similarity in size, sequence, and repeat content, *N. amplexicaulis* displayed unique tandem duplications of genes related to sulphate and phosphate transport, potentially contributing to its Se hyperaccumulation trait. Additionally, *N. amplexicaulis* showed a notable expansion of the pentatricopeptide repeat (PPR) protein family, which could be related to species-specific adaptations or organellar functions. Transcriptomic analysis revealed similar expression patterns between the species, with the main differences observed in the regulation of sulphate transporters in response to Se exposure in the root. This study reports the first insights into the molecular mechanisms of Se accumulation and tolerance in *N. amplexicaulis* and provides the basis for further study of Se metabolism.

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

Chamilka Ratnayake[1], Satomi Hayashi[1], Julia Bally[1] and Peter Waterhouse[1]

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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. However, the current plant reporter systems rely heavily on transgenic approaches, which pose significant limitations for field applications and commercial deployment. 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. In this study, 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 in nature. 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 have identified and characterised specific flavonoid hydroxylase genes in *Nicotiana benthamiana* which contribute to the different shades of anthocyanins. Subsequently, we have silenced the above genes using CRISPR/Cas9 technology, generating stable transformants that exhibit a spectrum of anthocyanin shades. These colours will be linked to specific metabolic pathways of interest through CRISPR/Cas9-mediated homologous recombination. This novel reporter system, utilizing endogenous anthocyanin-based visual markers, will enable real-time monitoring of gene regulatory networks in plants without the need for transgenes. Further, it will provide readily observable visual cues to detect and assess the activation and 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 field-based plant monitoring, overcoming the constraints of existing transgene-based systems and advancing agricultural research towards sustainable crop development.

Forward genetic screening in *Nicotiana benthamiana* to unravel genes responsible for successful anthocyanin production

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Abstract:

Nicotiana benthamiana, a native Australian species belonging to the agronomically valued Solanaceae family, is the species of choice for host-pathogen interactions and gene function analysis in plants. The popularity of *N. benthamiana* as a model plant system has seen exponential growth; however, its utilisation in forward genetics studies has been limited to date. Therefore, we developed a platform for high-throughput trait mapping and gene discovery pipeline based on the recently assembled high-quality genomes of two phenotypically distinct *N. benthamiana* isolates, LAB and QLD. We then utilised our platform to investigate the contrasting response to anthocyanin production observed in the two *N. benthamiana* ecotypes. Transient over-expression of AcMYB110, an activation regulator of anthocyanin biosynthesis, enhances anthocyanin production in the wild QLD ecotype, but is detrimental in the LAB strain. Here, we utilised a cross-population between LAB and QLD ecotypes to investigate the segregation of these contrasting parental phenotypes in its progeny. The First Generation (F1) produced from the cross exclusively exhibited the purple QLD phenotype, and the progeny of F1 (F1S1) segregated in a 1:3 ratio for LAB and QLD phenotype, respectively, suggesting that the inability to accumulate anthocyanin in the *N. benthamiana* LAB strain is recessive in nature. We carried out bulk segregant analysis by utilising a pool of 40 individuals exhibiting the phenotype of LAB for whole genome sequencing followed by mapping of F1S1 reads to the respective parental genomes to identify the causal region and gene of interest responsible for their phenotype. Furthermore, we investigated the depth of sequencing coverage required to successfully map the region of interest, providing insights into how to rapidly identify the genes or genetic loci responsible for a given trait in *N. benthamiana*.