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ORAL AND LIGHTNING TALKS

ABSTRACT BOOK

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Improving Food Security in West Africa: The Case of an Insect-Resistant Cowpea in Nigeria and Ghana

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Cowpeas (*Vigna unguiculata*) are an essential crop in West Africa, providing vital protein for over 200 million people. However, their production faces severe yield losses due to insect pests, particularly the destructive pod-borer *Maruca vitrata*. Traditional breeding methods have been ineffective in developing resistant varieties due to the lack of natural resistance, so farmers have relied on chemical insecticides, posing health and environmental risks. To address this problem, we developed a Bt pod-borer resistant (PBR) cowpea variety, the world's first genetically modified cowpea, which provides full protection against Maruca. It was released in Nigeria in 2020, providing farmers with an affordable, safe, and accessible solution, which is projected to yield over USD$336 million in benefits over the next 25 years. This project has triggered a transformative effect on seed systems in the region, with the establishment of national seed companies aiming to reach 25% of farmers within five years. The impact of this breakthrough extends beyond Nigeria, as the PBR cowpea was recently approved in Ghana in 2022, and it is expected in Burkina Faso soon. The release of a GM food crop in Africa is a significant achievement and it highlights the pivotal role of biotechnology in addressing global food security challenges. Moreover, the project is a great example of Australian science contributing to the UN Sustainable Development Goals. Our team is now focused on developing a second-generation PBR cowpea, incorporating two different Bt genes to prevent insect resistance and an additional gene to combat a grain storage pest.

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Genome wide methylome and CRISPR/dCas9-based gene activation in Medicago truncatula

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Tobacco retrotransposon, *Tnt1*, has been used to mutagenize and tag the whole genome of *Medicago truncatula*. We studied the somatic embryogenesis of *M. truncatula* R108 using leaf explants and explored the dynamic shifts in the methylation landscape from leaf explants to callus formation and finally embryogenesis. Robust cytosine methylation in all three contexts of CG, CHG and CHH patterns was observed during somatic embryogenesis compared to the controls. Differentially methylated promoter region analysis showed a stronger CHH methylation in embryogenesis samples when compared to CG and CHG methylation. Strong correlation (89.71 %) was identified between the differentially methylated regions and the site of *Tnt1* insertions in *M. truncatula* R108 and stronger hypermethylation of genes correlated with higher number of *Tnt1* insertions in all contexts of CG, CHG and CHH methylation.
We generated stable transgenic *M. truncatula* lines expressing the chimeric fusions of CRISPR/dCas9 with a transcriptional activator and single or multi-guide RNAs targeting the long-terminal repeats of *Tnt1* to activate the gene expression in the *M. truncatula Tnt1* mutants. These transgenic lines were crossed with *Tnt1* insertion lines wherein the insertions are in gene promoter regions. A significant upregulation of six-fold to 15-fold was observed in genes located at modular positions of ~500 bp upstream of the transcriptional start sites in the *Tnt1* insertion lines NF21042, NF11121 and NF8355. Overall, this novel approach could be utilized to overcome the necessity to overexpress individual genes that may result in misexpression of genes in undesired tissues when constitutive promoters are used.

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Induced genetic variation: Precision breeding utilizing conventional breeding technologies

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An innovative, accelerated trait development process, enables consumer accepted precision breeding, utilizing non-GM technologies

TRAITOMIC is a Carlsberg spin-out that is specialized in developing vast libraries of genetic variants in elite germplasm to identify traits of interest in plants and microbes. We recently expanded our library portfolio in Australia also in legumes. We have established successful collaborations with both, universities, and the agri-food industry. Utilizing only traditional breeding techniques, TRAITOMIC fast-tracks the identification of traits by combining large genetic libraries with an innovative and patented screening method. The method was validated in various crops, including several legumes as well as tropical plants for the identification of valuable traits. The platform technology is also routinely used to develop microbial strains for applications in food, feed, and agricultural bio-solutions. This non-GM approach can lead from seed to trait or cell to trait in a few weeks, thereby accelerating project deliverables and time to market for commercial products.

References on TRAITOMIC technology:

- Website: Traitomic | Empowering Nature
- Portfolio of available libraries: https://www.traitsource.com/
- Traitomic technology reference: FIND-IT: Accelerated trait development for a green evolution | Science Advances
- An example of academic collaboration on lupin https://doi.org/10.1126/sciadv.adg8866

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Genetic Characterisation of Plant Genetic Resources at the Australian Grains Genebank

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Genebanks play a pivotal role in conserving and promoting the genetic diversity of grain crops, which are essential for ensuring global food security and sustainable agriculture. The Australian Grains Genebank (AGG) Strategic Partnership is a joint investment by Agriculture Victoria and the Grains Research and Development Corporation (GRDC) that aims to unlock the genetic potential of the AGG to accelerate pulse, cereal and oilseed crop improvement for the benefit of Australian grain growers.

Plant genetic resources conserved within the AGG are widely accessed by the Australian and international research and breeding communities. So far, more than 18K chickpea, lentil, field pea and lupin accessions have been genetically characterised using the multispecies pulse 30K SNP array. The genotype data has been released publicly through an online repository under a CC BY 4.0 license. Bioinformatic tools being developed in the Partnership are enabling industry to use this data effectively for specific research and breeding questions. For example, the diversity of chickpea accessions conserved within the AGG has been assessed relative to ICRISAT accessions, diversity sets have been selected and visualised in the context of worldwide diversity for several pre-breeding projects, and the analysis of accession genotype data has enabled the detection of admixture within seed packets.

By integrating genomics into traditional genebank processes, the Partnership is developing a dynamic bio-digital resource to enable researchers and breeders to harness the full genetic potential of legume crop biodiversity. Genebank users can now make more informed selections to meet research and breeding objectives, enabling accelerated trait discovery and variety improvement for future generations.

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A role for gene editing in breeding chickpea for future protein.

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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, both to better meet the needs of established export markets as well as to grow new domestic markets.

One approach to support growth in the Australian chickpea industry is the use of gene editing, a technique for the targeted modification of genomes, for both direct application to precision breeding as well as in support of predictive breeding programs as a tool for validation *in planta*. Progress towards establishing robust techniques for the generation of gene edited chickpea plants will be presented, alongside a discussion of the prospective application pathways into breeding programs. An application for gene editing to support predictive breeding programs will be highlighted within the CSIRO Artificial Intelligence (AI) for Missions program which seeks to utilise AI in the analysis of large genomic data. This project aims to generate data driven predictions about traits of value which can be modified and tested by gene editing, such as the techno-functional properties of chickpea protein, assisting the development of new varieties tailored to individual markets.

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Genetic diversity in nutritional and phytochemical compositions of cowpea [*Vigna unguiculata* (L) Walp] germplasm tested under dryland farming system in South Africa

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The identification of potential cowpea (*Vigna unguiculata* (L) Walp) genotypes with dense grain nutritional and phytochemical compositions is key to improve global food and nutrition security. This study aimed at identifying cowpea genotypes possessing suitable grain nutritional and phytochemical compositions for consumption, production and breeding. The responses of 50 cowpea genotypes cultivated under dry land farming system were studied using grain nutritional [i.e., calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), zinc (Zn), protein, and fat] and phytochemicals [i.e., phenols, flavonoids and condensed tannins (cond. tannins)]. The genotype-by-environment interaction effect was significant ($p \leq 0.05$) for all studied traits except flavonoid contents. Pearson's correlation (r) analysis revealed the following positive and significant ($p \leq 0.001$) correlations: Ca correlated with Mg ($r = 0.92$), P with Zn ($r = 0.33$) P with Na ($r = 0.83$) and Fe with Ca ($r = 0.69$). Negative and significant ($p \leq 0.001$) correlations were recorded between phenolic content and Ca ($r = -0.40$), Na with K ($r = -0.16$), total phenolics with P ($r = -0.42$) and condensed tannins with K ($r = -0.35$). Based on principal components analysis (PCA), the genotypes G10, G12, G24, G29 and G47 were superior for Ca, Fe, Mg, Na and P contents, while G14, G23, G25, G27, G30, G34, G45 and G50 associated with increased phenolics content. The genotypes possessing desired grain nutritional and phytochemical compositions were recommended for consumption, cultivation and breeding.

Key words: Cowpea, genotype-by-environment interaction, grain, macro- and micro-nutrients, phytochemicals,

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Development of SNP based marker in urdbean for MYMIV resistance using transcriptome and candidate gene analysis

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Urdbean (*Vigna mungo* (L.)Hepper) is a highly consumed pulse crop in India. India is the largest producer and consumer of urdbean or blackgram. This crop faces many biotic stresses, among them, disease caused by the Mungbean Yellow Mosaic India Virus is one of the most prominent one. It may cause 100 percent failure of the crop if resistant varieties are not grown.

An experiment was conducted to find out the differential gene expression in a resistant variety (PU31) and a susceptible variety (LBG17). LBG17 is resistant to the Mungbean Yellow Mosaic Virus but showed susceptibility to the Mungbean Yellow Mosaic India Virus. These two varieties were tested for differential gene expression under control (disease free) and treatment condition (disease condition) each with three biological replications. Hence, RNA-Seq data was generated for 12 samples and *de novo* assembly was developed and differential gene expression analysis was conducted.Transcripts with FDR < 0.05 and absolute Log Fold Change value > 1 were considered as differentiallyexpressed (DE).Top 20 genes which are common and differentially expressed are taken to generate heatmaps.

A set of sixteen transcripts were analyzed for gene expression using RT-PCR. Among these two validated transcripts were used to develop DNA based PCR marker. Using Sanger sequencing of PCR amplicons SNPs were found between resistant and susceptible parents for these two genes. Using these SNPs polymorphic PCR usable primers were designed and validated in a panel of urdbean genotypes. These markers will be useful in marker assisted selection in urdbean.

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Expression profiling studies for eelucidation of drought tolerance mechanism in horsegram (*Macrotyloma uniflorum*)

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Horsegram (*Macrotyloma uniflorum*) genotypes were explored for its drought tolerance potential through physio-biochemical evaluation. Genotypes, IC-94637 (tolerant) and IC-426521 (sensitive) were selected for the identification of DEGs using RNA-Sequencing. cDNA libraries were prepared by reverse transcription of oligo-filtered RNA samples and subjected to high throughput sequencing on Illumina Hiseq platform for raw reads generation using Rsubread r-cran package. Of 28385 transcripts obtained 3977 and 1581 genes were found to be upregulated and 18015 and 4809 genes were downregulated in two lines under normal and stressed conditions. 30 highly expressed genes were identified, characterized and validated. These annotated genes encoded the proteins during stress that enhances drought tolerance by regulating cuticular wax biosynthesis (3-ketoacyl-CoA synthase (EC 2.3.1.-), enhancement of drought resistance by increasing the levels of osmotic adjustment (C2H2-domain). Study also conducted for identification and characterization of circular RNAs in the same genotypes of horsegram and comparative analysis of differentially expressed genes (DEGs) in response to drought stress, identifying 8 key DEGs. In, 2 and 8 genes were upregulated respectively under control conditions in IC-94637 and IC- 426521, while 2 and 1 genes were downregulated under stress for these lines. Therefore, it can be concluded that downregulation of genes exhibited a similar trend, emphasizing the complex regulatory mechanisms involved in stress response in each genotype. Distinct defensive mechanisms could be employed by horsegram genotypes while dealing with water stress. The superior genotype was able to withstand drought primarily through heightened expression of stress-related genes as compare to sensitive genotype.

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From Orphan Crop to Cutting-Edge Genomics: a Journey with Lentil

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At the first ICLGG meeting in 2002 those of us working in pulse crops could only dream of having the genomic resources that Medicago had and the ability to deploy genomic technologies in a breeding program. Fast forward to today where we are now facing down what some days feels like too much genomic data, and we are using genomics to tackle many different breeding targets in a more efficient way. We can now strategically access more diverse cultivate germplasm without compromising adaptation, giving us the ability to make wider crosses. We have moved from assaying single SCAR or SSR markers, often tenuously linked to a trait of interest to genotyping whole genomes. This allows us to look at not just at single nucleotide polymorphisms but also structural variation that can be associated with phenotypic variation. We also have a better picture of what wild lentil genomes look like and can predict how they will interact with the cultivated genome in interspecific crosses. Identifying both beneficial and deleterious regions of wild genomes allows breeders to access useful genetic variability only found in the wild accessions – e.g., disease resistance, while leaving behind the variability that would lead to trouble - shattering or dormancy, for instance. We have gone from being in awe of the Wheat geneticists in the Department to now having them look over our shoulder at some of the tools and approaches we have developed to make the most of technologies in the breeding programs.

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Unusual mergers, acquisitions, and diversity in the legume family

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The legume family, with approximately 770 genera and close to 20,000 species, is one of the largest and most diverse plant families. Following the family's origin around 70 million years ago, it diverged into six lineages—the largest of these in terms of species count being the Papilionoideae, with close to two thirds of the family's species, and the smallest being Duparquetioideae, containing the single eponymous genus Duparquetia. Among those six subfamilies, symbiotic nitrogen fixation (SNF) is present in most genera in the Papilionoideae but is found in only a scattered minority of genera in the second largest subfamily, the Caesalpinioideae. Adding to the complexity of the family—while also contributing to the potential for evolutionary innovation—is the presence of whole genome duplications (WGD) early in most of the subfamilies (though interestingly, skipping two early-diverging genera). Resolving the timing of the early evolutionary events has been difficult, as both the diversification and the WGD events occurred within 10-20 million years following the family's origin. Adding to the difficulty is the apparent delayed allopolyploid origin of Caesalpinioideae, probably involving progenitors of the Papilionoideae and one of the other subfamilies. This allopolyploidy likely contributed to the great diversity seen in the Caesalpinioideae, including the variation in capacity for SNF in that subfamily. A finding of allopoloyploidy along the legume backbone also means that the legume phylogeny cannot properly be represented by a standard bifurcating phylogenetic model but needs to be considered as a more complex reticulate history.

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Faba bean: From orphan crop to genomic trailblazer

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Faba bean (*Vicia faba*) is a globally adapted protein crop with a high yield potential and an exceptional nitrogen fixation capacity. Just a decade ago, faba bean was an orphan crop with scarce genetic and genomic resources. However, the development of the first reference genomes [1-2] has paved the way for high-resolution gene mapping and molecular genetics. I will illustrate how this new genomic information is enabling us identify markers and candidate genes robustly associated with agronomic traits across populations. I will also share our emerging understanding of the genetics that control genotype by environment interactions for yield, and how this is allowing us to predict the performance of specific genotypes in unseen environments. Lastly, I will discuss the ongoing efforts to further enhance faba bean genetic and genomic resources.

References:

*[1] Jayakodi, M. et al. The giant diploid faba genome unlocks variation in a global protein crop. Nature **615**, 652–659 (2023).*
[2] https://projects.au.dk/fabagenome

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On the pulse - enhancing the quality of grain protein for future food

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There is a growing demand for plant-based protein sources to meet future global nutritional requirements. Pulses provide protein, fibre and nutrient-dense seeds and are an excellent source of bioactive components that can have positive health outcomes, controlling cardiovascular disease, diabetes, and obesity. Soy has been a mainstay of the plant protein ingredient market, but new plant protein sources are emerging. For instance, mung bean is a vitamin-rich legume that offers unique functionality including emulsification and gelling properties and hence has seen use as an egg alternative. Likewise, chickpeas are a popular protein source for many people and in many cuisines. Chickpea has seen recent application as an emulsifier, as an ingredient in both hot and cold beverages based on its neutral flavour, viscosity, and mouthfeel. Another example is lupin whose seeds possess high protein content and can lower cholesterol and blood pressure. Despite their potential and the health-enhancing properties of the seedborne proteins, pulses remain under-utilised as a human food, in part due to food allergies or intolerances. Food processing treatments, including protein fractionation, isolation or modification including via microbial fermentation can reduce or degrade allergenic proteins, toxic components (like alkaloids) or carbohydrates (like raffinose) and thus play a role in the development of innovative plant-based food products. The opportunity exists to select pulses to meet specific end use applications, to inform breeding programs to enhance food related traits, or to apply processing steps or treatments to enhance the final products.

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Toward soybean molecular design breeding

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Soybean (*Glycine max* [L.] Merr.) is a major crop of agronomic importance as a predominant source of protein and oil. To meet the needs of the rapidly increasing human population, soybean breeders are challenged with finding a high-efficiency breeding strategy for developing soybean varieties with higher yield and improved quality. Molecular breeding has been proposed to be a powerful and effective approach for crop breeding, but requires a better understanding of the genetic architecture and networks underlying agronomical traits.

To develop molecular design breeding in soybean, we first comprehensively investigated the genetic information and evolution of large number of representative soybean accessions from worldwide, including individual de novo genome assemblies for 27 representative soybeans that were selected from 2,898 deeply sequenced accessions, and investigation the genetic variation, epigenetic variation, and 3D-genomic variations among the accessions. Meanwhile, we developed an integrating method to identified the causal genes conferring important agrominic traits based on population genetics and multi-omics data, and identified a number of genes. Through systematic genome-wide association analysis and genetic network construction, we established multi-trait genetic regulatory coupling network, and bred some elite cultivars by molecular design breeding.

References:

[1] Liu Y. et al, Pan-genome of wild and cultivated soybeans. Cell 2020, 182: 162-176.
[2] Wang M. et al, Parallel selection on a dormancy gene during domestication of crops from multiple families. Nature Genetics 2018, 50: 1435-1441.

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Exploring the mungbean pan-genome

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Mungbean (*Vigna radiata*) is a short-season tropical pulse grown on over six million hectares worldwide, mostly in Southern Asia with increasing importance in Australia and East Africa. Current commercial varieties show little genetic diversity with breeding focussed on a limited number of agronomic traits like disease resistance and flowering time. Luckily, we have access to diverse core collections and an Australian NAM (Nested Association Mapping) population.

Eight genetically diverse NAM parent accessions were selected based on available DArTseq SNP data including commercial varieties and an Australian wild accession (*V. radiata* var. *sublobata*). High molecular weight DNA was extracted for long-read HiFi PacBio sequencing (71-136x coverage). These reads were assembled into high quality genomes (contig N50 17.6-32.8 Mb) with a total assembly length varying from 512.8 to 577.2 Mb and a BUSCO completeness score of over 99% for each of the eight accessions. RNAseq data was generated using Illumina paired-end sequencing for shoot, root, and nodule tissues to allow for evidence-based gene annotation and differential gene expression analysis. Overall, this approach allowed for improved assembly statistics relative to the publicly available genomes VC1937A and Vrad_JL7.

This high-quality pan-genome makes it possible to investigate diverse topics from crop improvement to evolutionary biology. It can be utilised by researchers and breeders to explore and improve complex traits like nitrogen fixation, yield stability, seed quality and adaptability to climate stresses. Integration of this sequence data with other data types like diverse phenotypes will help us move towards genome-based predictive breeding and crop improvement.

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PanFaba: The Pangenome of faba bean

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Faba bean (*Vicia faba* L.) is one of the first domesticated plants at the dawn of agriculture. Its high yield potential and higher grain protein content hold promises for sustainable regional protein supply worldwide. The giga-size chromosomes (~13 Gb) bloated by repetitive sequences had hampered its study and crop improvement. Recently, with international collaborative effort, a high-quality reference genome was released [1]. With a revolutionary new low-cost high accurate long-read sequencing and a chromosome-scale physical map in hand, it is now possible to undertake investigations into the pangenome of faba bean. We characterized the faba bean germplasm collections (> 2000 accessions) representing global diversity using genotyping-by-sequencing (GBS). We selected a representative set of over 45 genomes to develop chromosome-scale genome assemblies. The faba pan-genome will contain a more accurate and diverse representation of global genomic variation, improve trait mapping and serve as the permanent genomic resource for sustainable breeding and research.

References:

[1] Jayakodi M. et al, The giant diploid faba genome unlocks variation in a global protein crop, Nature, 615, 2023, 652-659.

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Genomic changes shaping domestication traits of soybean

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During soybean domestication, genetic variations were selected to improve agronomic traits such as growth habit, flowering time, and crop yield. Genomic studies in crops not only advance our understanding about crop domestication, but also enable the identification of novel alleles for crop improvement.

To understand domestication-related events using wild germplasms, we have constructed a reference-grade genome of wild soybean W05. By comparing between the wild and cultivated soybean genome, a complex structural rearrangement which led to the expression of a subtilisin-anti-CHS1 chimeric transcript was found to cause the seed coat color change during domestication. Using a recombinant inbred population between wild soybean W05 and cultivated soybean C08, we have performed QTL mapping and successfully identified genomic variations regulating different domestication traits. Making use of the optical mapping data, a copy number variation region of the *gibberellin 2-oxidase 8* genes was found to be strongly selected during domestication to repress trailing growth and long shoots. Also, several QTLs for root system architecture-related traits was found to co-localize with flowering-time loci in soybean. Knocking down the maturity gene *E1* led to a diminished root system, suggesting that the selection for flowering time has indirectly shaped the root system architecture in soybean. We also identified that a loss-of-function allele of a transcriptional repressor, GmbHLH113, contributed to a longer root hair that has potential beneficial effect on nutrient acquisition. To facilitate breeding, we also developed a universal panel of DNA markers including domestication traits-associated markers based on the genomic data from popular soybean varieties.

References:

[1] Xie M. et al, 'A reference-grade wild soybean genome', Nat Commun, vol. 10, 2019, p. 1216.
[2] Wang X. et al, 'Increased copy number of gibberellin 2-oxidase 8 genes reduced trailing growth and shoot length during soybean domestication', Plant J, vol. 107, 2021, p. 1739-1755.

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A high-quality genome assembly of an Australian chickpea variety

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We have generated a genome assembly for an inbred line (i4) of the Australian desi chickpea variety, PBA HatTrick. PBA HatTrick (Jimbour/ICC14903, released 2009) is adapted to Australian conditions. It has been used to generate bi-parental and mutant populations for research, and as a parent for newer varietal releases.

The assembly was developed using PacBio HiFi sequencing data for contig generation, and alignment to Bionano consensus maps. Employing the hifiasm algorithm [1], we generated two assemblies: the first by using all HiFi reads, and the second from chloroplast-free HiFi reads. The latter was aligned to Bionano maps, and the former was used to close gaps between scaffolds. Scaffolds were anchored to two genetic maps and assigned to chromosomes by sequence alignment to CDC Frontier genome assembly v3 [CicerMine: Data Categories (legumeinfo.org)].

For six chromosomes we created pseudomolecules that contain only small gaps (regions with N bases). The other two chromosomes (3 and 5) remain fragmented as ordered scaffolds, due to the presence of large repetitive regions. Two scaffolds, containing mainly repetive DNA, could not be allocated to chromosomes. The total estimated genome size of 710.44 Mb is close to what has been published for CDC Frontier (738.09 Mb) [2].

Our PBA HatTrick assembly, which is a significant improvement over earlier genome assemblies, is a valuable new resource for chickpea research. It is available for download or for BLASTing with sequences of interest, via the webpage https://hatchiblap.adelaide.edu.au.

References:

[1] Cheng H. et al, 'Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm', Nat Methods, vol. 18, 2021, p. 170.

*[2] Varshney R. et al, 'Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement' Nat Biotechnol, vol. 31, 2013, p. 240.*

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Medicago truncatula FLOWERING LOCUS T genes *FTb1* and *FTb2* function redundantly to control the induction of flowering in response to long-day photoperiods.

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Flowering time significantly impacts plant yield and adaptability to diverse climates and day lengths. The FLOWERING LOCUS T (FT) genes play pivotal roles in the transition from vegetative to reproductive stages in plants. In the legume *Medicago truncatula*, an important forage crop, six FT orthologs - *Mt FTa1, FTa2, FTa3, Ftb1, Ftb2,* and *FTc* - are present. Currently, only the role of *MtFTa1* has been described[1], with a strong involvement in promoting flowering after prolonged exposure to cold, known as vernalisation, but the functions of the other five are not fully understood. In this study, we demonstrate that the genes *MtFTb1* and *b2* together are essential for the induction of flowering only under long-day (LD) photoperiod conditions. Using CRISPR/Cas9, we generated single and double mutants for *MtFTb1* and *MtFTb1/MtFTb2* genes. We found that they function redundantly and are required for the upregulation of the *MtFTa1* gene under LD conditions. While the *Mtftb1* mutants displayed normal flowering behaviour under both short day (SD) and long day (LD) conditions, the *Mtftb1*/2 double mutant exhibited delayed flowering exclusively under LD conditions and retained its response to vernalisation (V). To identify genes that act downstream of *MtFTb1* and *MtFTb2*, we performed a transcriptomic analysis comparing wild-type and *Mtftb1/2* plants. This analysis revealed differentially expressed genes (DEGs), including both known and novel transcription factors that act to promote the floral transition. This study sheds light on the genetic control of flowering time in legumes and could have practical implications for increasing forage productivity.

References:

[1] Laurie, Rebecca E., et al. "The Medicago FLOWERING LOCUS T homolog, MtFTa1, is a key regulator of flowering time." Plant physiology 156.4 (2011): 2207-2224.

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Genomic analysis of alfalfa for the development of salt and drought tolerant germplasm for breeding programs

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Alfalfa (*Medicago sativa* L.), also known as the queen of forages, holds significant importance as a legume forage crop due to its high yield and nutritional quality. Cultivated alfalfa (*Medicago sativa* ssp. sativa L.) is a perennial, self-incompatible, autotetraploid species. In Canada, alfalfa is cultivated across various climatic regions, facing challenges such as salt and drought stress which adversely affect its production. This project aims to enhance alfalfa breeding programs by developing genomic resources for higher-yielding cultivars with increased tolerance to salt and drought stress. Utilizing PacBio long-read sequencing, contig-level assemblies were created for PI212798 (diploid) and Beaver (tetraploid) lines, yielding genome sizes of 889 Mb (N50: 26.75 Mb) and 2064 Mb (N50: 10.23 Mb) respectively, with BUSCO analysis indicating >99% completeness for both assemblies. Currently, a HiC-based scaffolding approach is underway to develop pseudo-chromosome scale assemblies, followed by gene annotation and repeat analysis. Additionally, Illumina short-read sequencing was employed for genotyping by sequencing (GBS) to assess genetic diversity across a broad panel of alfalfa germplasm. Pre-screening for salt stress identified 8 potential salt-tolerant lines. The outcomes of this project will facilitate the development of highly contiguous and high-quality genome assemblies and a pangenome, serving as foundational genomic resources. These resources will expedite the future breeding efforts and identify key genes/QTLs associated with salt and drought tolerance, thereby expediting the development of salt and drought-tolerant alfalfa cultivars.

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One Century of Discovery in Mendel's Pea Genes

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Pea, Pisum sativum, is an excellent model system that enabled the establishment of the foundational principles of inheritance by Gregor Mendel through the studies in seven pairs of contrasting traits. However, the molecular nature of the genetic differences underlying Mendel's pea traits remains incompletely understood, as indeed is the case for many agronomic traits which are targets in today's pea breeding. In this talk, I will present a genomic and phenotypic variation map coupled with an extensive haplotype-phenotype association analyses across a wide range of traits in a widely-used Pisum diversity panel. I will focus on the genomic and genetic dissection of each of the seven traits that Mendel studied in detail, revealing significant genetic loci and many previously undescribed alleles particularly for the three remaining uncharacterized pea genes. I will also look back into the research history of Pea and genetics in the past one century, and share a vision on the genomics-driven '21st-century modern synthesis' to understand traits and genes both for fundamental research and for applied practices in crop breeding. (this is a collaborative project (Mendel Pea G2P) between CAAS and JIC, between I with Noel Ellis).

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Genomic and nutritional analyses of bitter vetch, a traditional grain legume adapted to marginal semi-arid regions

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Bitter vetch (*Vicia ervilia*, 2n=14), is a traditional Mediterranean/west Asian forage and grain legume with no economic significance to industrial economies. Despite being listed among the Neolithic Near Eastern 'founder crops'[1], it is still understudied. Due to its adaptation to drought and shallow soils bitter-vetch is cultivated in semi-arid steppe regions and given climate-change scenarios may be considered as a climate-resilient forage and alternative protein source. A germplasm collection of wild and domesticated genotypes was assembled and is being evaluated for agronomic and nutritional-quality traits. Pod-shattering and hardseededness are typical of wild forms while non-shattering and free germination characterize the domestic genepool[2]. The range of seed protein and of amino acids levels in the various genotypes was tested. Likewise, metabolomics based analysis shows consistent separation based on grain metabolic features. Pollen fertility of domesticated x wild F_1 hybrids were above 90% in certain combinations, and 50% in others, indicative of chromosome order variation among the wild genepool. QTL and GWAS analyses of agronomic, grain quality and domestication traits will be based on a high-quality chromosome level (96.34% completeness; BUSCO) reference genome of 3.6 Gb. RAD-seq genotyping of ~1300 accessions comprised of the entire germplasm array and hybrid progeny populations will expose the structure of both domestic and wild genepools and identify selective domestication associated sweeps. Understanding the evolutionary trajectory of bitter vetch in the wild and under domestication may help discover the genetic basis of its adaptation profile and provide clues for future breeding and yield and quality improvement.

References:

[1] Abbo S, Gopher A (2022) Plant Domestication and the Origins of Agriculture in the Near East. Cambridge University Press.
[2] Ladizinsky G, van Oss H (1984) Genetic relationships between wild and cultivated Vicia ervilia (L.) Willd. Bot J Linn Soc 89: 97-100.

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Environment as a limiting factor of the range expansion of cultivated mungbean

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After being domesticated, the cultivation range of crops expanded to different agro-ecological zones. How did this crop globalization occur, and how did crops adapt to diverse natural environments? Using mungbean (*Vigna radiata* var. *radiata*) as a test case, we examined the factors shaping the expansion route of a crop. The Asian mungbean cultivars could be separated into four genetic groups associated with distinct geographic regions, including South Asia (SA), Southeast Asia (SEA), East Asia (EA), and Central Asia (CA). Despite the geographic proximity between Central and South Asia, we showed that after initial domestication in South Asia, the mungbean first expanded to Southeast Asia, then East Asia, and reached Central Asia the latest. We showed that environmental factors may be more important than geographical barriers, and local water availability and growing season length might be the critical factor limiting the direct expansion of South Asian cultivars into Central Asia. This hypothesis is supported by their phenotypes, suggesting distinct types of selection in regions of Asia, with artificial selection likely maximizing yield in the south and natural selection for environment adaptation in the north. The results suggested that human activities might not solely dictate the patterns of crop range expansion, and environmental adaptation might be important.

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Combination of Phenomics, Genomics and Transcriptomics to Dissect Genetic Basis of Soybean Compact Plant Architecture

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Planting soybeans (*Glycine max* (L.) Merr.) at high densities has been shown to significantly enhance crop yields. To achieve this, we harnessed omics-based solutions to pinpoint genes that facilitate successful high-density planting practices. Our study began by collecting time-series phenotypic data using an unmanned aircraft system, focusing on canopy coverage variation in a diverse panel of soybean varieties. Through a genome-wide association study (GWAS), we identified 35 loci that displayed dynamic associations with canopy coverage across developmental stages. Among them were 10 known QTL related to flowering time and plant height, as well as novel QTL influencing canopy coverage. One of these novel loci named *GmARF1* showed evidence of adaptive selection during domestication, with a low canopy coverage haplotype favored in high-density planting within high latitude regions. Furthermore, we conducted GWAS and a transcriptome-wide association study (TWAS) on plant height, leading to the identification of *GmPH13*, which encodes a WD30 protein. A 5.5kb transposon insertion responsible for a truncated GmPH13 protein results with reduced plant height and improved lodging resistance, making it suitable for high-density planting. Confirmation of these findings was achieved through gene editing mutants of *GmPH13*, which exhibited reduced height, while over-expression of *GmPH13* led to increased plant height. Furthermore, we identified 57 soybean varieties harboring favorable haplotypes for both *GmARF11* and *GmPH13*. In summary, our research not only provided valuable insights into genes but also furnished a collection of materials with an expanded genetic basis for soybean cultivation under high-density planting conditions, ultimately enhancing crop productivity.

References:

[1] Qin et al. 2023, Nat Commun, vol. 14, p. 6813

[2] Li et al. 2023, J Integr Plant Biol, vol. 65, p. 117–132

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The Genome-Wide Association Mapping reveals new insights into genome diversity of wild Old World Lupins

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Grain legumes are an important source of nutrients for both animal feed and human food production. Among these legumes are native to the Mediterranean Old World Lupins (OWLs), which are of particular interest to researchers due to their complex flowering regulation, including the requirement for vernalization to promote flowering.
Only three out of 12 OWL species have undergone the domestication process, which required the identification of early-flowering, thermoneutral lines that can be sown independently of temperature and photoperiod. However, this process has reduced their genetic variability, limiting their ability to adapt to climate changes. **Therefore, a major goal of modern lupin genetics is to find new sources of genetic variability that can help improve current varieties and create new ones better suited for the climate of the future[1].**
Our research focuses on understanding the mechanisms of flowering induction and regulation in wild OWLs by analyzing homologs of the *FT* gene and potential indels in their promoter region. To achieve this, we conducted a series of glasshouse experiments for 10 species over two years, during which we recorded the dates of the first bud, flower, and pod emergence for approximately 700 accessions. This was supplemented by Genome-Wide Association Mapping using DArT-seq markers generated for three species with at least 100 accessions/genotypes. The next step will involve sequencing both mRNA and whole-genome DNA, as well as conducting Differental Gene Expression analysis to identify the key regulators in the flowering induction pathways of this species.

Funding source:

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References:

[1] Bohra, er al., 2022, Trends Biotechnol. 40, 412-431.

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Domestication genetics in the mysterious Ethiopian pea *Pisum abyssinicum*

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Studies of crop domestication provide valuable insight into the history of agriculture and the genetic basis for adaptation. In the temperate legume pea (*Pisum* spp.) two independently-domesticated forms are known: the widespread *P. sativum,* one of the world's oldest crops, and *P. abyssinicum*, a minor form restricted mainly to Ethiopia [1]. Little is known about the origin of *P. abyssinicum,* or the genetic control of key domestication traits such as pod indehiscence and reduced dormancy, relative to *P. sativum.* However, given that wild *Pisum elatius* does not flower under short photoperiods, the low-latitude distribution of *P.abyssinicum* implies that the ability to flower early under short photoperiods may have also been important in its domestication, unlike *P. sativum*.

Analysis of a *P. abyssinicum x* wild *P. elatius* cross identified three QTL for flowering time and four for dormancy, all of which co-located with QTL in corresponding analyses of domestication traits in *P. sativum* [2]. Initial sequence-level analysis has revealed contrasting histories of key candidate flowering time genes in *P. abyssinicum*, with *FTa3* and *TFL1c* more similar to *P. elatius* and *P. sativum* groups, but *FTa1* showing surprisingly high affinity with a second wild species *P. fulvum*. This work implies a complex origin of *P. abyssinicum* independent of *P. sativum,* and further highlights a central role of *FT/TFL1* genes in crop legume evolution.

References:

[1] Kosterin O. (2017) Abyssinian pea – a problematic taxon Act. Biol. Siber. 3, 97-110
[2] Williams, O. et al. (2022) The genetic architecture of flowering time changes in pea from wild to crop. J. Exp. Bot. 10.1093/jxb/erac132

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Unravelling Chickpea's Genetic Landscape with the Cicer Super-Pangenome

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Chickpea (Cicer arietinum L.) - an important legume crop cultivated in arid and semiarid regions—has limited genetic diversity. Efforts are being undertaken to broaden its diversity by utilizing its wild relatives, which remain largely unexplored. In this direction, we developed the Cicer super-pangenome based on the de novo genome assemblies of eight annual Cicer wild species. We identified 24,827 gene families, including 14,748 core, 2,958 soft-core, 6,212 dispensable, and 909 species-specific gene families. The dispensable genome was enriched for genes related to key agronomic traits. Structural variations between cultivated and wild genomes were used to construct a graph-based genome, revealing variations in genes affecting traits such as flowering time, vernalization, and disease resistance. These variations will facilitate the transfer of valuable traits from wild Cicer species into elite chickpea varieties through marker-assisted selection or gene-editing. This study offers valuable insights into the genetic diversity and potential avenues for crop improvement in chickpea. Furthermore, to enhance the accessibility and utilization of these genomic resources, we developed a user-friendly, public repository 'CicerPanDB'. This comprehensive database hosts all genome assemblies, gene models, and structural variations identified between wild and cultivated Cicer species, supporting global research and breeding programs aimed at enhancing chickpea's agronomic traits and resilience to climate variability.

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Medicago truncatula Mutant Resources for Legume Genomics

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Medicago truncatula is widely used model legume species for genetics, genomics, and functional genomics studies. The genome sequencing of the ecotype Jemalong A17 has been completed and published in 2011. To better facilitate the characterization of gene functions in *M. truncatula* and other legume species, we spent more than 10 years and generated more than 21,700 *Tnt1* retrotransposon insertion lines, which encompass approximately 500,000 insertions in the genome of *M. truncatula*. The *Tnt1* mutant population is estimated to cover about 90% of genes in the *M. truncatula* genome. To make the maximal utilization of the mutant resources, we generated a searchable web-based database (https://medicago-mutant.dasnr.okstate.edu/mutant), which contains photos of all lines, phenotype description of lines exhibiting visible phenotypes during forward screening, and more than 400,000 *Tnt1*-flanking sequence tags (FSTs) from 21,000 lines. So far, we have distributed more than 17,000 *Tnt1* lines to scientists from 45 laboratories in 24 countries and more than 260 papers resulting from *Tnt1* mutants have been published. These publications cover many areas of plant biology, including plant physiology, nutrition, metabolism, growth and development, and plant-microbial and plant-environmental interactions.

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Translational Research in Grain Legumes: Results, Applications and Perspectives

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Translational research is an opportunity to translate basic discoveries into applications quickly and efficiently. In plants, basic science can indeed be translated into methods and innovations to develop and improve crop varieties and thus achieve food security in a sustainable and safe way. It is therefore important to generalise the application of translational research and make it accessible to a large community of researchers and breeders. Grain legumes, including pea, faba bean and lentil, are an important source of protein for animal and human nutrition. As the demand for plant-based protein continues to grow, more land is being devoted to these crops, but many challenges still limit their productivity. Improving the response of grain legumes to limiting factors is key to ensuring the stability of grain yield quantity and quality. Here, we report on the use of OrthoLegKB, a new knowledge graph database for grain legumes, to query large available genetic, genomic and transcriptomic datasets. We show how relevant biological questions can be addressed and how information from a single species or group of species can be simply transferred to other species. OrthoLegKB is an important step towards translational approaches in grain legumes and a great tool for the legume research and breeding community. Current results related to seed quality and stress resistance will be highlighted and future developments to accommodate new data types will be discussed.

Reference:

[1] Imbert B. et al., Development of a knowledge graph framework to ease and empower translational approaches in plant research: a use-case on grain legumes, Front Artif Intell., 6:1191122, 2023. doi: 10.3389/frai.2023.1191122.

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Reference genome sequence and population genomic analysis of peas provide insights into the genetic basis of Mendelian traits and beyond

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Peas are important sources of starch, protein, dietary fiber, and other nutrients for humans. As the genetic model used to discover Mendel's laws of inheritance, the study of agronomic traits in peas served as the foundation of modern genetics. In this study, we reported a de novo assembly of the chromosome-level genome for the elite vegetable pea cultivar 'Zhewan No.1' and used 314 accessions encompassing wild relatives, landraces, and cultivars resequencing data to resolve the genetic variation map of pea. We uncovered 235 candidate loci associated with fifty-seven important agronomic traits using genome-wide association studies (GWAS). In particular, we anchored the causal gene haplotypes of *PsGA3ox1*, *PsbHLH*, *PsSGR*, and *PsSBE1* associated with four Mendel's traits of stem length (*Le*/*le*), flower color (*A*/*a*), cotyledon color (*I*/*i*), and seed shape (*R*/*r*), respectively. Furthermore, *PsCLE42* and *PsPPO1* encoding a CLAVATA/ESR (CLE)-related protein and a polyphenol oxidase were mapped as causative genes controlling the resultant traits of pod form (Mendelian *P*/*p*) and hilum color by integrating GWAS and bulked segregant analysis (BSA) approaches. In addition, we constructed a spatio-temporal gene expression atlas via transcriptome analysis across twenty-two tissues and highlighted the gene modules involving in pod and seed development. These findings provide valuable pea genomic information and will facilitate the future genome-informed improvement of pea crops.

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Differential selection of yield and quality traits has shaped genomic signatures of cowpea domestication and improvement

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Cowpeas are important tropical legumes in ensuring food and nutritional security in developing countries especially in sub-Saharan Africa. Herein, we reported two high-quality genome assemblies of grain and vegetable cowpeas and resequenced 344 accessions to characterize the genomic variations landscape. We identified thirty-nine loci for ten important agronomic traits and more than 541 potential loci underwent selection during cowpea domestication and improvement. Particularly, the synchronous selections on the pod-shattering loci and their neighboring stresses relevant loci likely led to the enhancement of pod-shattering resistance and the compromise of stresses-resistance during the domestication from grain to vegetable cowpeas. Moreover, differential selections on multiple loci associated with pod length, grain number per pod, seed weight, pod / seed soluble sugars and seed crude proteins shaped the yield and quality diversity in cowpeas. Our findings provide genomic insights into cowpea domestication and improvement footprints, enabling further genome-informed cultivar improvement of cowpeas.

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Towards a complete phylogenetic tree of angiosperm genera, including legumes!

Bailey PC[1] & the PAFTOL Community

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The plant component of the Plant and Fungal Trees of Life (PAFTOL) project at the Royal Botanic Gardens, Kew aims to reconstruct a phylogenetic tree comprising at least one species representative of each genus of all (~13,700) angiosperm genera. This tree will be an essential tool for comparative evolutionary studies such as the analysis of traits and distribution data. Such a goal is now feasible due to the efficiency of methods used to extract DNA from herbarium specimens and the development of targeted enrichment approaches that allow the sequencing of a set of orthologous nuclear genes from species across all angiosperm families using a universal probe set. To date, the PAFTOL project has sourced material for 92% of genera and representative specimens have been sequenced for 60% of them. An angiosperm tree of life was reconstructed from the data which is available in the Kew Tree of Life Explorer data portal (https://treeoflife.kew.org) along with all supporting data[1]. In a recently published study, we have refined these analyses to explore the relationships of key groups in angiosperms and, by scaling the tree to time with fossils, have investigated the dynamics of diversification during angiosperm evolutionary history[2]. We are now finalising our search for the remaining genera with collaborators and from public resources for our final data release in the second half of 2025. As part of PAFTOL, we have made a particular effort at securing material for all the 796 genera of the legume family, which has been largely achieved.

References:

[1] Baker et al (2022) A Comprehensive Phylogenomic Platform for Exploring the Angiosperm Tree of Life. Systematic Biology 71, 301–319
[2] Zuntini et al (2024) Phylogenomics and the rise of the angiosperms. Nature 629, 843–850

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Tailoring more nutrient and healthy beans: from lab to fork

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Current eating habits, primarily based on animal protein sources, are not sustainable and impact both planetary and human health. This has led to the recognition of the need to shift to alternative protein sources. Legumes are an excellent alternative to animal protein. Common bean (*Phaseolus vulgaris* L.) is a significant legume in the human diet and a major source of key nutritional components. However, its consumption and use in developing novel food products are limited by a number of bioactive components such as lectins, α-amylase inhibitor, trypsin inhibitor, raffinosaccharides, and phytic acid that have both negative (ex. limit nutrient availability, cause intestinal discomfort) and positive (ex. prebiotic effect, reduce glycemic index, anticancer activity) impact on human health and nutrition.

To address these challenges and better exploit the nutritional and health potentials of the common bean, we have utilized natural and induced genetic variability. We identified useful nutritional traits such as a low phytic acid (*lpa*) mutant, a lectin null mutant (*lec*-), and a phaseolin null/α-amylase inhibitor enriched mutant (*phsl*-, *αAI++*). These traits have been combined, and the resulting improved genotypes, along with wild-type beans, have been used to develop bean-based bakery products with enhanced nutritional properties[1,2], also utilizing fermentation as a beneficial process to improve technological properties.

References:

[1] Sparvoli, F. et al, 2016, Front. Plant Sci. 7, 928
[2] Sparvoli, F. et al, 2021, Nutrients, 13, 4517

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Pea Seeds with Reduced Antinutritional Proteins: A Techno-functional and Nutritional Study

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Sustainable and nutritious protein sources are becoming increasingly essential due to a growing global population and the rising popularity of plant-based diets. Legumes, such as pea (*Pisum sativum* L.) emerge as promising alternatives due to their nutritional richness and environmental sustainability. Nonetheless, their full utilization is hindered by suboptimal techno-functional performance and the presence of antinutrients. This research aimed to evaluate the techno-functional properties of proteins isolated from two distinct pea lines: a wild-type control pea and a mutant line carrying null mutations for three proteins with poor nutritional characteristics.

The seeds of the wild-type and mutant pea lines were milled, and protein was isolated in an alkaline buffer. Next, the protein isolates were used to create emulsions and foams. The emulsions were characterized based on their particle size, zeta potential, microstructure, and stability over time, whereas foams were evaluated based on their foamability and stability. The pendant drop method was used to measure their surface tension and interfacial properties.

Both wild-type and mutant pea proteins showed effective emulsion stabilization over a 24-hour period, although the low zetapotential values indicated potential instability over time. The mutant pea line exhibited higher foamability, suggesting improved foaming properties compared to the wild-type. However, the mutant line had a higher surface tension as compared to the wild-type indicating that the foams could be prone to instability over time. Future work will assess the impact of the null mutations on the bioaccessibility of starch and proteins, in addition to the reduced allergenic potential of pea.

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Chickpea grain composition for future markets

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Chickpea is a key agricultural crop which has been consumed by humans for millennia, and is currently ranked third among pulses in global production. There is an increased focus on its use as a source of plant-based protein due to its nutritional value and potential applications to functional ingredients. Chickpeas are categorized into two market classes, desi and kabuli according to grain size and colour. Despite its potential application as a nutritious ingredient, variation in grain composition within and between classes remains largely unexplored. In this study, we characterise the variation in seed content for several key macronutrients (sugar, starch, protein, lipid classes, fibre) in a global chickpea diversity panel, to identify drivers of grain composition changes and relationships between the different nutritional traits. Assays performed on equal quantities of ground chickpea flour revealed extensive variation in almost every seed composition trait, with no relation to market class or geographic origin. Correlations between macronutrient content also ranged from moderate to absent, suggesting independent genetic control amenable to breeding. To gain insight into this genetic control, we genotyped the panel using a SNP chip and conducted a Genome Wide Association Study, which identified several loci of interest and provided promising avenues for future work. Taken together, these results highlight important opportunities for developing breeding strategies aimed at creating chickpea varieties with enhanced nutritional profiles and better suitability for emerging markets in the food industry.

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Varietal Selection For Life-Saving Ready to Use Therapeutic Foods (RUTF)

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NUTRISET was created in 1986 to fight malnutrition and nourish kids in need. For more than 35 years, NUTRISET and its PlumpyField partners have been revolutionizing the treatment and care of malnutrition thanks to the invention of the Ready-to-Use Food (RUF) concepts in which peanut is the main ingredient. PlumpyNut®, the essential life-saving food treatment is produced in 11 countries, reaching over 10 million persons in need since 2020. In 2022, close to 100 000 metric tons have been produced from PlumpyField factories, in the US, the Caribbean, France, Africa and India. The Nutriset Group today encompasses a network of producers, PlumpyField who transform peanut's in 9 countries.

From the beginning, peanut has been crucial to the development of the product and the localization of the production in countries with vulnerable populations. Thousands of farmers are benefiting from the procurement of PlumpyField's producers and we had to invest in the whole value chains from the field to roasting facilities. We will present the multi-sector and pluri-disciplinary approach to tackle children malnutrition challenges through varietal selection and agriculture with the promising results of peanuts varieties exhibiting nutritional traits adapted to RUTF conducted in Haiti, Guinea, Burkina Faso, Nigeria, Ethiopia, Madagascar, and Sudan.

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Genome editing to refine soybean as a plant-based protein source

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Soybean is among the best sources of plant-based protein in human diets and is given high hopes to substitute animal meat and milk and help fill the forecasted global protein gaps in a sustainable manner. Nevertheless, various barriers, such as the off-flavor, anti-nutrient factors, and poor texture, can hinder the promotion of soy-products. Genome editing technology could rapidly and precisely modify multiple genes in plants, making it a revolutionizing tool in molecular breeding. We have developed a highly efficient genome editing system in soybean [1,]. To improve the sensory profile of soybean produce, we have generated "beany-flavor-free" varieties by multiplex genome editing. We also reduced anti-nutritional factors by generating soybean with ultralow RFO, and reduced phytic acids. To improve the functional property of soy-protein, we customized storage protein composition for ultra-high emulsibility or gelling ability [2]. Now, we are simultaneously editing dozens of genes, aiming to "re-design" soybean with sensory profile, nutritional composition, and functionality that is preferable as a protein source in human diets.

Reference:

1 Bai, M. *et al.*. (2020) Generation of a multiplex mutagenesis population via pooled CRISPR-Cas9 in soya bean. *Plant Biotech. J.* **18**, 721-731

2 Bai, X. *et al.* (2022) Combination of two multiplex genome-edited soybean varieties enables customization of protein functional properties. *Mol.Plant* **15,** 1081-1083.

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Identification of sweet alkaloid genes in narrow-leafed lupin

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In wild narrow-leafed lupin (*Lupinus angustifolius*) seeds, the quinolizidine alkaloid (QA) content was around 2%. In contrast, the QA content in sweet lupin cultivars decreased 100 times to ~0.02% to meet the industrial limit.

To identify the gene for QA content, we used three recombinant populations, and fine-mapped the major bitter locus to a 0.46 Mb interval region on chromosome 7. The DEG gene *RAP2-7* in this region was considered to be the candidate gene. By investigating >300 lupin re-sequencing data and their phenotypes, one SNP within the gene region was identified to be associated with alkaloid content. Virus-induced gene silencing of *RAP2-7* was conducted in bitter wild lupin plants, and we successfully converted the bitter leaves to sweet leaves in the VIGS plants. It indicated that *RAP2-7* was the gene controlling the sweetness in narrow-leafed lupin cultivars.

In addition, the Australian sweet lupin cultivar Kalya contains 0.015% QA, while the QA content of another sweet cultivar Tallerack contains 0.00166%, around 10 times difference. DNA analysis indicated they carried the same sweet allele of the gene RAP2-7, and different genes should control the sweeter gene in Tallerack. Two new QTLs for sweeter QA in Tallerack were identified on chromosomes 13 and 16, explaining 25% and 12% of phenotypic variations, respectively. Aphid tolerance analysis showed that the QTL for aphid tolerance was mapped to Chr.13, and this QTL was consistent with the relevant alkaloid QTL on Chr.13.

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Three decades of playing around with legume genetics

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Legumes have unquestionable importance in both in nature and agriculture, and made significant historical contributions to plant genetics and physiology, but this has not always been reflected in their prominence as study systems. However, as the ICLGG enters its third decade, we are seeing their contribution to fundamental plant biology continue to grow steadily, in parallel with improved resources and techniques. These changes have been driven by collective effort of colleagues around the world committed to legume crops and models, many of whom have attended and guided this conference since its inception.

Our part in this effort has centred on peas as a model system, and has had four broad aims: gaining genetic insight into flowering time control, understanding how flowering pathways may intersect with the control of other traits, and revisiting the genetics of domestication. In parallel, we have been interested in exploring the extent to which these processes may be conserved across other crop and model legumes. This presentation will highlight some of our recent progress.

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Climatic ambitions of neglected underused legumes

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The Mediterranean region, including Portugal, is expected to become a climate change hotspot, exacerbating existing vulnerabilities. Temperature rise will exceed global averages, along with reduced winter rainfall and more frequent, intense local rain events. This poses significant challenges, particularly for agriculture.

The improvement of plant responses to these challenges is therefore an important open field of research, namely in legumes, which have not kept pace with the advances made for cereals. However, selection for these abiotic stresses tolerance is complex due to its quantitative nature, with difficult-to-score phenotypes in large populations.

The success in future breeding strategies may reside on an integrated approach combining plant physiology, biochemistry, genetics and genomics at a whole-plant level to identify key phenotypic traits and develop cost-effective, accurate molecular selection tools.

This presentation will highlight the most significant findings from studying natural variation under water stress in underused grain legumes using different high and medium-throughput physiological phenotyping approaches such as gas exchange, thermal imaging, chlorophyll fluorescence and hyperspectral measurements. The discussed results come from national (Portuguese) and European legume projects with a particularly focus on outcomes from the H2020 DIVINFOOD project, relevant for understanding drought and flood tolerance genetics and mechanisms in grass pea (*Lathyrus sativus*).

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From pangenomes to traits – linking genome variation with phenotype variation

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The ability to assemble genomes at low cost is revolutionising our understanding of biology. Sequencing multiple individuals has identified significant structural genomic variation within a species, with as many as 40% of genes being absent in some individuals. These differences limit the application of single genome references as they may not contain the genes associated with observed heritable traits. This has led to the growth of pangenomics, with pangenomes representing the genomic diversity of a species or higher taxonomic group rather than a single individual. It has been demonstrated that pangenomes capture more heritability for traits than single reference genomes.

Pangenomics is still in its infancy, new approaches for pangenome construction and analysis are being developed as long read DNA sequencing improves. Costs continue to reduce, permitting population level analysis. The analysis of pangenomes within and between species is providing a greater understanding of species diversity, evolution, adaption and supporting the acceleration of crop improvement.

Here I will present our findings from constructing and analysing pangenomes for several species and demonstrate how they can be applied to identify haplotypes in populations that confer favourable traits.

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Unveiling the mechanisms underlying photoperiod sensitivity

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In 1920, Garner and Allard first elucidated the phenomenon of photoperiodism, a discovery that shed light on how plants regulate flowering in response to daylength. While photoperiod sensitivity is crucial for seasonal growth in plants, it also imposes limitations on the global adaptability of crops. To overcome these limitations, agronomists and plant breeders have been working to reduce the photoperiod sensitivity of crops. But what are the regulatory mechanisms underlying photoperiod sensitivity? Soybean, a short-day plant with photoperiod sensitivity, has complicated mechanism controlling its flowering time. Our research has revealed that the *J* gene, a key component of the evening complex (EC), plays a decisive role in soybean's photoperiod sensitivity. Employing reverse genetic approaches, we discovered that the simultaneous knockout of two *LUX* genes in soybean results in an extremely late flowering phenotype under any photoperiod, highlighting the central role of the EC in regulating photoperiod sensitivity. Furthermore, the *PHYA* gene, a pivotal regulator in the photoperiodic response, interacts directly with LUX proteins within the EC and promotes their degradation. The interaction between the E2 protein and the EC forms a complex regulatory loop, known as the photoperiod Taijiloop, which is essential for maintaining soybean's sensitivity to photoperiods. We have identified an increasing number of components involved in the regulation of photoperiod sensitivity and are unveiling the intricate web of their regulatory relationships...

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Update on the interplay of signals controlling shoot branching in plants – challenges and solutions for predicting network perturbations

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The number of branches on a plant is a highly plastic trait that impacts yield and ornamental value of most plant species. An overview of the stepwise development of the current model/network for shoot branching will be presented [1]. Apical dominance, a form of branching control by the shoot tip, is regulated by the shoot's demand for resources for growth, particularly sugars. Auxin produced by the shoot tip acts to reinforce this dominance. Acting in an opposing manner, sugars and auxin regulate the levels of cytokinins that move upwards into buds to promote bud outgrowth. The strigolactone pathway act antagonistically with cytokinins and is also suppressed by major plant resources such as nutrients and sugars. Once a bud is induced to grow, local auxin content sustained at high enough levels will maintain vascular development and sustained growth through promoting auxin canalisation and ensuring gibberellin levels required for sustained growth [2]. The current knowledge of this network is quite complex and it is not trivial to interpret the effects of multiple perturbations. As crops and natural selection rely on the emergent properties of networks, such as the branching network, our current work focusses on how to understand the structure of network and its connectivity - the details of which are critical but not individually sufficient to solve wicked problems such as predicting what gene perturbations and combinations will successfully improve crops in particular environments.

References:

[1] Beveridge CA, Rameau C, Wijerathna-Yapa A. 2023. Lessons from a century of apical dominance research. Journal of Experimental Botany, 74:3903-3922.

[2] Cao D, Chabikwa T, Barbier F, Dun EA, Fichtner F, Dong L, Kerr SC, Beveridge CA. 2023. Auxin-independent effects of apical dominance induce changes in phytohormones correlated with bud outgrowth. Plant Physiology 192, 1420–1434.

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afila: The Whole Story, From Identifying Potential Candidate Genes to Detecting the Responsible Megabase-Scale Deletion

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The *afila* (*af*) mutation in *Pisum sativum* L. (pea) induces the conversion of leaflet primordia into midrib-like primordia, resulting in bipinnate leaves. The phenotype was first reported in 1953 and has since become a highly desirable trait to improve lodging resistance and facilitate mechanical harvesting. Despite its widespread introgression into pea cultivars, the molecular basis of *af* has remained unknown. We have shown that deletion of the *Medicago truncatula PALMATE-LIKE PENTAFOLIATA1* co-orthologs, namely *PsPALM1a* and *PsPALM1b*, is responsible for the af phenotype. Different origins for *af* in released cultivars have been identified and up to seven haplotypes, determined by the size of deletions including *PsPALM1a-b*, have been detected in leafless and semi-leafless pea accessions. This previously unrecognised diversity sheds light on important chapters of pea breeding history and highlights unexplored potential for current and future programmes.

Reference:

[1] Tayeh N. et al., afila, the origin and nature of a major innovation in the history of pea breeding, New Phytol., in press, 2024. doi: 10.1111/nph.19800

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Gene-edited Medicago *Mtsoc1* or *Mting* mutants do not flower

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Optimised flowering is a key trait for enhanced crop productivity. In the eudicot Arabidopsis, genetic pathways are known to converge on floral integrator genes like *FT* and *SOC1* to elevate their expression and lead to flower development. *Medicago truncatula* (Medicago), like winter annual Arabidopsis, is induced to flower by extended cold (vernalisation) followed by warm long day photoperiods. Interestingly Medicago lacks key Arabidopsis flowering regulators including CO and FLC. However, the *FT-like* gene *MtFTa1* is upregulated by floral inductive conditions and promotes flowering.

We analyse Medicago flowering using gene editing to knock out single Medicago candidate genes or multiple duplicated homologs simultaneously. Previously, a *SOC1-like* gene *MtSOC1a* was implicated as it was elevated by *MtFTa1* expression, while there was a moderate delay to flowering in the single mutant. We also showed that the novel *MtING2* gene, encoding an ING domain and a PHD finger, promotes growth and flowering in Medicago.

Here we present results of analysis of triple *soc1* mutants, or double *Mting* mutants. Both mutants have striking phenotypes - because they do not flower. We use RNA-seq to compare their differential gene expression to other non-flowering Medicago plants. We test if homologs of targets of Arabidopsis SOC1 are misexpressed in the *soc1* triple mutants. We ask if targets of the NuA4 histone acetyltransferase complex in Arabidopsis, which ING2 is part of, are misexpressed in the Medicago *ing* mutants. Our work provides insights into flowering regulation in legumes and points to increased forage production including by using non-flowering mutants.

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Mutations in the *AFB4/5* auxin receptor gene confer altered shoot architecture and improved grain yield in field pea (*Pisum sativum*)

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Hormonal regulation of plant architecture is a fundamental component of how plants respond to their environment to optimise resource capture and ensure their survival in the face of biotic and abiotic stress. We recently identified a series of herbicide tolerant field pea (*Pisum* sativum) lines with mutations in an auxin receptor, AUXIN SIGNALLING F-BOX PROTEIN 4/5 (AFB4/5). Alongside offering new weed management options, the mutants had unusual plant growth and development phenotypes. Substantial phenotypic variation was observed for shoot architecture, seed size, and phenology between different *afb4/5* alleles. *AFB4/5* played a role in seed size determination and grain yield potential. While null mutants were severely dwarfed, hyper-branched, and exhibited reduced seed size and yield, a unique hypomorphic mutant had a moderate effect on plant architecture and increased seed size and yield. Field trials in South demonstrated 27.5% increase in grain yield in a long-season, high-rainfall environment, and no yield penalty in a short-season, low-medium rainfall environment.

There is clear opportunity to target genes in hormone signalling pathways to develop a new crop ideotype for field pea and potentially other species to provide a step change in grain yield potential.

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The role of CLE peptide signalling in shoot meristem development of pea

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The CLV3/EMBRYO-SURROUNDING REGION (CLE) peptides control plant development and response to the environment. Key conserved roles include the regulation of shoot apical meristems and the long-distance control of root colonisation by nutrient-acquiring microbes, including the widespread symbiosis with arbuscular mycorrhizal fungi and nodulation with nitrogen-fixing bacteria in legumes. At least some signalling elements appear to operate across both processes but clear gaps in our understanding remain. In legumes although CLE peptide signalling has been examined in detail in symbioses, the role of this pathway in SAM development of legumes is poorly understood.

We found that in pea both genetic and environmental buffering of CLE pathway influences SAM development. In pea, the CLAVATA2 CLE receptor and the unknown gene product encoded by the K301 gene are required to limit SAM size and floral organ production under cool temperatures. In contrast, the CLAVATA1 receptor-like kinase actually promotes SAM proliferation and appears to do so via a CLV2-independent pathway. In contrast, we found no role for RDN1 enzyme, capable of arabinosylating CLE peptides, in SAM development. Future studies in other legumes are required to examine the role for other CLE peptide signalling elements in SAM control and studies in non-vascular mycorrhizal hosts could explore if the cross-over in SAM control and symbioses limitation reflects a conserved ancestral role for this signalling pathway.

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The circadian clock modulates bud outgrowth in pea via the strigolactone signalling pathway

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Shoot architecture is an important determinant of yield in several crops. Outgrowth of axillary buds is altered by changes in photoperiod and in photoperiod-insensitive mutants. The mechanism by which photoperiod regulates bud outgrowth is unknown, but photoperiod effects are observed in many plants including garden pea (*Pisum sativum*). We utilised the photoperiod-insensitive circadian clock mutant *die neutralis* (*dne*) to study photoperiodic and circadian clock effects. *DNE* is orthologous to arabidopsis *EARLY FLOWERING4* that encodes a member of the Evening Complex, a core circadian clock component. We show that circadian clock regulation of bud outgrowth involves long-distance signalling and activation of genes involved in the strigolactone response and downstream pathway. Experiments with *dne* and strigolactone double mutants showed that strigolactone response, but not strigolactone synthesis, is required for circadian clock regulation of bud outgrowth. It is likely that the circadian clock pre-conditions buds for differing growth response capacities to endogenous signals through modulating strigolactone signalling. In this study, we observed effects of the absolute photoperiod (the number of hours within a 24 hour period during which the plant receives light), separately to the metabolic photoperiod (the period across the day during which a plant has a net carbon gain). Experimentation with different light intensities and photoperiods suggest that the circadian clock and DNE enables plants to adapt to environmental conditions by predicting energy availablity. These insights will help future endeavours to predict and optimise flowering and branching responses in different environments, providing knowledge relevent to crop yield and reproductive success.

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Characterisation of Defensin-Like peptides (DEFL) family members involved in cowpea reproduction

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Cowpea (*Vigna unguiculata [L.] Walp*.) is a valuable legume crop, but its yields are often suboptimal, particularly in regions like sub-Saharan Africa. Effective communication between male and female reproductive tissues is essential for successful plant reproduction and seed formation. Recent evidence from studies in *Brassicaceae sp.* and maize indicates that small, secreted Defensin-Like (DEFL) peptides act as signaling molecules in these critical interactions [1]. However, in cowpea and other species, gene annotation and functional understanding of these peptides remain limited [2]. Using bioinformatics, the aim is to identify the complete family of cowpea genes encoding DEFL peptides and pinpoint candidate genes acting in plant reproduction. Two highly and specifically expressed DEFL genes in the female embryo sac before and after fertilization have been identified. Validation of DEFL1 and DEFL2 gene expression was conducted by qPCR analyses of pre and post-fertilization whole gynoecia samples. Additionally, cowpea was transformed with a nominal 3Kb portion of DEFL1 and DEFL2 promoters fused to ZsGreen fluorescent protein. However, results suggest that the selected promoter regions lack critical elements. RNA in situ hybridization assays is currently being performed to examine DEFL1 and DEFL2 mRNA localization within the ovule. Finally, DEFL1 and DEFL2 knockout mutants were successfully developed through plant transformation using CRISPR-Cas9. T1 generated mutants are currently growing in our glasshouse facilities, awaiting flowering for phenotypic analysis. Through microscopy, we aim to determine whether these mutant plants can undergo normal fertilization and normal seed development.

References:

[1] Kim, M-J. et al. (2021). Peptide Signaling during Plant Reproduction. Trends Plant Sci. 26(8), 822-835.
[2] Feng, YZ., Zhu, QF., Xue, J. et al. (2023). Shining in the dark: the big world of small peptides in plants. aBIOTECH 4, 238–256.

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How is early bud growth driven at the cellular level?

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Axillary bud initiation and outgrowth is a major factor that impacts plant architecture. Shoot branching is a result of the stimulation of axillary buds to grow into lateral branches and is controlled via a complex interplay of genetic, molecular and metabolomic networks in response to both the environment and endogenous ontogenetic cues. Great progress has been made in discovering and describing many of the hormonal and genetic pathways underlying branching, yet we have little understanding of how these hormonal and molecular changes are translated into growth at the anatomical and morphological levels. Here we are using the model plant *Pisum sativum* (garden pea) to characterise early bud growth using time-lapse photography, SEM microscopy and cell proliferation assays. Together with the identification of early transcriptional changes we aim to build a morphological and cellular framework for how hormonal and sugar signalling cues are translated into bud outgrowth and branching.

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Genotype x environment interactions affecting flowering time and flowering duration in mungbean.

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Mungbean (*Vigna radiata L.*) is a high value pulse crop cultivated in the subtropics and the primary summer legume rotation option in Australia. Understanding phenology, particularly flowering, is key to achieving adaptation of mungbean to varying production environments. Despite its importance, the traits that underpin flowering behaviour in mungbean such as days to flowering and flowering duration are not well understood. This includes a lack of knowledge about how these physiological traits interact with each other and the production environment, and the underlying genetic mechanisms. Utilising a diverse mungbean nested association mapping population, key flowering traits (days to flowering and flowering duration) were evaluated across four field experiments conducted in Queensland, Australia in 2022 and 2023. Genotype by Environment interactions (G x E) were observed, and extensive genotypic variation was noted particularly in traits such as days to flowering and flowering duration. Fluctuations in flowering time have been observed, with high yielding varieties typically flowering at ~30-35 days and lower yielding varieties flowering earlier Flowering duration in mungbean was observed to be substantially influenced by the environment with a high degree of genetic variation within the mungbean germplasm studied. To dissect the genetic mechanisms controlling days to flowering and flowering duration, genome wide association studies were conducted. Ten QTL were identified to have significant associations with flowering traits on several chromosomes. This research provides new knowledge of novel flowering traits in mungbean and provides genetic mechanisms which lay the foundation for further investigation.

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Opportunities to improve grain legumes for future climates

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Grain legumes, such as mungbean and chickpea, play a valuable role in improving the sustainability of farming systems. Despite the potential value to production systems, grain legumes are often perceived by farmers as a risk, as production varies year on year, primarily due to abiotic stress. New breeding technologies provide opportunities to incorporate genetic variation into breeding programs and accelerate the development of pulse varieties better suited to sustainable farming systems. In this presentation we share the application of new breeding technologies through two case studies conducted in grain legume crops mungbean and chickpea. Firstly, we tackle the phenotyping bottleneck by developing and applying high throughput unmanned aerial vehicle platforms and longitudinal modelling approaches to predict canopy development. This was validated using a nested association mapping population evaluated in multi-environment field trials conducted in the Northern Grains Region of Australia. In the second case study, we share a new framework for fast tracking the deployment of chickpea to high temperature which brings together a multidisciplinary team across agronomy, crop physiology, plant breeding and genetics from major research entities working to advance chickpea production. By integrating phenomics, GxE modelling, genomic prediction tools, the approach can be applied more broadly to a range of pulse crops, to ensure farmers can maintain their competitive edge and maintain future growth in a changing climate.

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Impact of nitrogen deficiency on plant growth and development: discovering conserved mechanisms for legumes and non-legumes

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Efficient nitrogen uptake and root development are crucial for robust plant growth and high crop yields. Small signalling molecules called CEP and CLE peptides play a vital role in regulating these processes. Produced in response to nitrogen availability or limitation, these peptides travel from roots to shoots, triggering responses that enhance nitrogen absorption and root structure.

In legume plants, the formation of nitrogen-fixing root nodules is carefully controlled by a negative feedback mechanism known as Autoregulation of Nodulation (AON) that is mediated by CLEs as well as positive feedback mediated by CEPs. However, the detailed molecular processes underlying these regulation remain unclear.

Here, we present overview and recent discovery of how plant peptide signalling modulate plant development in response to nitrogen availibility. Ultimately, this knowledge can contribute to developing crops with improved nitrogen efficiency and higher yields, addressing global food security challenges.

References:

[1] Imin N, et al. CLE peptide tri-arabinosylation and peptide domain sequence composition are essential for SUNN-dependent autoregulation of nodulation in Medicago truncatula. New Phytologist. 218 (1), 2023, p. 73-80.

[2] Saur I, et al. Crosstalk between the nodulation signaling pathway and the autoregulation of nodulation in Medicago truncatula. New Phytologist. 190, 2011, p. 865-874.

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Unlocking Chickpea Potential: AtBAG4, the Cytoprotective Co-Chaperone, Enhances Drought Tolerance, Nodulation and Seed Protein Content

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Drought and extreme temperatures significantly limit chickpea productivity worldwide. The regulation of plant programmed cell death pathways is emerging as a key component of plant stress responses, maintaining cellular homeostasis, and enhancing crop resilience to environmental challenges. Arabidopsis thaliana Bcl-2 associated athanogene 4 (AtBAG4) is a cytoprotective co-chaperone implicated in plant stress responses. In this study, we explore the effects of exogenous expression of AtBAG4 on chickpea stress tolerance, nodulation and nitrogen fixation. Transgenic chickpea lines engineered to constitutively express AtBAG4 are more drought tolerant and produce higher yields under drought stress compared to non-transgenic controls in the field. Additionally, the AtBAG4 expressing transgenic lines supported higher nodulation, increased photosynthetic activity and elevated nitrogen fixation. The increased photosynthetic activity and nitrogen fixation resulted in seeds with higher nitrogen content. These findings suggest that using protective chaperone genes like AtBAG4 has the potential to significantly improve crop performance, especially under unpredictable climate conditions. Importantly, these improvements were achieved with minimal impact on yields under both well-watered and drought conditions. This research highlights a promising approach for sustainable agriculture in the future.

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GmPIF4 and soybean high-temperature tolerance

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Soybean is a major oilseed legume crop produced primarily for human and animal feed. Increasing consumer demand for plant protein has led to recent growth in the soybean market. As a nitrogen-fixing plant, soybeans are used in rotational agriculture to replenish soil nitrogen. However, the rise of global temperature and heat waves associated with climate change threaten crop productivity as the reproductive system is most susceptible to heat stress. To expand soybean cultivation, we need to understand and breed soybeans that can adapt to changing climates and significant increases in temperature and heat waves. Our lab used an integrated transcriptome, bioinformatic and experimental approach to address this gap in our knowledge. Paleopolyploid legumes such as soybean have multiple gene copies. Using RNA sequencing in soybean, we identified key transcription factors that could affect plant morphology and reproductive stages and found that flowering genes were differentially expressed and functionally divergent. We will present our exciting work on Phytochrome-interacting factor 4 (PIF4, a signalling hub), showing altering expression of *GmPIF4* reduced heat-induced loss of seed yield in soybean plants that can be exploited for soybean improvement programmes to meet future demands.

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Precision phenotyping reveals beneficial drought responses in faba bean

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Drought stress is one of the most crucial abiotic stress factors threatening worldwide cultivation of faba beans. In the face of climate change, breeding for drought stress tolerant genotypes is gaining further importance under increasing temperatures and prolonged drought periods. Here we screened a diverse *Vicia faba* genotype panel under controlled, field-like conditions, using a unique precision drought phenotyping platform to identify morphological and physiological traits associated with relevant drought stress tolerance characters, along with interesting genotypes as crossing partners for future for breeding.

80 diverse genotypes were grown in 240 large containers with a 90 cm deep soil profile in a fully-automated drought phenotyping platform (*DroughtSpotter XXL*) which accurately measures the weight of each container every five minutes 24/7, enabling precise gravimetrical recording of water use patterns and stress responses throughput the entire plant lifecycle. Furthermore, an automated watering system individually maintains containers at a preset field water capacity, allowing simulation of specific drought stress scenarios. Simultaneously, high-resolution plant images are captured daily throughput the entire vegetation period by a fully automated, vertical, multispectral PlantEye 600 dual 3D scanner. The scanner not only enables three-dimensional assessment of temporal changes in plant morphology and architecture, but also of spectral indices indicative of stress responses (NDVI, NPCI, PSRI, Hue). Connecting precise phenotypic and plant performance data obtained under relevant drought stress conditions help pinpoint useful target traits for efficient field selection and identification of breeding lines capable of maintaining high yield under limited water availability.

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Enhancing chickpea tolerance to soil acidity through genomics

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Australia is the second largest producer of chickpeas, with >95% of production exported. Acid soils with aluminium toxicity is the single largest production constraint for chickpea in Australia after Ascochyta blight. Among the pulse crops, chickpea is the most sensitive to acid soil conditions. Current Australian chickpea varieties do not possess sufficient genetic variation for acid soils tolerance, which has hampered breeding for improved yield and local adaptation beyond the traditional areas of cultivation in southern and northern Australia.

We evaluated 1,100 chickpea accessions including wild cicer introgressions, cultivars and bi-parental crosses using hydroponic assays at 15-20 µM Al^{3+} concentration, where comparison of root length under normal and stress conditions was used as a measure of acid tolerance. Plant materials were genotyped using the multispecies pulse 30K SNP array. Genomic breeding values for root length and root length index (rootlength_stress / rootlength_normal) were estimated using the GBLUP method. Prediction accuracies for root length under stress condition and root length index were 0.42 and 0.32, respectively. Genome-Wide Association Studies (GWAS) and BayesR analysis identified major genes associated with Al^{3+} toxicity tolerance. Significant trait-marker associations were observed on chromosomes 1, 4, 7 and 8 using GWAS and were consistent with strong marker effects on the same chromosomes in BayesR analysis.

Overall, we observed remarkable genetic variation across accessions for Al^{3+} toxicity tolerance and achieved a level of genomic prediction accuracy to facilitate early trait selection in breeding. Data obtained from the hydroponic assays will be validated through soil assay and field trials. The novel sources of acid tolerance identified hold promise for providing valuable germplasm to breeding programs aiming to expand chickpea adaptation to acid soils.

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Unvelling Phenotypic Diversity for Future Climate Risks: First Large-Scale High-Throughput Charecterisation of the 5050 Chickpea Global Diversity Panel

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Chickpea (*Cicer arietinum* L.) ranks second globally among legumes, yet faces substantial yield losses from abiotic stresses. This study aims to investigate whether enhanced biomass accumulation under stress conditions can improve chickpea productivity (Zhang et al 2022). The research objectives include characterizing chickpea germplasms (n=5050) for drought and heat stress adaptation, evaluating germplasms (n=968) for salinity stress response, and conducting detailed characterization of selected germplasms focusing on water use efficiency and agronomic traits using advanced phenotypic platforms. Drought investigation revealed significant variation in plant canopy traits, particularly 3DLA, with a 37-fold difference under irrigated conditions. Selected genotypes exhibited 2.2-fold variation in transpiration efficiency and 15-fold variation in seed yield under water stress. Furthermore, seed yield correlated significantly with harvest index under both well-watered (R^2 = 0.63**) and water stress (R^2=0.86**) conditions. Heat stress caused a 30% reduction in biomass accumulation, while salinity stress resulted in a 45% decrease. Interestingly, approximately 10% of germplasms exhibited superior biomass accumulation under both stresses compared to controls, emphasizing the importance of selecting for reduced biomass loss in crop improvement efforts. In conclusion, maximizing early shoot growth is crucial for enhancing seed yield, especially in water-limited environments. Early-stage biomass accumulation can enhance seed yield even under salinity and heat stress, findings to be validated in field trials. Efficient assessment of large germplasm collections using HT-Phenomics platforms is critical for developing resilient varieties, particularly in chickpeas, to enhance production and food security amidst changing climatic condition.

References:

[1] Zhang H et al. Abiotic stress responses in plants. Nat Rev Genet 23, 2022, p. 104–119.

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Improving chilling tolerance in chickpea using genomics and computational approaches

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Chickpea (*Cicer arietinum L.*) is one of the most important legume crops, grown primarily as a protein source. Low temperature (chilling) stress significantly impacts the yield and profitability of chickpeas, particularly during the reproductive growth stage. The development of chilling-tolerant chickpea varieties is a priority for growers and breeding programs in areas where chilling stress is prevalent. This requires developing methods to measure chilling stress tolerance and selective breeding to improve it while keeping yield and other desirable traits intact. Our research aims to investigate the genetic architecture of chilling tolerance using Genome-Wide Association Studies (GWAS) and understand the efficacy of genomic selection (GS) to breed for improved tolerance. About 270 lines were phenotyped at Dale, Western Australia over two years using a novel 'pod marking' method to assess pod viability, which serves as an indicator of cold tolerance. Pod viability is defined as the ratio of viable pods (those filled with grain) during the chilling period (average daily temperature below 13°C) to the total number of pods, with a higher ratio indicating greater tolerance.

GWAS analysis indicated pod viability is a moderately complex trait governed by a few major and many minor genes. This was further confirmed by moderate (0.25) narrow sense heritability (0.25) for the trait. Moderate genomic prediction accuracy (0.39 ±0.06) was obseved for pod viability from 10-fold cross validations, providing evidence for the reliability of GS to guide selection decisions.

Both the novel screening method for chilling tolerance and genomics-assisted breeding are being used to accelerate breeding toward the development of chickpea varieties with improved chilling tolerance.

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Does early growth estimation using remote sensing help our understanding of genotypic stability for lentil varietal selection under heat stress?

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Genotype by environment interaction (GEI) affects genetic gain in developing varieties under abiotic stress. Understanding this interaction at early crop growth stage is essential to identify the most stable breeding lines across a wide range of production environments. With this objective, we evaluated 156 lentil genotypes across contrasting sowing dates and environments for heat stress tolerance in 2023. Using Unmanned Aerial Vehicle, we collected phenotypic data, such as ground cover, crop growth and development and plant stress levels. Early vigour was calculated using digital ground cover, NDVI and OSAVI indices. Significant variation for the indices were observed among the genotypes evaluated. GEI for early vigour trait was also significant. This identified several genotypes with higher early vigour and broader environmental adaptation and vice versa. Based on the Additive main effects and multiplicative interaction model, the genotypes were grouped into four clusters. Genotypes in cluster 4 were classified as the most unstable and poor in early vigour. These genotypes were highly sensitive to GEI. Using early vigour data, we also evaluated the performance of top 20 and 30% of the population and compared these to three commercial varieties and population mean. In general, higher genetic gain was observed compared to the released varieties and mean population, which suggests a genetic advantage associated with germplasm evaluated in these trials. In some of the environments, early vigour was significantly associated to seed yield. Overall, vegetation indices derived from aerial based sensor were capable of ranking genotypes for stability and early vigour performance across different environments and sowing times.

Keywords: Broad adaptation, Early growth, GEI, Genetic Gain, Specific adaptation, Stability

References:

[1] Abeya TT. et al, Estimating early season growth and biomass of field pea for selection of divergent ideotypes using proximal sensing, Field Crops Research, Vol. 277, 2022. https://doi.org/10.1016/j.fcr.2021.108407
[2] Atena H. et al, Application of unmanned aerial systems for high throughput phenotyping of large wheat breeding nurseries, Plant Methods, Vol 12, no.35, 2016. https://doi.org/10.1186/s13007-016-0134-6

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Mining alleles of agronomic value from wild *Cicer* species, with implications for soil health.

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In agricultural systems, aluminum toxicity and nitrogen status of soils are linked. Prolonged use of inorganic nitrogen fertilizers can lead to decreased soil pH, which solubilizes aluminum that is, in turn, toxic to dividing plant cells. Legume biological nitrogen fixation (BNF) offers an alternative to nitrogen fertilizers. BNF is more sustainable than inorganic nitrogen fertilizers, in part because BNF is less perturbing to soil pH. However, in soils that are already acidified and where BNF might have added benefit, legume nitrogen fixation is compromised and dependence on fertilizer application is increased, perpetuating a vicious cycle. Working with chickpea's wild progenitors we identified both aluminum tolerance and more effective nitrogen fixation. Aluminum tolerance is controlled by single wild *Cicer* gene, enabling marker-based selection to create new *C. arietinum* crop varieties. By contrast, efficient nitrogen fixation, for which beneficial alleles also derive from wild *Cicer* species, is a highly complex trait that depends on both host and bacterial genetics. Complexity of nitrogen fixation in cultivated chickpea may involve the accumulation of deleterious alleles in crop germplasm, requiring non-traditional breeding strategies. In addition to the obvious agronomic benefits of aluminum tolerance and efficient nitrogen fixation, we are interested in combining these two traits as a possible means to remediate soil health, reducing the aspirational goal of "regenerative agriculture" to mechanism.

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Progress and Prospects of CMS based Hybrids in Pigeonpea: Redefining plant architecture, resistance, and yield attributes.

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Pigeonpea is an important pulse crop serving as food and livelihood for about 1.6 billion people across Africa and Asia. The average productivity of the crop has ranged from 0.7 to 2.0 tonnes/ha for the past 6 decades. The advent of hybrid technology is seen as a prospect in breaking the yield plateau. Hybrids show 20-56% yield superiority over the checks. The prioritized breeding on short duration hybrids maturing in 150 days with well characterized maintenance and restorer programs strengthens the breeding scheme. Stable CMS for maintainers and high pollen reproducibility for restorers are prime focus. Whereas open flower type, photo-insensitivity, annuality, non-shriveling seeds, increased pod clusters, 4-6 seeds per pod, multiple resistance for biotic and abiotic stresses are the key traits characterized for hybrid breeding. A2 and A4 are the two CMS systems aiding in hybrid development. Fertility percentage based heterotic pooling at ICRISAT resulted in more restorers at A4 system. Redesigning the plant architecture in hybrid pigeonpea has a potential to increase yield by 25 to 33%. ICPH 2671, ICPH 2740, IPH 15-03 IPH 09-5 are the released hybrids. Whereas IPH 21-06, ICPH 2222, ICPH 2211 are the short duration hybrids depicting the superiority of 35 to 56% over the ruling checks in testing pipelines. Pigeonpea hybrids with novel plant architecture, higher yields and multiple resistance are envisioned to break yield barriers adopting in multi-cropping system and increasing the global production.

Key words: Hybrid, pigeonpea, Restorer, Heterosis

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Enhancing Lupin Seed Quality for Food Market: Integrated Multi-Omics and Genetics Approaches

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Current projections indicate that the global population, expected to reach 9.8 billion by 2050, will require approximately 70% more food. Plant-based protein is poised to fulfill over half of this protein demand. Narrow-leafed lupin (NLL) stands out as a prime candidate due to its high protein content (35-40%). Not only is it a valuable source of animal feed, but it's also gaining traction as a premium plant-based protein for human consumption. Lupins boast high fibre, low starch, low fat, and a low glycemic index, holding promise in addressing prevalent health concerns like diabetes, cardiovascular diseases, and obesity. However, current lupin varieties harbor undesirable traits such as thick seed coats, grain alkaloids, a beany flavor, and specific seed storage proteins that can trigger food allergies in some individuals.

CSIRO's research endeavors to enhance seed quality by exploiting the intricate programming of seed development and composition in NLL. Toward this end, we have re-sequenced a diverse panel of wild and domesticated NLL germplasm, established large transcriptome and proteome datasets for lupin grain development, along with a reverse genetic (TILLING) platform. This integrated genetic and multi-omics approach enables us to delve into crucial developmental and biosynthetic pathways in seed biology, pinpointing targets for precision genome engineering to expedite future breeding efforts aimed at redesigning seed composition in lupins. It paves the way for the discovery of novel genes to remove or mitigate undesirable traits, thereby enhancing the suitability of lupins for plant-based protein food production.

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Maturity And Adaptation of Sub-Tropical x Temperate Soybean Populations in Zamabia And Zimbabwe

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Soybean (*Glycine max* (L.) Merrill) is a pivotal crop within the global food and feed industries due to rich protein and oil content. Early maturity in soybean allows the crop to escape drought. Introgressing temperate germplasm into subtropical improves subtropical populations for earliness among other traits. The objective of this study was to assess maturity and adaptation of F_4 subtropical x temperate soybean populations to the subtropical environments in Zimbabwe and Zambia. Thirty-seven tropical x temperate populations and 13 checks were evaluated across 14 sites in Zimbabwe and Zambia in the 2020/21 and 2021/22 cropping seasons. AMMI analysis showed that the Genotype, environment and genotype by environment interactions were significant for yield, maturity and other traits measured, signifying differences in performance of genotypes across environments. GGE comparison biplots and Cultivar Superiority Index identified S1735-4 and S1724-4 as superior genotypes yielding comparably with the highest yielding subtropical check varieties: Status, PAN1867, Lukanga and Sentinel. Additionally, these exhibited early maturity with S1735-4 maturing in 109 and S1724-4 in 112 days after planting, both earlier than the trial mean of 116 days. S1735-4 yielded 3808 kg/ha while S1724-4 achieved a yield of 3688 kg/ha. Test environments were divided into 3 mega environments with Africa University in the 2021/22 emerging as the most discriminating and representative. Selections from advanced generations of S1735-4 and S1724-4 are expected to isolate early maturing and high yielding lines. Should the isolated lines demonstrate superior performance in the preliminary testing, they can be recommended to be recycled as parents.

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Contribution of INDEL polymorphism in *FLOWERING LOCUS T* genes to vernalization and photoperiod responsiveness of yellow lupin (*Lupinus luteus* L.)

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Plants have evolved a complex molecular signaling network that regulates flowering in response to environmental factors. Wild yellow lupin accessions require prolonged cold period during juvenile phase to fulfill vernalization requirements and induce flowering. Moreover, they considerably delay flowering under non-inductive, short-day photoperiod. The convergence point of vernalization and photoperiod pathways in plants is the transcriptomic regulation of the floral integratory gene, *FLOWERING LOCUS T* (*FT*). While *Arabidopsis* has only two *FT*-like genes, partially sub-functionalized into temperature and photoperiod responsiveness, legume genomes encode a higher number of FT-like genes, divided into three subclades: *FTa*, *FTb* and *FTc*. Lupins during evolution lost the whole *FTb* clade, but accumulated duplicated *FTa* and *FTc* genes.

The presence of multiple *FT* copies in the genome reduced the selection pressure, facilitating environmental adaptation. It resulted in the loss of vernalization and/or photoperiod requirements, naturally as a drought escape strategy or artificially during domestication process. The causal mutations underlying day-neutral and thermoneutral phenotypes were identified in our study [1] as INDEL polymorphisms in the promoter regions of *FTa1* and *FTc1* genes, carrying the sole candidate binding sites in the whole promoters for the major repressive transcription factors, TARGET OF EAT 2 (TOE2) and AGAMOUS-like 15 (AGL15), respectively. Moreover, a very late flowering phenotype was associated with a *Copia*-like retrotransposon insertion in the third intron of the *FTc2* gene. PCR markers designed for these loci and positively validated in the yellow lupin diversity panel await implementation in marker-assisted selection.

Funding: National Science Centre, Poland, project OPUS21 2021/41/B/NZ9/02226.

References:

[1] Plewiński P. et al, 'FLOWERING LOCUS T indel variants confer vernalization-independent and photoperiod-insensitive flowering of yellow lupin (Lupinus luteus L.)' Horticulture Research, vol. 9, 2022, uhac180.

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Genetic variability and identification of genomic regions associated with stem rot disease resistance in cultivated and interspecific derivatives of peanut (*Arachis hypogaea* L.)

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Peanut is an important food and oilseed legume crop. Stem rot of peanut is a major soil-borne disease causing pod yield loss of 20-80%. Genetic variability of 160 peanut genotypes including interspecific derivatives was assessed by screening at ICRISAT-Hyderabad and ICAR-DGR-Junagadh. The genotypes showed a variability of 13-80% for percent mortality (PM) at ICRISAT sick field with ten resistant (13-19% PM) and fourty moderately resistant (20-29% PM) genotypes. Fourty four of these, (9 resistant and 35 moderately resistant) were interspecific derivatives from wild *Arachis* species *A.villosa*, *A.correntina*, *A.helodes*, *A.diogoi*, *A.cardenasii*, *A stenosperma*, *A.paraguariensis, A.kempff-mercadoi*, *A.hoehnei* belonging to AA, EE genomes. To identify the genomic regions governing stem rot disease resistance, an RIL population (from the cross, ICGV 02266 x NC 3033) with 192 lines (F_9) was studied. Genotyping the RILs using 2.5K *Arachis* mid-density panel revealed the presence of 441 polymorphic markers between the parents, of which 426 high resolution SNPs were mapped across the 20 linkage groups. These SNPs spanned a map-length of 0 to 592.71cM. QTL analysis revealed the presence of one major and several minor QTLs governing disease resistance. We presume that the QTL qIC-3-1, located on chromosome 3, might be a potential stem rot disease resistance QTL, owing to its consistency and highest PVE value (14%) among all the QTLs identified. This study highlights the importance of interspecific derivatives as potential sources of stem rot resistance in peanut, and the identified genomic regions can be utilized to develop diagnostic markers for their use in peanut breeding.

References:

[1] Cui R. et al, 'Quantitative trait loci sequencing–derived molecular markers for selection of stem rot resistance in peanut', Jounal Crop Sci Jul, vol. 60, 2020, p. 2008-18

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Integrating Advanced Speed Breeding Strategies in Pulse Pre-Breeding

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Speed breeding techniques hold significant promise for expediting the genetic improvement of legume crops crucial for global food security. We have integrated various innovative approaches into our speed breeding pipeline for lentil and chickpea to accelerate plant growth and development.
Deploying embryo rescue techniques to recover immature embryos from cross hybridisations has reduced generation advancement time by up to 4 weeks. Similarly, the application of plant regulatory hormones such as Gibberellic Acid 3 (GA3) encourages flowering and pod maturity to contribute to a shorter breeding cycle and faster generation turnover. Optimisation of nutrient regimes to enhance plant vigour, health and resilience also maximizes flowering and productivity. Our holistic approach to speed breeding has improved chickpea and lentil hybridisation efficiency to more than 50% and 65% respectively, reduced the plant growth cycle by 2-fold and enabled us to achieve fast generation turnover and to complete a single seed descent cycle within 8 weeks (seed to seed).
The integration of these advanced speed breeding techniques offers immense potential to accelerate genetic gain. By shortening breeding cycles, speed breeding – in conjunction with other tools such as genomic selection – facilitates the utilization of untapped genetic diversity to breed more resilient and productive crop varieties.

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Pan-genomes and graphs: new ways to explore genetic variability within Lens spp.

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Advances in sequencing technology have made it more affordable than ever to assemble high-quality genomes, and we have assembled at least one of every wild species within the genus Lens. However, even before the pan-genomics age, it was clear than a single reference genome was insufficient to represent an entire species, and that the new approaches and software currently being developed are required to fully examine the breadth of variation and make it accessible to geneticists and breeders. To avoid issues in computational time and complexity, we have applied different pan-genomic analyses to solve different problems within the breeding program. These include a gene-based graph to examine synteny, unique genes within each genome and duplications, as well as a full, de novo assembly-derived structural and SNP-level variation graph of two different species: *L. culinaris* and *L. orientalis*. This graph is being used to map reads in an interspecific MAGIC population as a means to reduce ascertainment bias caused by using a single reference. Finding that most graph viewing software has difficulties handling larger graphs, we have developed software to better understand some of the more complex regions of the graph and visualize them in detail. We have also examined 10x coverage PacBio HiFi of diverse lentil lines and compared de novo assembly and mapping approaches for making maximal use of high-quality, long read data.

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Resources and tools for legume research at the Legume Information System

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The Legume Information System (LIS, https://legumeinfo.org) has been under development for more than 20 years, but has seen especially rapid changes in the last five, corresponding with rapid improvements in sequencing technology. LIS now hosts genomic data for more than 25 legume genera and 60 species, including more than 150 assemblies and annotations. Collections reflect research community focus—for example, with 57 and 33 genomes for *Glycine* and *Medicago* respectively; but there is also good taxonomic coverage, with representatives from four of the six legume subfamilies. Data management and tool development is coordinated with sister projects SoyBase (https://soybase.org) and PeanutBase (https://peanutbase.org).

Pan-gene collections (sets of allelic genes across a set of annotations) are available for six genera (*Arachis, Cicer, Glycine, Medicago, Phaseolus, Vigna*), and rigorously constructed gene families are available that incorporate most of the genic data at LIS. Methods are provided for adding new annotations of interest to the families.

LIS provides many exploratory tools, including: InterMine instances for well-represented genera (InterMine is a data warehouse system that permits powerful queries); BLAST against all genome assemblies and gene sequences; the Genome Context Viewer for exploring synteny; search tools for genes by name or publication or description; JBrowse instances for viewing assemblies; ZZBrowse for exploring GWAS and QTL studies - and comparing marker-trait associations between two species of interest; a gene family viewer; comparative visualization tools for expression data; a tool for displaying germplasm origin in a geographic information system; and for annotating user-supplied sequences.

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AI- Augmented Rapid Allele Stacking to Breed for Durable Ascochyta Blight Resistance in Chickpea

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Ascochyta Blight (AB) is one of the most devastating fungal diseases of chickpea. The genetic architecture of AB resistance is moderately complex, involving a combination of a few major genes (qualitative) and numerous minor (quantitative) genes. Breeding for AB resistance has traditionally involved pyramiding major genes, however it is prone to failure due to the rapid evolution of the pathogen, which results in pathogen shifts every 5-7 years. Chickpea varieties experience a decline in AB resistance over time. A different approach is therefore needed to breed for durable AB resistance in chickpea. One potential solution is the use of genomic selection (GS), which considers both major and minor genes to predict breeding values. In this study, we implemented an AI-augmented and genomic-assisted speed breeding approach to develop germplasm with enhanced qualitative and quantitative AB resistance. Phenotypes were collected from 2,962 chickpea lines using terrace screens (outdoor pot-based, screen from 2016 to 2023, using different single *Ascochyta rabiei* isolates) and field nurseries (2020-2023, using a mixture of isolates). All material was genotyped using a multi-species pulse 30k SNP chip. A strong genetic correlation (0.91) was observed between the terrace and field data, indicating a close relationship. High genetic correlations (c. 0.8) were also observed between different isolates. The results demonstrate the potential of developing durable AB-resistant chickpea lines that can withstand multiple isolates. Using our fast-track breeding approach, we generated improved germplasm in < 3 years with c. 30% less disease severity than the diverse germplasm as predicted from genetic values. Our study offers a fast approach to breed for durable AB resistance in chickpea that can be applied to any breeding program to breed for durable disease resistance.

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Genomic tools for *Pongamia pinnata* enable comparisons of nuclear and organellar genomes, diversity assessments, and potentially point to historical seed movements.

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Pongamia pinnata (syn. *Millettia pinnata*) is an economically important sub-tropical arboreal legume producing a bean with many uses similar to those of soybean (e.g. oil and meal). Proprietary methods now also allow production of human food. Further, as a tree, pongamia provides additional ecological benefits such as carbon sequestration.

To explore the genomic biology of pongamia, we assembled a high-quality reference genome comprising ~999.3 Mb across 11 chromosomes. Completeness of the genome assembly was assessed with a Fabales BUSCO dataset of 5,366 genes. Comparative mapping of the BUSCO dataset with three other species within the Millettioid clade (*Phaseolus vulgaris*, *Vigna radiata* and *Cajanus cajun*) revealed chromosomes with highly conserved structures, and other chromosomes with various rearrangements.

Organellar genome sequence as well as transcriptomic data of *P. pinnata*, *Lotus japonicus* and P. *vulgaris* were also compared (Kazakoff et al., 2012) showing extremely high sequence and structural homology (range 1.6 to 9.0%).

To compare relationships among diverse seed sources, we genotyped 199 accessions from Asia, Australia, and the United States using 1,089 single-nucleotide polymorphisms. Principal component analysis revealed several related clusters amongst Australian and U.S. collections, possibly indicative of historical seed movements.

As shown here, genomic resources from nuclear and organellar genomes can facilitate a better understanding of Millettioid evolution, while likewise illustrating genetic relationships among pongamia seed sources to facilitate germplasm improvements.

Reference:

[1] Kazakoff et al., 2012, PLoS ONE:e51687, 7(12) https://doi.org/10.1371/journal.pone.0051687

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Image Analysis for Mapping of Lentil Branch Architecture

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Crop architecture traits such as increased plant height and leaf size, and reduced branching are known to be positively correlated with yield in lentil. Glasshouse-grown plants were imaged regularly to evaluate lentil branch structure using a LemnaTec Scanalyser phenomics platform. A novel method for mapping and quantifying individual branch structures at regular intervals in young plants was developed using open-source software.

Branch structures (angle and length) were accurately quantified, forming novel phenotypes useful for further analysis. After the first four weeks of development, occlusions reduced the ability of the method to detect individual branch structures, but the information collected remains useful for trait estimation and prediction.

A pipeline was developed that analysed morphological skeletons generated from images of lentil plants. This program was incorporated into a PlantCV image analysis pipeline that measured the number, angle, geodesic and Euclidian lengths of individual branches, using queue-based data structure algorithms.

The accuracy of the method was determined against the ground truth obtained from a manual count of the same images; a visual inspection of the skeletons representing the main branches overlaid against the original RGB images and correspondence between images of the same plant taken from two angles 90-degrees apart.

We further envisage that this method, using image analysis, and the subsequent detection of novel phenotypes could be applied to other dicotyledonous species to further understand plant development and responses to environmental change.

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Mechanisms of plant-microbe symbiosis and applications

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Nitrogen is the fundamental building block of key organic molecules in life processes, such as proteins, nucleic acids, and chlorophyll. It is essential for all living organisms. In the natural environment, nitrogen gas makes up 78% of the air. Plants, as primary producers in ecosystems, cannot directly utilize atmospheric nitrogen and rely on external nitrogen-containing compounds for their growth. To meet the demands of crop production, approximately 1-2% of global fossil energy is used for nitrogen fertilizer production. The excessive use of chemical fertilizers has led to severe environmental pollution.

In the symbiotic nitrogen fixation process, as seen in leguminous plants and rhizobia, nitrogen-fixing bacteria convert atmospheric nitrogen into forms of nitrogen compounds that are readily available for plant growth. This biological process reduces the dependence of leguminous plants on external nitrogen fertilizers. In my presentation, I will focus on mycorrhizal symbiosis and the nitrogen-fixing symbiosis between leguminous plants and microorganisms. I will discuss the nutrient exchange and signal recognition between plants and their symbiotic microorganisms, as well as the potential applications and challenges of plant-microbe symbioses in agricultural production.

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Breeding pea for improved disease resistance

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Improving biotic stress resistance is a key objective in the University of Saskatchewan pea breeding program. From ~1990-2015 our main emphasis was on improving resistance to the ascochyta blight complex. Putative sources of resistance to this quantitative trait were evaluated and used in crosses. Allele diversity for ascochyta blight score and associated single nucleotide polymorphisms were explored. Newer varieties tend to be more resistant than older pointing to gradual progress after recurrent selection. Since ~2015 our emphasis has shifted to improving resistance to root rot with key pathogens being *Aphanomyces eutieches*, *Fusarium avenaceum*, and *F. solani.* We utilized marker-assisted backcrossing to pyramid known quantitative trait loci (QTL) associated with *Aphanomyces* resistance into locally adapted cultivars. The first cohort of arising lines has improved field root rot resistance. We conducted genome wide association analysis for *A. eutieches* and *F. avenaceum* with the objective of identifying new sources to build durable resistance. Recombinant inbred lines arising from one promising accession gave rise to new insights into the relative importance of Aphanomyces QTL for resistance against western Canadian isolates. We developed a multi-parent advanced generation inter-cross (MAGIC) population of 850 $F_{7:8}$ lines which are being assessed in field trials. Our current goal is to pyramid and fine map the QTL associated with root rot resistance. A synergistic goal is the improvement of nitrogen fixation. We developed lines from crosses with nodulation mutants that have improved nitrogen fixation capacity and have discovered marker-trait associations for nitrogen fixation traits in the GWAS-2 panel.

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From Sequence to Selection: Genomic and Predictive Breeding for Pulse Crop Improvement

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With escalating demand for food legumes amidst global population growth, climate change challenges and a shift towards plant-based diets, there is an urgency to enhance genetic gain in pulse crops. Genomic and predictive breeding tools offer promising solutions to expedite crop genetic improvement. This plenary session will delve into the integration of a diverse range of approaches aimed at trait improvement and accelerated crop breeding including genomic selection (GS) and advanced predictive analysis techniques such as environmental covariates combined with genomic selection (EC-GS) and crop growth model enabled whole genome prediction (CGM-WGP). EC-GS is particularly useful for connecting genotype-environment interactions with environmental covariates, while CGM-WGP can enable prediction for unobserved (latent) traits underlying crop growth models, providing the opportunity to select on component traits. This talk will also include the use of simulation modelling to optimize genomic selection deployment, speed breeding techniques to shorten generation times, and AI-augemented trait stacking for variety development. The successful implementation of these tools in the Australian lentil breeding program has notably increased the rate of genetic gain. These tools are also deployed in our pre-breeding research to enhance heat stress tolerance during flowering in lentils and disease resistance in chickpeas. By leveraging such techniques, breeders can develop pulse crops better suited to a changing environment, with enhanced yields and improved tolerance. Join us in this session to explore the transformative potential of genomic and predictive breeding in revolutionizing pulse crop improvement.

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A genetic strategy to enhance nitrogen fixation in legumes

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Plants adapt to fluctuating environmental conditions by adjusting their metabolism and gene expression to maintain fitness. In legumes, nitrogen homeostasis is maintained by balancing nitrogen acquired from soil resources with nitrogen fixation by symbiotic bacteria in root nodules. I will outline our approaches to identify novel regulators of nitrogen fixation in legumes. Unexpectedly, we found that zinc, an essential plant micronutrient, acts as an intracellular second messenger connecting environmental changes to transcription factor control of metabolic activity in root nodules. We identify a novel transcriptional regulator, FIXATION UNDER NITRATE (FUN), that acts as a sensor, with zinc controlling the transition between an inactive filamentous megastructure and an active master regulator. Lower zinc concentrations in the nodule, which occur in response to higher levels of soil nitrate, dissociates the filament and activates FUN. FUN then directly triggers breakdown of the nodule. The zinc-dependent filamentation mechanism thus establishes a concentration readout to adapt nodule function to the environmental nitrogen conditions. Our genetic and biochemical analysis thus identifies an unexpected regulatory mechanism and opens new possibilities for translation to the field.

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The common bean (*Phaseolus vulgaris*) –*Rhizobium etli* N-fixing symbiosis: unraveling novel plant regulators through genetic/genomic approach

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The symbiotic N_2-fixation process (SNF) in the legume-rhizobia interaction, relevant for sustainable agriculture, is a complex process that is tightly regulated in both symbionts. Advances in legume genomics and genetics, mainly the characterization of symbiotic mutants, have been instrumental for the understanding of legume genes required for effective symbiosis with rhizobia [1].

Common bean (*Phaseolus vulgaris*) is the most important grain legume for human consumption in the world. The focus of our current research is to characterize or decipher relevant symbiotic genes in common bean using genetic approaches.

Only one common bean EMS symbiotic mutant has been genetically/molecularly characterized [2]. It is evident that isolation, characterization and mapping of symbiotic common bean mutants is required to expand the knowledge of the fine regulation (SNF) in this important crop. To this end, we have screened 1,692 M4 lines of an EMS-generated common bean mutant population derived from the BAT93 genotype [3]. After the population screening, we aim to characterize three stable non-nodulating *(nnod*) mutant lines, that appear to be recessive and monogenic. Microscopic analysis of inoculated mutants revealed that each line is altered in a different early step of rhizobial infection: effective root hair deformations, formation of infection chambers and infection thread formation and development [4]. Comparative whole genome sequence analysis was undertaken for the prediction of responsible mutated genes in each mutant. In this talk we will present advances aiming to decipher mutated genes as well as further genetical/molecular characterization of selected common bean mutants.

References:

[1] Roy S. et al, Celebrating 20 Years of Genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation. The Plant Cell. Vol.32, 2020, p. 15.
[2] Ferguson BJ. et al, The Soybean (Glycine max) Nodulation – Suppressive CLE peptide, GmRIC1, Functions Interspecifically in Common Bean (Phaseolus vulgaris) but Not in a Supernodulating Line Mutated in the Receptor PvNARK. Plant Biotechnology Journal. Vol. 12, 2014, p. 1085.
[3] Porch TG. et al, Generation of a Mutant Population for TILLING Common bean Genotype BAT93. Journal of American Horticultural Science. Vol. 134, 2009, p. 348.
[4] Reyero-Saavedra R, et al. Identification and Characterization of Common Bean (Phaseolus vulgaris) Non-Nodulating Mutants Altered in Rhizobial Infection. Plants. Vol. 12, 2023, p. 1310.

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NF-Ys affect iron and nitrate homeostasis in *Medicago truncatula*

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Most legumes can interact with beneficial N-fixing bacteria called rhizobia. Shortly after contact with the rhizobia, components of the heterotrimeric Nuclear Factor Y (NF-Y) transcription factor complex, comprised of A, B and C subunits, are induced in the roots of the host. In *Medicago truncatula*, these subunits are encoded by multiple genes, 8 *NF-YA*s, 19 *NF-YB*s and 11 *NF-YC*s, of which *NF-YA1* has been implicated in rhizobial infection and nodule formation [1]. We investigated the role of *NF-YB7*, which is induced in *M. truncatula* root hairs in response to rhizobia and Nod factors [2]. We determined that NF-YB7 can interact with NF-YA1, and that loss of NF-YB7 reduced the formation of rhizobial infection threads. Contrary to expectation, *M. truncatula* roots overexpressing NF-YA1 and NF-YB7 (NFYox) showed suppressed nodule formation. Investigated of this phenomenon using RNAseq revealed that abscisic acid signaling is activated in NFYox roots. In addition, NFYox roots showed increased expression of *Ferritin* and deregulation of other iron related genes, and accumulated iron. Genes involved nitrate uptake and nitrate signaling were strongly repressed in NFYox roots, and comparison with ChIP-seq data revealed that amongst these, *NIN-like protein 1* and *Nitrate Transporter 1.1B* were direct targets. Co-expression of the ABA degrading enzyme CYP707A restored the ability of NFYox roots to nodulate. Our results suggest that ABA may serve as a negative feedback mechanism directly downstream of the NF-Ys during nodulation and reveals a potential role for NF-Ys in alleviating nitrate's negative effect on nodulation and in nodule iron homeostasis.

References:

[1] Laloum T., et al. Two CCAAT-box-binding transcription factors redundantly regulate early steps of the legume-rhizobia endosymbiosis. Plant J. 2014, 79:757-68.
[2] Breakspear A., et al. The root hair "infectome" of Medicago truncatula uncovers changes in cell cycle genes and reveals a requirement for auxin signaling in rhizobial infection. Plant Cell. 2014 26:4680-701.

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Nodule-specific PLAT domain (MtNPD1) - dependent host-strain compatibility in the *Medicago truncatula* – *Sinorhizobium* sp. symbiosis

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Symbiotic nitrogen fixation (SNF) is a complex process regulated by thousands of legume host and nitrogen fixing symbiont genes. Tobacco retrotransposon (*Tnt1*)-insertion mutagenesis has been instrumental in the discovery of new genetic controls of SNF in the model legume *Medicago truncatula*. A foward genetic screening identified the Nodule-specific Polycystin-1, Lipoxygenase, Alpha-Toxin (PLAT) Domain-encoding gene, *MtNPD1*, which is essential for root nodule development, survival of *Sinorhizobium* sp. inside infected cells, and efficient nitrogen fixation.

We previously reported that, in *npd1* nodules, *S. meliloti* Sm1021 fail to mature and undergo early senescence, while *S. meliloti* Rm41 differentiate and fix nitrogen, thus reversing the mutant phenotype into wild type. *MtNPD1* is predicted to play a role in facilitating or restricting effective nodulation in *M. truncatula* [1].

A systematic analysis of host strain compatibility using *Sinorhizobium* strains representing a range of secretion systems uncovered three *S. meliloti* strains that induce wild type-like nodules in *npd1*: Rm41, T073, and M10. In contrast, *S. meliloti* Sm1021 and *S. medicae* A321 and WSM419 induce deffective *npd1* nodules. A phenotype-informed pangenome analysis to identify relevant bacterial gene clusters will be discussed. Unique insights into paired transcriptional responses in the *MtNPD1*-mediated host-strain compatibility were gained by dual RNA-Seq. To facilitate the tracking of infections in single inoculations and in competition studies involving multiple strains, we developed a collection *Sinorhizobium* sp. strains constitutively expressing green-, red, cyan-, and yellow-fluorescent proteins. How reporters influence nodulation will also be discussed.

References:

[1] Pislariu C.I. et al. (2019) The nodule-specific PLAT domain protein NPD1 is required for nitrogen-fixing symbiosis, Plant Physiology, vol. 180, no. 3, p. 1480.

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Sugar signaling acts as a proxy for cytokinin signaling for de novo meristem formation during nodule organogenesis.

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Symbiosis between plants and diazotrophs require formation of a *de novo* meristem for endocytic accommodation of symbionts, a process that is tightly regulated by plant hormones cytokinin and auxin. Cytokinin signaling through CRE1 receptor causes auxin accumulation by regulating its transport or biosynthesis to initiate cell division for nodule organogenesis. Accordingly CRE1 mutant (*cre1*) is unable to undertake symbiosis and our objective was to strategize and restore functional symbiosis in *cre1* for understanding the downstream events. Our findings are :- (i) Sucrose signalling can restore functional symbiosis in *cre1*. (ii) Sucrose signalling upregulated an auxin conjugate hydrolase *MtIAR33* that converts IAA-asp to IAA. Overexpression of *MtIAR33* could also restore symbiosis in *cre1* indicating deconjugation of auxin conjugates to be a potential pathway of auxin accumulation during nodule organogenesis. (iii) Sugar signaling significantly upregulated an auxin responsive homeobox transcription factor WOX5 well known for its role in meristem maintenance. While *AhWOX5* from *Arachis* having determinate meristem could completely restore symbiosis in *cre1, MtWOX5* from *Medicago* having indeterminate nodule meristem failed to do so. We could show that *MtWOX5* function as a repressor whereas *AhWOX5* acts as an activator and swapping a single amino acid is sufficient to functionally convert *MtWOX5* to *AhWOX5* and vice versa. Based on these evidences, we propose a model where we show CRE1-independent deconjugation of auxin to be a potential contributor to auxin accumulation and activation of NIN-WOX5 axis, a step forward toward having an integrated view of how organogenesis starts during root nodule symbiosis.

References:

1. Molla, F., Kundu, A. and DasGupta, M., 2023. Sucrose-induced auxin conjugate hydrolase restores symbiosis in a Medicago cytokinin perception mutant. *Plant Physiology*, *191*(4), pp.2447-2460.
2. Kundu, A., Molla, F. and DasGupta, M., 2019. Turanose mediated WOX5 expression rescues symbiosis in cytokinin perception mutant cre1. *bioRxiv*, p.830661.

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Root architecture is regulated by miR2111 and TML in response to soil Pi

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Plants control their root system architecture in response to irregular and fluctuating nutrient availability in the rhizosphere. This includes localised morphological changes and drastic shifts in overall root system structure to enhance soil foraging and nutrient acquisition. Previously, we reported that enhanced miR2111 expression led to an increase in the density of emerged lateral roots (Zhang et al., 2021). Such a phenotype is often observed in Pi-deplete plants. This prompted us to hypothesise that the miR2111 and TML regulatory module that controls nodule organogenesis may have been hijacked and evolved from a pre-exiting regulatory mechanism that acts to alter root development in response to environmental factors, such as nutrient availability. We tested this hypothesis by functionally characterising miR2111 and TML in the context of Pi deprivation-induced root adaptive responses. Three *GmmiR2111* encoding genes were transcriptionally upregulated in leaves, but not roots, of Pi-starved plants, resulting in increased accumulation of mature miR2111 and a concomitant decrease in the transcript abundance of *GmTML1* homologous in roots. Overexpression of *GmTML1* encoding genes reduced lateral root density and root thickness. This was consistent with phenotypic alternations observed in Pi-starved root systems, which exhibited diminished root growth, enhanced root branching and increased root diameter compared with control plants. Collectively, these findings demonstrate that miR2111 and TML have a critical role in the systemic manipulation of root system architecture in response to Pi availability, and subsequently appear to have been co-opted into the nodulation control mechanism of legumes.

References:

Zhang MB, Su HN, Gresshoff PM, Ferguson BJ (2021) Shoot-derived miR2111 controls legume root and nodule development. Plant, Cell & Environment 44: 1627-1641.

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Genetics and genomics of symbiotic nitrogen fixation in legumes: past, present, and future

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Legumes are a large and diverse family of plants that provide us with food, feed, fuel, and feedstocks for industry. They can use atmospheric di-nitrogen for growth, via symbiotic nitrogen fixation (SNF) with bacteria called rhizobia, making them key to sustainable agricultural systems. There are opportunities to increase SNF in legumes to help tackle critical challenges related to the overuse of fertiliser-N in agriculture. The last two decades have seen enormous progress in our understanding of the genetics of SNF, although this is yet to be leveraged to improve SNF in legumes. In principle, two main plant-based approaches exist to improve SNF, one involving genetic engineering and the other using existing natural variation for this complex trait. These approaches are not mutually exclusive and now is an opportune time to attempt to increase SNF in legumes via plant genetics and genomics. This presentation will briefly review current knowledge of SNF genetics before outlining potential pathways to SNF improvement in legumes. Importantly, there is a new national initiative in Australia to develop genetic resources and knowledge for predictive plant breeding to improve SNF in chickpea, lentil, field pea, fababean, lupin, and mungbean. An outline of this project will also be presented.

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Advancing predictive breeding methods and capacity building to develop future pulse crops

Prof Lee Hickey

Plant breeders are under increasing pressure to improve crop productivity and sustainability to meet future demand. This calls for more efficient breeding approaches to accelerate the introgression and stacking of genetic variation for target traits into elite breeding germplasm. A great source of genetic variation is the genebank, but trait introgression is a time-consuming process and is particularly challenging for multiple traits controlled by multiple genes. To help meet this challenge, we have developed 'FastStack', an AI-guided breeding toolkit. Here, we demonstrate the potential of the approach in a pulse crop for the first time. In a mungbean case study, we draw on a global dataset for the minicore collection evaluated in field experiments by collaborators throughout Asia and Africa through the International Mungbean Improvement Network (IMIN) project. Using the FastStack approach we applied a genetic algorithm to identify parental lines for crossing that maximise desirable haplotypes. Then, using genetic simulation we identified the optimal crossing path to rapidly stack haplotypes to improve yield potential by increasing pods per plant and seed weight in mungbean. The FastStack mungbean populations are being created under speed breeding conditions and seed of the lines with stacked haplotypes will be distributed to researchers and breeders to support global yield improvement of mungbean. The toolkit is now being applied and extended in several new investments by the Grains Research and Development Corporation in Australia to accelerate genetic gain for a range of traits in key pulse crops, including chickpea, lentil and faba bean. Finally, we highlight the importance of training and building human capacity in predictive breeding technologies to develop our future pulse crops.

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Simulation guided establishment of heterotic pools for breeding of synthetic cultivars in faba bean

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Synthetic cultivars of faba bean comprise a mix of two or more parental genotype components. Before release, the genotypes are grown together and undergo open pollination, hence the seeds sold to farmers are a mixture of homozygous parental genotypes and cross-pollinated F1 hybrids. Consequently, the performance of synthetic cultivars can be attributed to the per se performance of the synthetic components along with heterosis of F1 hybrids. It´s widely recognized that heterosis is high when the parental genotypes are genetically distinct, and breeders of major crops such as maize exploit heterosis systematically. Preliminary results of test-synthetics between faba bean genotypes indicated that the successful synthetics originated from crosses between distantly related parental components. Hence, development of genetically distinct heterotic pools is a useful strategy to optimize heterosis and synthetic cultivar performance in faba bean, which presently arises predominantly from specific combining ability rather than general combining ability (GCA). To optimize faba bean breeding by fixing heterosis and improving GCA, we apply a simulation-based pipeline that utilizes genome-wide SNP data to design crossing schemes that rapidly create new, distinct gene pools from an admixed founder population. The pipeline employs a chain crossing scheme that maximizes recombination and maintains diversity within pools, but promotes pool separation. To simultaneously enhance per se performance of parental components, additional crosses are conducted based on genomic predicted per se performance of cross offspring. Based on successful implementation in hybrid breeding programs in other crops, we expect this strategy to accelerated genetic gain in faba bean breeding.

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Genomic prediction for Grain yield and other key traits in field pea

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Field pea (*Pisum sativum* L.) is an important pulse crop grown worldwide for its protein-rich seeds and economic value. Pea breeding programs have used traditional methods to improve yield and yield stability. However, the rate of genetic gain is not enough to meet the increasing demand for protein, especially with the changing climate and increasing population, necessitating the deployment of modern tools and technologies in breeding programs. Genomic selection (GS) is an innovative approach that uses whole genome markers to predict the breeding values of individuals early in the breeding cycle to reduce the generation interval and increase the genetic gain per unit of time. In this study, we developed and implemented GS for the Australian National Field Pea breeding program. The training population comprised 2,889 advanced breeding lines and was evaluated in multi-environment trials over 2013-2023 for 15 traits, including grain yield, phenological traits, biotic resistance, abiotic stress tolerance, and seed quality. The narrow sense heritabilities ranged from 0.26 - 0.67, confirming the polygenic inheritance. We used 39,659 high-quality SNPs and phenotypic data to train GBLUP model for genomic prediction. Three prediction accuracy validation scenarios, leave-one-out, forward, and five-fold cross-validations, were used: leave-one-out, forward, and five-fold cross-validations. The prediction accuracies for all traits ranged from 0.12 to 0.78. The GS was utilized in two phases within the field pea breeding program, namely, F1 and F4:5. These stages aimed to enhance the selection process for parental lines in the subsequent breeding cycle and selecting lines for field trials. Our results showed that GS could improve prediction accuracy and intensity and help in early parental selection. Implementing GS would enable an increased rate of genetic gain and the development of better-adapted pea varieties.

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Breeding for rapid cooking biofortified bean cultivars for East Africa through novel breeding strategies

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Common bean (*Phaseolus vulgaris* L.) is a key food to combat anemia, stunting and wasting in diets of East African women and children. Long cooking time discourages consumption due to the significant amounts of water and fuel required, and health hazards to women and children from smoke inhalation and firewood collection. The study aims to breed biofortified bean cultivars with 30% less cooking time (CKT), 15% higher seed iron and 10% higher seed zinc compared to current commercial cultivars, while safeguarding grain yield, and seed size and colour. Innovative breeding strategies guided by BRIO principles were implemented (Cowling et al. 2023; Saradadevi et al 2021) in an East African breeding program based at the Alliance of Bioversity International and CIAT, Uganda, with regional testing at national agricultural research systems in six countries. BRIO in common bean involves two-year cycles of recurrent selection, accurate breeding values from genomic and pedigree information, selection indices composed of weighted breeding values of key traits, and optimised crossing designs through optimal contribution selection. Up to 30% reduction in CKT was achieved while increasing grain yield from cycle 1 to cycle 2. However, detrimental genetic correlations prevented improvements in iron and zinc in large-seeded beans, and the first rapid-cooking biofortified bean cultivars will have small seeds. Adjustments to BRIO will permit selection of large-seeded biofortified beans in future. The progress marks a significant stride towards rapid cooking biofortified bean cultivars which will enhance future health outcomes for women and children and improve nutritional security in Africa.

References:

[1] *Cowling, W. A., et al. (2023). "Optimal Contribution Selection Improves the Rate of Genetic Gain in Grain Yield and Yield Stability in Spring Canola in Australia and Canada." Plants 12(2): 383.*

[2] *Saradadevi, R., et al. (2021). "Multivariate genomic analysis and optimal contributions selection predicts high genetic gains in cooking time, iron, zinc, and grain yield in common beans in East Africa." Plant Genome 14(3): e20156.*

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Leveraging Genome-Wide Association Studies (GWAS) for Enhanced Understanding and Breeding of Faba Bean: Insights from Two Distinct Panels on Agronomic Traits and Stress Tolerance

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Faba bean (Vicia faba L.), a crucial legume crop for sustainable agriculture due to its nitrogen-fixing ability and high protein content, faces production limitations and instability due to various biotic and abiotic stressors. Furthermore, breeding efforts have been limited due to underdeveloped genomic resources.

To enhance breeding efficiency, this study employs a comprehensive GWAS approach on two distinct faba bean panels to identify candidate genes associated with key agronomic traits and stress tolerance, followed by cross-panel validation using genomic prediction.

The two panels, consisting of around 200 diverse faba bean accessions, with an overlap of around 50, was analyzed to uncover genetic markers linked to agronomic traits such as plant height, time to flowering and lodging, as well as disease-related traits. Using high-density SNP arrays, we identified several significant marker-trait associations that provide insights into the significant genetic components of these traits.

To validate these findings and predict their utility across different genetic backgrounds, we applied genomic prediction techniques. Cross-panel validation was conducted, where significant SNPs from one panel were used to predict the other panel. The integration of GWAS findings with genomic prediction not only confirms the robustness of the GWAS findings but also underscores the potential of identified candidate genes for targeted breeding programs. This strategy promises to accelerate the development of faba bean varieties with improved agronomic qualities, thereby supporting global food security and sustainable agricultural practices.

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Genomic Selection: a new frontier in Mungbean Breeding

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Genomic selection (GS) is a breeding strategy based on a statistical model that uses molecular markers across the genome to predict the performance of individuals for a trait of interest. The introduction of GS promised to accelerate animal and plant breeding processes while improving their individuals' performance. However, utilising genomic data to predict the yield potential in plant breeding has some additional challenges that need to be addressed in a GS model. Plant genotypes are tested in replicated trials with spatial variation and genotypes are tested in multi-environment trials (MET) generally, in an unbalanced way, to assess genotype by environment interaction (GxE).

The Australian Mungbean Improvement Program has been testing genotypes for a range of agronomically important traits with primary focus on yield across multiple environments throughout Queensland and New South Wales for many years. Selections for grain yield have relied on factor analytic linear mixed models that use pedigree information and explain GxE [1], helping deliver superior grain yield, reliability, and adaptation to growers. To build on this success, genomic data was collected and implemented in an extension of the previous model which incorporates genomic data in a MET scenario [2]. In this study, we share the details of the GS model as well as the outputs driving selections such as GxE insights, genomic predictions for tested genotypes at all the environments and the potential to predict new genotypes that have not been tested for yield yet only using their genomic marker data profile.

References:

[1] Oakey, H., et al (2007) Joint modeling of additive and non-additive (genetic line) effects in multi-environment trials. Theoretical and Applied Genetics, 114, 1319-1332.

[2] Tolhurst, D. J., et al (2019) Genomic selection in multi-environment plant breeding trials using a factor analytic linear mixed model. Journal of Animal Breeding and Genetics, 136(4), 279-300.

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Getting to the root of symbiotic root nodule development

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Legumes grow specialized root nodules to host beneficial nitrogen-fixing bacteria that provide the plant with ammonia in exchange for carbon. These symbiotic nodules are distinct from lateral roots in morphology and function as they comprise of cells that can accommodate nitrogen-fixing rhizobial bacteria intracellularly and provide favorable conditions for the biological nitrogen fixation process.
Nodules initiate from the inner tissue layers in response to the perception of rhizobial bacteria at the root surface via cytokinin-mediated upregulation of the nodulation-specific transcriptional regulator *NODULE INCEPTION (NIN)*. Our previous findings that the initiation of lateral roots and nodules converges at a common developmental program [1] led to the hypothesis that an additional nodule-specific program is required to determine nodule organ identity on top of the shared root-like initiation program.
Recently, we have shown that two members of the *LIGHT SENSITIVE SHORT HYPOCOTYL (LSH)* transcription factor family (*MtLSH1* and *MtLSH2*), predominantly known to define organ boundaries and meristem complexity in the shoot, function as regulators of nodule organ identity [2]. *MtLSH1*/2 are upregulated during early stages of nodule development in a cytokinin- and *NIN*-dependent manner and are expressed in dividing cells. Our loss of function analysis of *lsh1/2* demonstrated that these regulators are required for the development of functional nodule primordia that can support the intercellular cortical infection, the intracellular colonization, and nitrogen-fixation by the bacteria. Furthermore, molecular functional analysis revealed that *LSH1/2* control components of the auxin-cytokinin cross talk and function upstream of and together with the previously identified nodule organ identity genes nuclear factor *Y-A1* (*NF-YA1)* and *NODULE ROOT1/2 (NOOT1/2)* to recruit a program with pleiotropic functions in the shoot to differentiate nodules from lateral roots and to determine nodule organ identity. The principal outcome of *LSH1/LSH2* function is the production of cells able to accommodate nitrogen-fixing bacteria, the unique nodule feature.
These findings provide a framework at molecular and cellular level to investigate how the coordinate recruitment of pre-existing organ development and identity programs can underpin the morphological and functional divergence between lateral roots and nodules, in parallel to a root initiation program.

REFERENCES:
[1] Schiessl et al., Curr Biol (2019).
[2] Lee, Orvosova et al., Curr Biol (2024).

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Genetic diversity and symbiotic effectiveness of *Mesorhizobium* and *Bradyrhizobium* strains nodulating selected annual grain legumes growing in Ethiopia

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Cicer arietinum L., Vigna unguiculata, Vigna radiata, and *Arachis hypogaea* growing in Ethiopia are nodulated by a genetically diverse group of rhizobia where chickpea (C. arietinum L.) being nodulated by Mesorhizobium genus while the latter three host legumes are by Bradyrhizobium strains. A collection of 167 test strains originating from the root nodules of respective hosts was investigated using multilocus sequence analyses (MLSA) of core genes including 16S rRNA, *recA, glnII, gyrB, atpD* and *dnaK*. Sequence analysis of *nodA* and *nifH* genes along with tests for symbiotic effectiveness were undertaken. The MLSA grouped most test strains into several well-supported distinct positions. We found similar grouping for the *nodA* and *nifH* gene sequences of strains from Vigna unguiculata, Vigna radiata, and Arachis hypogaea where most of the test strains were clustered on one of a well-supported large branch that comprise Bradyrhizobium species from the tropics. Similarly, the *nodC* and *nifH* gene sequences of strains from C. arietinum showed a monophyletic origin and related to a clade representing three symbiovars. The symbiotic effectiveness of selected test strains revealed the presence of highly effective nitrogen fixers. It was concluded that Ethiopian soils are a hotspot for rhizobial diversity. This calls for further research to unravel as yet unknown rhizobia nodulating legumes growing in the country. In this respect, prospective research should also address the mechanisms of symbiotic specificity that could lead to high nitrogen fixation for legume production that could help to sustainably intensify cropping systems.

References:

[1] Degefu T. et al, Genetic diversity and symbiotic effectiveness of Bradyrhizobium strains nodulating selected annual grain legumes growing in Ethiopia. International Journal of Systematic and Evolutionary Microbiology, 2018.
[2] Gunnabo A.H. et al, Phylogeography and Symbiotic Effectiveness of Rhizobia Nodulating Chickpea (Cicer arietinum L.) in Ethiopia, Microbial Ecology, 2021.

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Uncovering The Role Of Gibberellin In Nodulation: Gibberellins Restrict Rhizobial Infection In The Epidermis And Promote Nodule Organogenesis In The Endodermis And Regulate Key Nodulation Genes

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Legumes associate with rhizobia to access to atmospheric nitrogen in root nodules. This symbiosis requires the precise coordination of rhizobial infection in the epidermis and nodule formation in the inner root layers. Plant hormones play important roles in regulating these processes, with their spatial and temporal activation dictating nodulation progression. We employed a powerful approach of specific epidermal and endodermal complementation of severely gibberellin-deficient *na* pea mutants, coupled with a novel comparison strategy of RNA-seq gene expression data from tissue-specific complemented roots, which allowed the dissection of genes possibly regulating specific nodulation stages. Our findings reveal that gibberellins restrict epidermal infection, limiting bacterial entry and regulating infection thread progression and branching in the cortex. Moreover, we found that gibberellins are essential in the endodermis to promote nodule and lateral root development. In contrast, gibberellin presence in the epidermis does not affect nodule or root development. Careful comparison of RNA-seq data of epidermal and endodermal complemented and control *na* mutants allowed the identification of genes potentially involved in the regulation of infection and nodule organogenesis downstream of gibberellin. These include the transcription factors *NSP1* and *NSP2*, ethylene response factors *ERN1* and *ERN2* and flavone synthase *FNSII*, along with genes important for nodule organogenesis and symbiosome formation. Future studies could target selected candidate genes to determine their precise role in nodulation and their regulation by gibberellin. This knowledge will facilitate the integration of the roles of plant hormones in nodulation and root development processes and the gene expression networks behind these processes.

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A novel nucleotide-binding domain leucine-rich repeat receptor (NLR) involved in soybean nodulation

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Legumes can exploit their relationship with nitrogen-fixing rhizobia to thrive in low nitrogen environments via the formation of symbiotic organs on their roots, termed nodules. The plant innate immune system has been shown to be a major determinant of nodule formation. For example, soybean nucleotide-binding domain leucine-rich repeat receptors (NLRs) have been shown to restrict symbiosis with certain strains of rhizobia upon recognition of specific rhizobia proteins. Here, we report the characterisation of a novel NLR of soybean which responds to Nod factors unlike previously reported NLRs. Interestingly, a promoter::*GUS* fusion revealed this gene is induced from early infection through to mature nodules. When overexpressed, the *NLR* reduces nodule number and size. When knocked-out via CRISPR genome editing, nodule diameter significantly increased but there was no change in nodule number. mRNA of the *NLR* undergoes alternative splicing to produce at least four different protein isoforms. NLRs in plant-pathogen interactions, often called *R*-genes, have recently been shown to form resistosomes capable of degrading nucleotides and facilitating cell death. Sequence analysis suggests the soybean NLR involved in nodulation has the necessary catalytic sites for this enzyme activity. New insight into this gene and our current understanding of the interplay of plant immunity in legume nodulation will be presented.

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Nodule organogenesis in Medicago truncatula requires local stage-specific auxin biosynthesis and transport

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The importance of auxin in plant organ development including root nodule formation is well established. Using auxin reporter constructs the spatiotemporal auxin distribution pattern during nodule development has previously been illustrated. However, our understanding of how this pattern is built-up and maintained still remains elusive.

To this end, we studied how the auxin gradient visualized by DR5 expression patterns at different stages of nodule development in Medicago truncatula (Medicago), is correlated with the spatiotemporal expression patterns of known auxin biosynthesis and auxin transport genes. In addition, we record the MtPIN10-GFP expression pattern and polar positioning on the cell plasma membranes during nodule primordium development to investigate the auxin flux. RNA interference and the application of auxin synthesis blockers were used to demonstrate the relevance of biosynthesis and transport at the initial stages of the nodulation process.

Our results show that upon rhizobium inoculation, preceding the first mitotic activity, a specific set of MtYUCs and MtPINs as well as MtLAX2 are expressed in the pericycle contributing to the creation of an auxin maximum. Overall, we demonstrate that dynamic spatiotemporal expression of both, MtYUCs and MtPINs, result in specific auxin outputs in subsequent stages of nodule primordia and nodule meristem formation.

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Exploring potential benefits of biostimulant treatments in lupin cultivation

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Lupin is a valuable alternative protein source for both human food and animal feed. White- and narrow-leafed lupins are cultivated in Germany alongside traditional protein crops such as broad bean and pea and represent an attractive alternative to (GMO-) soybean from national- and international markets. Our project "P³roLucas" (Optimization of plant performance and products for lupin cascade use) aims at promoting and improving lupin cultivation in Germany and encompasses a wide range of research topics ranging from exploration of lupin alkaloids for industrial use to sequencing of the Andean lupin genome as a resource for future breeding programmes [1]. Central topic is the smart use of so called biostimulants as (partial) alternatives to traditional plant protection products which are increasingly being phased out under European and national regulatory policies. Therefore, we are exploring effects of commercially available biostimulants in narrow-leafed lupin (*Lupinus angustifolius*) on plant growth and protection against biotic- and abiotic stresses. Products based on *Bacillus* spec. were identified as the most promising candidates. We implemented a combined approach of seed treatments including the so-called seed-priming strategy, which is close to agricultural practice. In addition, the localisation of bacteria after seed treatments and their influence on plant development and induction of resistance to lupin anthracnose, were investigated. We present results from lab-scale experiments, currently being validated in field experiments and analyses at the metabolic- and transcriptomic level, using consolidated and newly generated genomic resources within this project.

References:

[1] https://www.biosc.de/p3roLucas_en

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Non-rhizobial bacteria exhibit persistent colonization in the roots and nodules of chickpea cultivars across diverse environments

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Rhizobia are known for forming stable symbiotic relationships within the endosphere of legume hosts. However, the colonization of non-rhizobial bacteria in legume roots has been less explored. Firstly, we investigated the mechanisms for the microbiome establishment in the chickpea (*Cicer arietinum* L.) root across different cultivars and soils. Deterministic effects were more important than the stochastic processes in shaping the endophyte community structure for all the cultivars tested. The cultivar with higher symbiotic potential had greater secretion of benzoic acid (BA) and consistently colonised by 3 core ASV (Amplicon Sequence Variant) from *Burkholderia* clade in the root. Using a selective media, DNA identification, and colonisation testing, two of 98 isolated strains were confirmed as the culturable candidates of the core ASV. The two core strains were able to survival and grow in higher concentration of BA than the other strains, but had no plant growth promotion potential. In the second study, we analysed the nodule microbiome of four chickpea cultivars across five different growing environments spanning 1,400 km in Australia. Besides the symbiotic *Mesorhizobium*, we found two ASV from the *Burkholderia* clade and *Pseudomonas* sp. were persistently enriched in the nodules of all tested cultivars and environments. The culturable strains of these ASV were isolated, and demonstrated significant capability in producing Indole-3-Acetic Acid and enhancing chickpea nodulation and nitrogen fixation. The conserved colonisation of non-rhizobial endophytes is one target for future research developing beneficial strains to promote legume growth.

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Ethylene inhibits cell cycle progress in root hairs of *Lotus japonicus* infected by rhizobia

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The symbiotic bacteria *Mesorhizobium loti* typically gains entry to its host *Lotus japonicus* by means of infection threads, tubular invaginations that serve as transcellular passageways through root hairs, that form through the physical remodelling of the cell wall and membrane. During rhizobial infection numerous cell division genes are induced, suggesting at least partial engagement of cell cycle related processes to provide an invasive impetus, involving reorganization of the cytoskeleton and enlargement of the nucleus [1, 2].
Here we show that ETHYLENE RESPONSE1 (ETR1) is involved in cell cycle regulation during infection thread establishment. We found that the ethylene insensitive mutant LjETR1-1 displays an enlarged, irregular shaped nucleus in infected root hairs, which is sometimes associated with blocked infections. We show that despite the nuclear enlargement that occurs in wild type infected cells, no *de novo* DNA synthesis takes place, while in LjETR-1 these cells enter S phase, highlighting a new role for ETR1 in the direction of cell cycle processes during symbiotic infection.

References:

[1] Breakspear, Andrew et al. “The root hair "infectome" of *Medicago truncatula* uncovers changes in cell cycle genes and reveals a requirement for auxin signaling in rhizobial infection.” The Plant Cell vol. 26,12 (2014): 4680-701.
[2] Gao, Jin-Peng et al. “Intracellular infection by symbiotic bacteria requires the mitotic kinase AURORA1.” Proceedings of the National Academy of Sciences of the United States of America vol. 119,43 (2022): e2202606119.

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The NRT2.3 Nitrate Transporter Plays a Positive Role in Nodule Function Medicago truncatula

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Nitrate (NO_3^-) is the major inorganic form of nitrogen absorbed by plants and a key external environmental factor in regulating root nodule symbiosis and nitrogen fixation. As N_2-fixation is relatively energy intensive, low nitrogen levels promote nodulation while high nitrogen inhibits nodulation and nitrogen fixation in legumes. Here, we show that *Medicago truncatula NRT2.3* encodes a plasma membrane localized high affinity nitrate transporter plays an essential role in balancing nitrate transport in roots and in nodules. Loss of *NRT2.3* in non-symbiotic conditions caused nitrate accumulation in roots while decreasing shoot nitrate content. Under symbiotic conditions, *nrt2.3* mutants formed fewer nodules, and the nodules that formed had decreased acetylene reduction activity. Moreover, *nrt2.3* nodules were greenish in color, showed symbiosome degradation, and had higher expression of the senescence marker gene *Cysteine Protease 6*. Finally, both high nitrate (5 mM KNO_3) and low nitrite (0.5 mM KNO_2) treatments promoted the formation of green nodules in *nrt2.3,* which was associated with higher levels of nitrate and nitrite in the mutants. From these findings we conclude that *MtNRT2.3* mediated nitrate transport in nodules is important for optimal nitrogen fixation.

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Tyrosine Sulphated Root Meristem Growth Factor Peptides Regulate Root and Nodule Development in Soybean

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Legumes form a beneficial symbiotic relationship with soil bacteria that can fix atmospheric nitrogen into a plant usable form, resulting in a reduced reliance on synthetic nitrogen fertilizer and the associated negative environmental issues. This process is known as nodulation and involves complex molecular signaling pathways to control the physiological changes that are required to initiate and maintain nodule organogenesis. We have identified novel tyrosine sulfated peptides, called Root Meristem Growth Factor (RGF) peptides, in one of the most widely produced legumes, soybean (*Glycine max*)[1]. They are homologous to Arabidopsis RGF/GLV/CLEL peptides that play essential roles in regulating meristematic activity and immune responses[2]. We have identified conserved orthologs in other agriculturally important legumes, including *Phaseolus vulgaris*, *Pisum sativum* and *Lotus japonicus.* Within the soybean gene family, we have functionally characterized five soybean RGF-peptide encoding genes using multidisciplinary molecular biology techniques. Our results identified that they are expressed in the root tip region where meristem is located and during different growing stages of lateral root growth and nodulation. They also demonstrate a systemic signal transduction pathway, which is not seen in Arabidopsis and necessary for proper root growth and nodule development. These findings enhance our understanding of legume signaling and symbiotic nitrogen fixation and benefit future legume crop development programs which will improve the impact agriculture has on the environment, human health, economy, and biodiversity.

References:

[1] Hastwell et al. 2024, TIPS submitted, PLANTS-D-24-00070
[2] Matsuzaki et al., 2010, Science, 329, 1065-1067

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Pathogen fitness, disease dynamics and host plant resistance under the changing climate scenario with special reference to legumes (chickpea and pigeonpea)

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The significance of grain legumes like chickpea and pigeonpea in ensuring food and nutritional security, particularly in the context of climate change is critical. Globally, 30-40% of crop produce is lost to pests and diseases annually, with overall losses in yield far greater in Asia and Africa.
ICRISAT in collaboration with National Agricultural Research Systems (NARS), has been instrumental in developing high-yielding, disease-resistant varieties of these crops. These efforts are crucial as they address the substantial yield losses caused by pests and diseases, which are exacerbated by climate change.
Innovative approaches, including the use of artificial intelligence for early disease detection and risk mapping, are being employed to combat these challenges. The evaluation of 5000 chickpea germplasm accessions for resistance to diseases like Fusarium wilt, Ascochyta blight, Botrytis gray mold, dry root rot is a testament to the proactive measures being taken to address the increasing burden of crop losses. Similarly, advancements in pigeonpea breeding for resistance to diseases such as wilt, sterility mosaic disease and Phytophthora blight showcase the dynamic nature of agricultural research in response to evolving threats.
The use of novel sources of resistance and advanced breeding methods will not only enhance yield stability but also contribute significantly to sustainable food production. This aligns with global research indicating that a major refocusing towards grain legumes is required for food security and climate resilience. Additionally, the impact of climate change on legume physiology and ecosystem dynamics further emphasizes the need for such innovative approaches.

References:

1. *Sharma M, Avijit Tarafdar, Abhay Pandey, S. Ahmed, Vibha Pandey, Devashish R. Chobe et al. 2021. Biotic Stresses in Food Legumes: An Update and Future Prospects Pages 149-196 In Genetic Enhancement in Major Food Legumes - Advances in Major Food Legumes.*
2. *Delgado-Baquerizo, M., Guerra, C.A., Cano-Díaz, C. et al. The proportion of soil-borne pathogens increases with warming at the global scale. Nat. Clim. Chang. 10, 550–554 2020. https://doi.org/10.1038/s41558-020-0759-3*

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Genetics of Ascochyta Blight Resistance in Chickpea

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Ascochyta blight (AB) in chickpea, caused by the fungus *Ascochyta rabiei,* is a major endemic disease resulting in significant crop loss and management cost for growers. Limited genetic gains have been achieved through conventional breeding due to the complexity of genetics underlying AB resistance. To identify genetic regions associated with resistance, a diverse chickpea germplasm comprised of domestic x wild introgression material, Vavilov collections, ICARDA FLIP (Food and Legume International Program) lines, and Chickpea Breeding Australia (CBA) material was genotyped using a Multispecies pulse SNP chip and evaluated for resistance to AB. Two phenotyping environments were utilised i) an outdoor netted pot-based screening facility at the Waite campus, Adelaide, known as SARDI and ii) a field disease nursery at Horsham. A total of 1640 genotypes were phenotyped at the SARDI terraces and 1870 genotypes in the Horsham field nursery. Disease Index (%) and stem breakage (%) were used to rate disease severity in the SARDI terraces and in the field nursery. Broad genetic variation in AB disease severity was observed in both environments. Resistant and partially resistant lines, significantly better than the currently released moderately susceptible variety, Genesis™090, were identified. The SARDI terraces and Horsham field assays were highly correlated (r=0.7). GWAS conducted in GAPIT identified significant marker-trait associations for AB resistance in the field and in the SARDI terraces. Genomic regions on chromosomes 2, 4 and 5 were common between the two phenotyping platforms. Sequence capture of the significant regions has identified candidate genes potentially linked to AB resistance.

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Pan-genomic variation of *Pisum* immune receptors enabled identification of novel downy mildew resistances

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Downy mildew in pea, caused by the oomycete *Peronospora viciae* f. sp. *pisi* (*Pvp*), is a significant disease of peas and has been reported to cause annual yield loss of upto 30%[1]. Beyond the immediate impact, the pathogen's oospores can persist in the soil for up to 8 years, thus limiting available land for pea cultivation. This issue is further exacerbated by the diverse distribution of pathogen races and recent restrictions on seed treatments. To identify new sources of resistance, we screened 230 lines from the pea diversity panel with two recently emerged *Pvp* races reported at multiple sites in the UK. Phenotype screening revealed that 20% of the lines exhibited high resistance to both the races. To identify the molecular basis of this resistance, we sequenced and assembled a repertoire of disease resistance genes from the diversity panel using R gene enrichment sequencing[2], and their pan-genome variation was exploited using association genetics. We discovered a novel locus on chromosome 5 and a previously reported locus on chromosome 2. For the chromosome 2 locus, we implemented *k*-mers based haplotype analysis that reduced the mapping interval to a 300 kb region harbouring three R-genes. Using RNA sequencing of the host tisuse 48 hours post-inoculation with the pathogen, we found that one of the R-gene doesn't have any expression, while the other two R-genes have significantly different expression and sequence structure between resistant and susceptible lines. Future work will involve validation of these candidates using gene silencing and editing approaches.

References:

[1] https://www.fwi.co.uk/arable/crop-management/disease-management/downy-mildew-warning-for-pulse-crop-growers

*[2] Arora S. et al, Resistance gene cloning from a wild crop relative by sequence capture and association genetics, Nat Biotechnol, **37**(2): 139-143, 2019.*

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Identification of a QTL-hotspot region for resistance to Ascochyta blight in lentil

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Lentil, like all pulses, is an important crop that will help address global food security and environmental sustainability worldwide. However, pulses suffer from the devastating fungal disease known as ascochyta blight (AB). In lentil, *Ascochyta lentis* is responsible for AB which can infect all above-ground parts of the plant, reducing grain quality and yield, and resulting in yield loss of up to 40% under severe infection [1].
Three lentil recombinant inbred line (RIL) populations were generated to better understand the genetics of AB resistance. Resistant ILL7537 and Indianhead were independently crossed with highly susceptible ILL6002, and the Australian cultivars Nipper and PBA Bolt with each other. The resulting RIL populations were genotyped and phenotyped with Pathotype 1 and 2 isolates of *A. lentis*, and the results underwent QTL analysis. All three populations displayed significant QTLs at the beginning of chromosome 2, marking a hotspot for resistance-associated elements. This was the only major QTL within the ILL7537 x ILL6002 population, effective against both pathotypes and distinct from the Indianhead x ILL6002 population. As such, heterogeneous inbred families (HIFs) were generated to fine-map the location of the resistance gene. In addition to the major loci present on chromosome 2 in both Indianhead x ILL6002 and Nipper x PBA Bolt populations, which corroborates previous studies [2], QTL analysis identified multiple novel pathotype-specific loci in both populations. The markers associated with this resistance can be implemented in lentil breeding programs, allowing breeders to fast-track integration and stacking of resistance sources in future lentil varieties.

References:

[1] Gossen BD, Morrall RAA (1983) Effect of ascochyta blight on seed yield and quality of lentils. Canadian Journal of Plant Pathology 5, 168-173.
[2] Sudheesh S, Rodda MS, Davidson J, Javid M, Stephens A, Slater AT, Cogan NOI, Forster JW, Kaur S (2016) SNP-Based Linkage Mapping for Validation of QTLs for Resistance to Ascochyta Blight in Lentil. Frontiers in Plant Science 7.

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Exploring host – pathogen co-evolution rate in natural and agricultural ecosystems: A case study from *Cicer* spp. – *Ascochyta rabiei* pathosystem

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Ascochyta rabiei causal agent of the Ascochyta blight disease of *Cicer arietinum* limits chickpea cultivation worldwide. Southeastern Anatolia region of Türkiye takes place within the Fertile Crescent where chickpeas were first cultivated in human history and that it is considered to be the origin center of both the host and the pathogen (Abbo et al., 2003). Field studies conducted by our group for the last 10 years, *D. rabiei* isolates were obtained from annual (*C. reticulatum, C. pinnatifidum, C. bijugum, C. turcicum*), perennial (*C. anatolicum*, *C. isauricum*) and cultivated *Cicer* species (Nalçacı et al., 2021; Talapov et al., 2023). Structure of *A. rabiei* populations from the wild *Cicer* species revealed by mating type and pathogenicity exhibit ecotype difference when compared to that of *C. arietinum*, indicating that host adaptation of the wild *A. rabiei* population is driven differentially since there is no selection pressure in natural ecosystems. Consequently, the co-evolution rate of the wild and cultivated pathosystems differs from each other. Comparison of wild and cultivated pathosystems is important to reveal *A. rabiei* - *Cicer* spp. co-evolution rate and to predict the evolution of the agent under different ecological conditions. Comparative analysis of *A. rabiei* genomes obtained from perennial and annual *Cicer* spp., definition of population structures, and presentation of effector gene repertoire will contribute to its development as a model system to study. Understanding the mechanism by which wild species tolerate *A. rabiei* will enable the development of innovative methods to combat the pathogen in agricultural systems.

Abbo et al., 'Evolution of cultivated chickpea: four bottlenecks limit diversity and constrain adaptation', Functional Plant Biology,Vol. 30, no. 10, 2003, p. 1081.
Nalçacı et al., 'Epiphytotics of chickpea Ascochyta blight in Turkey as influenced by climatic factors'.Journal of Plant Diseases and Protection, Vol. 128, 2021, p.1121
Talapov et al., 'First Report of Ascochyta rabiei Infections on Endemic Turkish Populations of Cicer bijugum and C. turcicum'.Plant Disease, Vol.107, no. 12, 2023, p.4020.

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RNA-seq analysis of the molecular changes underlying defense responses in chickpea to infection by *Phytophthora medicaginis*

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In this study, we generated and analysed an RNA-seq dataset from biological replicate samples of chickpea roots, including treatments infected with phytophthora root rot (PRR), and without infection [1]. Chickpea genotypes were chosen representing sensitive and moderately-resistant cultivated chickpea, and a resistant genotype containing a wild *Cicer*-derived source of PRR resistance that is extensively utilised in the Australian chickpea breeding program. The genetic determinants underlying this critical source of resistance remain unknown, but our selected genotypes have previously been used for genetic studies to identify regions associated with PRR resistance.

The data were explored for potential mechanisms and gene candidates contributing to PRR resistance. We found limited evidence to support involvement of annotated R-genes. Different gene expression responses were observed between the wild *Cicer*-derived resistance source and the cultivated source, and supported earlier hypotheses that resistance derived from wild *Cicer* involves an ability to maintain primary root elongation and minimise initiation of lateral or adventitious roots in the presence of the pathogen [2]. We interrogated defined sets of differentially expressed genes for resistance gene candidates, by comparing their genomic locations with previously reported genetic regions. A number of candidates of interest were identified, providing a foundation for future research aimed at resolving the genetic basis of chickpea resistance to PRR.

References:

[1] Amalraj A. et al, 'Using RNA sequencing to unravel molecular changes underlying the defense response in chickpea induced by Phytophthora medicaginis', Physiol. Plant., vol. 176, no. 4, 2024, e14412.
[2] Dron N. et al, 'Phenotyping for waterlogging tolerance as a proxy for Phytophthora medicaginis resistance in chickpea', Plant Health Progress, vol. 22, no. 3, 2021, p. 287.

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Legume nutrition and quality in the context of climate change

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Embracing environmentally sustainable diets necessitates a shift towards plant-based food sources, especially in regions like the global north. However, various social, economic, and cultural obstacles impede progress towards more sustainable dietary habits. Despite being nutrient-rich, legume grains face certain nutrition-related challenges. This presentation explores three such challenges and stimulates contemplation:
1. While legumes are hailed as highly sustainable sources of plant-based protein, the prevailing protein-centric mindset in the global north markets (and beyond) warrants critical examination. Legumes offer more than just protein; their broader nutritional value merits attention.
2. Quality means fitness for the purpose. Despite being rich in phytochemicals, sometimes viewed negatively as anti-nutrients, legumes may confer health benefits by influencing the gut microbiome and possessing anti-inflammatory and antioxidant properties. Should we consider rebranding them to convey a more positive message?
3. The earth's climate change poses a fundamental threat to humanity, and the rise of atmospheric CO_2 is a main driver for most of these changes. Legumes have been with us for millennia. But has the nutritional value of legumes changed due to climate change, and will legumes be as nutritious in the future as they once were?

References:

[1] Duarte, RDC et al (2024) Frontiers in Plant Science 15, 1337653
[2] Deuchande et al (2024) Plant Soil 496:139–160

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VC2 regulates baseline vicine content in faba bean

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Faba bean (*Vicia faba*) is one of the highest-yielding cool-season legume crops and provides a valuable source of vegetable protein for human consumption in temperate climatic regions. However, its use for human nutrition is limited by the seed vicine and convicine (v-c) contents, which can induce favism in individuals with glucose-6-phosphate dehydrogenase deficiency. A bifunctional riboflavin gene, *VC1*, was identified as a major biosynthetic pathway gene. Although a 2 bp insertion in this gene results in a loss of function, this inactivation only partially eliminates v-c biosynthesis, thus indicating the involvement of other genes.

Here, we demonstrate that a novel *V. faba* riboflavin gene, *VC2*, is responsible for the residual v-c contents in faba bean. *VC2* shares nearly identical functional domains with *VC1* and has GTP cyclohydrolase II activity, catalyzing the conversion of GTP into an intermediate molecule in the biosynthetic pathway. Gene expression analysis revealed that *VC2* has a minor effect, accounting for approximately 5-10% of total riboflavin gene transcripts which significantly correlates with the baseline contents in low v-c cultivars. Our results illustrate that genotypes carrying the 2 bp inactivating insertion in *VC1* still have residual v-c levels due to *VC2* activity. Furthermore, *VC1* exhibits multiple alleles and copy number variations, complicating molecular marker development. Conversely, single nucleotide polymorphisms within *VC2* provide a reliable alternative for marker-assisted selection in faba bean breeding.

In conclusion, our study elucidates the complex genetic regulation of v-c biosynthesis and provides valuable insights to facilitate its elimination in faba bean.

Theme: Metabolism and quality traits

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Genomic variation in diverse pea accessions uncovers the genetic basis of seed protein content

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To enhance the understanding of genetic determinants of seed protein concentration in pea, we developed a high-quality, chromosome-level genome assembly of CDC Amarillo using long-read sequencing technology. The assembly spans over 4.1 Gb with contig N_{50} size exceeding 17 Mbp. We utilized a Genome-wide association study (GWAS) panel of 233 diverse pea accessions to evaluate the phenotypic diversity of seed protein concentration. The panel was initially genotyped using an Axiom® 90K SNP array followed by whole genome resequencing at 5x coverage. Through association mapping based on seed protein concentration data from multi-site, multi-environment field trials, we identified several genetic loci and SNP markers linked to this trait. We will present detailed findings on the genome assembly, and the trait-associated genetic markers, illustrating their implications for molecular breeding for high seed protein concentration.

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Proteomic approaches for engineering the protein composition of lupin grain

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Narrow-leafed lupin (NLL, *Lupinus angustifolius*) is the principal pulse crop grown in Western Australia. Due to its unique adoption to nutrient-poor soils and nitrogen fixing ability, it serves as a key rotation crop for sustainable farming systems. Currently, NLL seeds are mainly used as animal feed, but there is a growing interest in them as a human food, owing to their excellent nutritive value determined by high protein and fibre content.

The previous proteomics evaluation of NLL genotypes revealed distinct differences in the protein composition of the domesticated NLL cultivars compared to wild accessions. It also detected lower levels of allergenic seed storage proteins in a subset of Australian NLL cultivars, which instead contained higher levels of bioactive proteins. The observed diversity of proteome provides opportunities to improve the protein composition of lupin grain as a human food [1].

In this study proteome measurements were implemented to examine the protein profiles of a larger number of domesticated cultivars, aiming to understand how they related to the available pedigree information of these varieties. Furthermore, these assays were employed to study the changes in the protein composition of lupin grain caused by the environment. These enabled the identification of the breeding line which introduced divergence in the protein profiles of Australian NLL cultivars and revealed environmental influence in the protein pattens of the studied genotypes. The knowledge established from this study generates prospects to enhance the protein quality of lupin grain and encourage its utilisation as a complementary plant-based protein source.

References:

[1] Tahmasian A. et al, 2022, Front Nutr, 9, p. 842168

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Unlocking the Potential of Grass Pea: Improving Nutritional Traits Through Investigation of Natural Diversity

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Grasspea is a hardy legume with a rich nutritional profile, including high levels of protein and diverse micronutrients. However, a diet heavily reliant on grasspea can result in neurolathyrism and irreversible paralysis of the lower limbs due to the presence of β-ODAP, a non-proteogenic amino acid. Recent advances in grasspea research, including the development of a reference genome and greater understanding of the genes responsible for β-ODAP synthesis[1] will allow further investigation of traits in grasspea and its improvement as a resilient and versatile crop.

Using GWAS to investigate traits in grasspea

During this PhD project, **β**-ODAP content has been tested in 344 lines obtained from a diverse collection of resequenced grass pea germplasm. This identified a number of landraces low in **β**-ODAP and is being investigated further by k-mer based GWAS, to identify loci involved in **β**-ODAP content. This will be used to investigate **β**-ODAP synthesis further and generate markers for breeding low **β**-ODAP lines.

Understanding the genetic basis of a low β-ODAP variety

Prior to this project, a population of recombinant inbred lines (RILs) was created by crossing two parents with a large difference in ***β***-ODAP concentration: the high ***β***-ODAP European line LS007 and the low ***β***-ODAP Indian line Mahateora. The seed ***β***-ODAP content of this population has also been measured, and this is now being used to identify the genetic basis of the low **β**-L-ODAP trait in Mahateora.

References:

[1] Edwards A. et al, Genomics and biochemical analyses reveal a metabolon key to β-L-ODAP biosynthesis in Lathyrus sativus, Nature communications, 2023.

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Molecular Basis of Grain Calcium content in Pigeonpea through Comparative Proteomics Analysis

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Pigeonpea (*Cajanus cajan* (Linn.) Millsp.) is an important legume crop known for its high nutritional significance. Calcium, a vital mineral element in plants and animals, plays an indispensable role in structure and signaling. Understanding the genetic variation and molecular mechanisms underlying the uptake, transport, accumulation of calcium in grains is of utmost importance. A study was conducted to elucidate the molecular mechanisms underlying the calcium accumulation and transport thoroughly, between low and high calcium lines of pigeonpea through comparative proteomic analysis. The results revealed membrane protein (C24H6.13 and C2G11.09), calcium transporting ATPase, sarcoplasmic reticulum histidine-rich calcium-binding protein and calcium-binding protein for calcium uptake, transport, storage, and regulation in grains. A significant differential abundance was noted in proteins associated with calcium-binding, transporters, calcium-dependent signaling pathways, and calcium storage organelles. These findings help in the identification of genes for high grain calcium content in pigeonpea opening an avenue for calcium biofortification in Pigeonpea.

Keywords: Pigeonpea, Grain calcium, Comparative Proteomics, Biofortification

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Exploring the Lupin Genome: Uncovering Lipoxygenase Genes to Enhance Flavor

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Narrow-leafed lupin (*Lupinus angustifolius*) has high protein is currently a good source of animal feed but has untapped potential as a human food. However, the utilisation of lupin in food products is impaired partly due to the undesirable beany flavour caused by lipid oxidation. Reduction of the beany flavour will help increase the overall consumer acceptance of lupin-based products to help make it a valuable protein source for human consumption. Beany flavour in legumes is mostly derived from the catalyzed enzymatic oxidation of linoleic acid and linolenic acid by lipoxygenases. Volatile organic compounds (VOCs) are often associated with the beany flavour. However, the nature of lipid oxidation associated with the beany flavour in lupin is largely unknown.

Biochemical analyses using GC-MS showed that there were qualitive and quantitative variations in fatty acids and VOCs among NLL domesticated and wild accessions. Twenty-one *lox* genes were identified from NLL genomic database. Transcriptomic analysis showed that the expression profiles of the *lox* genes varied among the diversity panel of NLL accessions and among various tissue types and during grain development. Some *lox* gene members were predominantly expressed in lupin grains. Mutations in one or more of LOX isoenzymes were identified in our in-house EMS mutagenic population. These mutants are currently being characterized with regards to changes in lipid content and VOCs. This research deepens our understanding of lipid oxidation in lupins and provides new materials and opportunities to accelerate lupin breeding for flavour improvement to make lupins more attractive in the food market.

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Progress in understanding the *Sclerotinia sclerotiorum* pathosystem.

Sclerotinia sclerotiorum, a predominately necrotrophic fungal pathogen with a broad host range, causes a significant yield-limiting disease of soybean called Sclerotinia stem rot. Resistance mechanisms against this pathogen in soybean are poorly understood, thus hindering the commercial deployment of resistant varieties. I will discuss the use of a multiomic approach utilizing RNA sequencing, gas chromatography–mass spectrometry-based metabolomics and chemical genomics in yeast to decipher the molecular mechanisms governing resistance to *S. sclerotiorum* in soybean. The combined results show that resistance to *S. sclerotiorum* in soybean is associated in part with an early accumulation of JA-Ile ((+)-7-iso-jasmonoyl-L-isoleucine), a bioactive jasmonate, increased ability to scavenge reactive oxygen species, and importantly, a reprogramming of the phenylpropanoid pathway leading to increased antifungal activities. Using chemical genomics in yeast, we further show that this antifungal activity targets ergosterol biosynthesis in the fungus, by disrupting enzymes involved in lipid and sterol biosynthesis. Overall, our results are consistent with a model where resistance to *S. sclerotiorum* in soybean coincides with an early recognition of the pathogen, leading to the modulation of the redox capacity of the host and the production of antifungal metabolites.

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Introgression of Disease Resistance into *Phaseolus vulgaris* variety OAC Rex from *Phaseolus acutifolius*

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The white navy bean variety OAC Rex was developed from an interspecific cross with a wild relative *Phaseolus acutifolius* (tepary bean). The interspecific cross was made to introduce resistance to common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. phaseoli and its fuscan variant. *X. fus*cans subsp. fuscans into cultivated beans. The complete genome sequence for OAC Rex was obtained by Illumina HiSeq sequencing to give 136x coverage and supplemented by long-read PacBio™ data, resulting in a pseudochromosome-level draft genome. The genome coverage of OAC Rex comprises approximately 97% of G19833 and it contains partial or complete representations for 96% of the CEGMA conserved data set [1]. A comparison of a contig-stage assembly for the *P. acutifolius* accession in the pedigree of OAC Rex, with the OAC Rex and G19833 genome sequences, showed that there are regions on every chromosome that are shared between the *P. acutifolius* and OAC Rex but are missing from G19833. The regions of introgression from *P. acutifolius* include self-incompatibility, disease resistance, regulation of disease resistance, and Niemann Pick-like sterol transporter genes. A comparison of the OAC Rex sequence with the G19833 reference genome, which is susceptible to CBB, identified differences in gene content and structure on chromosome 8 near a marker (SU91) associated with CBB resistance. This region includes an introgression from *P. acutifolius* containing a Niemann Pick sterol transporter gene with a unique structure shared with *P. acutifolius* that has features of executor genes described previously in *Xanthomonas* resistant rice and pepper.

References

[1] http://www.ncbi.nlm.nih.gov/sra/?term=OAC+Rex; Accession: PRJNA237957 ID: 237957

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Metabolomics and proteomics integration unveil how secondary cell wall thickening in peanuts helps in resisting aflatoxin accumulation

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Aflatoxin contamination, caused by *Aspergillus flavus*, significantly threatens food safety and human health. However, the molecular and biochemical pathways need to be better understood. Different approaches have been explored previously, including genetic mechanisms, cellular defense mechanisms such as secondary cell wall thickening, utilization of biocontrol agents, and the identification of resistance or susceptibility-associated genes. These studies have unveiled potential targets for precision breeding in peanuts to resist *Aspergillus* infection and subsequent aflatoxin contamination. However, due to the complexity of this trait, these studies have yet to fully elucidate the overall resistance mechanisms during *Aspergillus* infection in peanut. Our study adopted an integrative approach using metabolomics and proteomics using contrasting peanut genotypes-transgenic peanut expressing 4RNAi (resistant) and WT-ICGV 91114 (susceptible), to uncover the underlying resistance mechanisms by identifying regulatory events governing cellular processes such as enzyme activity, post-translational modifications, and gene expression during *Aspergillus* infection. Notably, changes in protein expression often precede alterations in metabolite levels, indicating regulatory events at the proteomic level. Integrating metabolomics and proteomics provided complementary insights into the metabolic and protein profiles associated with the production of phenylpropanoids, flavonoids, and fatty acids, shedding light on crucial pathways involved in inhibiting aflatoxin production. Furthermore, our study delves into host-pathogen interactions at the molecular level, elucidating how plants recognize fungal pathogens, activate defense responses, and adjust their metabolism to counteract aflatoxin production. This comprehensive and multidimensional analysis lays the groundwork for breeding peanut with reduced aflatoxin levels and implementing sustainable solutions to agriculture-related challenges.

Keywords: Aflatoxin, Fatty acids, Flavonoids, Metabolomics, Phenylpropanoids and Proteomics.

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