ICLGG 2024

Brisbane / Meanjin, Australia

11th International Conference on Legume Genetics and Genomics (ICLGG)

30 September – 3 October 2024

Brisbane Convention & Exhibition Centre

POSTER ABSTRACT BOOK

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P100

Use of DNA fingerprinting to trace varietal purity in Groundnut Seed Value Chain

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Climate change significantly impacts agriculture due to unpredictable and extreme weather events. Increasing climate vulnerability highlights the need for crop varieties that can withstand the resilience to climate shocks. This necessitates the need for maintaining genetic purity in crop varieties to ensure the improvements imparted by the breeders are delivered to the farmers to gain optimum productivity and resilience. This helps in building trust in seed systems, ensuring that farmers are planting high-quality seeds that are capable of adapting to climate change. Odisha, India, a region highly dependent on agriculture faced groundnut seed shortage due to systemic challenges in seed production and farmer's concerns about high rejection rates during the certification process, which significantly contributed to a sharp decline in the groundnut area by 51.7% and production by 30.5% of what it was in 1990. This study with 104 farmers across four districts is focused on key challenges they face and evaluates the efficacy of DNA fingerprinting in improving seed quality standards. The study indicated that only 37.5% of farmers ultimately sold seeds to the the seed company (Public sector/OSSC), while 58.6% opted to sell in the local market, and 3.9% directly to consumers as food due to low risk of rejection and higher market prices. Further, the DNA fingerprinting revealed, 46.0% of foundation seeds and 52.0% of certified seeds did not match the parent varieties. Thus, implementing incentives aligned with seed purity standards could reduce the diversion of seeds, maintaining the traceability of the supply chain and farmers' loyalty to the formal seed systems.

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Accurate Detection of Heterogeneity in Chickpeas Using a SNP Array

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Genomic characterisation of germplasm is critical for making informed decisions in research and breeding. This can range from helping breeders to accelerate the selection of plants with desirable traits, through to asessing genetic diversity and trait dissection. Factors to consider when selecting a genotyping assay include required marker density, turn-around time and budget. For example, while whole genome sequencing provides the most comprehensive information, it is usually only applied for specific research purposes due to the increased complexity of data analysis and higher cost. On the other hand, SNP arrays are robust and cost effective for obtaining a snapshot of the genome.

At Agriculture Victoria, a genotyping tool regularly used by GrainDataGen™ is the Illumina Multispecies Pulse 30K SNP array. To investigate its accuracy for detecting heterogeneity within samples, we genotyped DNA from independent seeds and the same seeds bulked together. High genotype calling accuracy demonstrated its utility for applications such as detecting heterogeneity in genebank accessions, seed purity testing and bulked segregant analysis for QTL detection. Interestingly when utilising the array to genotype hybrid populations derived from crosses between cultivated and wild chickpea species, we observed in some instances higher heterozygosity than expected levels for the familial generation. We hypothesise structural variations between pan-genomes might cause these anomalies.

Our work highlights the high genotype calling accuracy achieved with the Multispecies Pulse 30K SNP array and its suitability for a wide range of applications in research and breeding including the detection of heterogeneity and admixture in plant genetic resources.

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Digital Imaging for Grain Quality in Mungbean

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Grain quality is an important factor in the production of mungbeans in Australia as price is dependent on quality traits and premium international markets quality specifications. Current grain quality assessments are based on visually scored of clean grain samples. This process is very time consuming, requires specialised skills and training and could be subjective.

In order to obtain objective grain quality data, we investigated digital image analysis using the SeedCount [1] machine. This machine takes an image of the sample of grains evenly distributed in a well plate and analyses the image to retrieve data on traits such as size (length and width), area, area/width ratio and colour (LAB coordinates). The standard results of the SeedCount machine are averaged across all grains in the sample.

When the sample is not clean, this averaging of the sample also includes outliers and noise such as broken, mishappen and discoloured grain. However, individual grain data from each sample can be extracted from the SeedCount machine allowing us the opportunity to investigate the individual grain data, remove the outliers and average the sample excluding the noise.

We have successfully imaged samples from six replicated trials from the National Mungbean Improvement Program (NMIP). The aim of investigation is to use this image data to define a simple algorithm to "clean" the grain sample from each plot and then to define grain quality traits that can be used in linear mixed model analyses to enhance the selection for grain quality traits in earlier stages of the mungbean breeding program.

References:

2. *[1] https://www.nextinstruments.net/index.php/products/seedcount*

P103

Improving genetic transformation efficiency in legumes

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In the 1990s plant genetic transformation emerged as an important technological advancement in modern science: enabled novel insights into plant biology and initiated a new era in crop improvement [1]. Yet, for legume crops, efficient transformation and complete plant regeneration remain a challenge and limit the application of gene-editing technologies [2].

Here, we explore two methods to increase genetic modification efficiency in subterranean clover and mungbean: i) plant growth regulators during in vitro multiplication and rooting, and ii) the effect of light during Agrobacterium – explant co-cultivation. The in vitro regeneration protocols developed here are modifications of current protocols. We achieved up to a 100% regeneration rate, with rooting achieved in 8 out of 10 isolated shoots: all survived transfer to ex vitro conditions. We implemented these protocols during genetic transformation of both species using plasmids harbouring Hygromycin or Kanamycin resistance genes. During co-cultivation three light spectra (fluorescent, red-enriched, and blue-enriched) were tested, after that period explants were cultured under fluorescence and/ or red-enriched light. In both species, higher regeneration rates (30% to 44%) were achieved under fluorescence and red-enriched after 14 days on selection media compare to blue-enriched (20%). In clover, the transformation efficiency (number of independent rooted shoots after selection per one hundred treated explants) was up to 15% under red-enriched and 4% under fluorescence. Further experiments are underway to corroborate these results.

This project will provide a cost-effective, reliable protocol for boosting fundamental studies on gene function and facilitate crop improvement of species previously regarded as recalcitrant to genetic modification.

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2. *Nivya VM and Shah JM. Recalcitrance to transformation, a hindrance for genome editing of legumes. Front Genome Ed., 21;5:1247815, 2023.*

P104

Optimization of Tissue Culture and Genetic Transformation Protocol for Faba Bean (*Vicia faba* L.)

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Faba beans (*Vicia faba* L.) are a valuable and economically important leguminous crop that contributes significantly to global food security. Nevertheless, their productivity is impeded by various impediments, such as vulnerability to diseases, unfavourable environmental conditions, sluggish growth, and restricted genetic variability. Furthermore, the existence of self-incompatibility presents an additional obstacle in the process of breeding. In order to fully maximise the capabilities of faba beans, it is imperative to improve the methods of tissue culture and genetic transformation.

The focus of our study was to enhance the techniques of tissue culture and genetic transformation for faba beans. In order to accomplish this objective, we utilised embryos as explants in our tissue culture experiments involving 37 distinct faba bean cultivars. Furthermore, we have devised genetic transformation procedures for the Tiffany and Hedin/2 faba bean cultivars by introducing a GFP construct into their embryos using particle bombardment. During the tissue culture process, we observed that the cultivars had a regeneration efficiency of around 95%. However, the transformation efficiencies of Tiffany and Hedin/2 were 75.5% and 66.66%, respectively. Our investigation focused on improving the rates of regeneration and transformation by analysing various parameters, with particular emphasis on Tiffany and Hedin/2.

The aim of this project was to offer plant breeders and researchers standardised methodologies to assist in the future advancement of faba bean cultivation. This study emphasises the importance of using strong tissue culture and genetic transformation methods to fully utilise the sustainable food production potential of faba beans.

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[2] Lyu et al., 2021, Sci Rep, vol. 11, no. 21094.

P105

Development of a biotech toolbox for bean research

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Research on common bean genetics and molecular biology faces challenges due to its complex genome, high genetic diversity (within and between Mesoamerican and Andean gene pools), and phenotypic plasticity. Hindered by limited forward genetic methods, the use of low-efficiency protocols (biolistic transformation) and recalcitrance to in vitro regeneration, identifying key genes and traits remains difficult. To face these challenges and to provide the scientific community a structured platform to look for mutants in common bean we recently developed an EMS-mutagenized populations for an approach of TILLING (Targeting Induced Local Lesions In Genomes) in the Meccearly dwarf borlotto type variety (Blumen). This population consists of 2345 M1 lines and we are currently reproducing 1360 M2 plants derived from 340 M1 lines. A Whole Genome Sequencing approach will be applied to exploit this population and a preliminar set of samples is under analysis to evaluate mutation efficiency. In parallel, we are working on the improvement of in vitro protocols and the development of next-generation vectors based on those already tested in other species with low regenerative potential (such as wheat), which would increase the efficiency of transformation and regeneration of bean embryonic axes after biolistics. Altogether, these efforts could be crucial in the frame of global food security, for enhancing crop productivity, genetic diversity and nutritional quality.

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Improving chickpea productivity by enhancing resistance to soil acidity

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Acidic soils pose significant challenges to crop production globally, particularly due to the toxicities of Al^{3+} and H^{+}. Soils with a pH below 5.5 are deemed acidic and can hinder growth and development of certain crop species, decreasing productivity.

Chickpea is an important legume worldwide, but highly sensitive to acidic soils. Farmers can apply lime to increase topsoil pH, but this approach is expensive and often takes years to correct subsoil acidity. In Australia, chickpea cultivation is primarily centred in the northern grain-growing region with high pH soil (above 6.0). There is a growing interest in expanding chickpea cultivation to Southern New South Wales and Western Australia, particularly due to the scarcity of non-cereal break crop in these regions and the prevalence of acidic soils.

The genetic bottleneck in chickpea resulting from domestication impedes breeding efforts to combat abiotic and biotic stresses. Utilizing the genetic diversity in chickpea wild progenitors through genomics-assisted breeding offers a promising approach for developing acid-soil-resistant varieties. A genome-wide association studies panel comprising 350 wild *Cicer* lines will be assessed for H^{+} and Al^{3+} resistance on the bases of the length of the longest root, dry root and shoot weights. DNA samples from each line will be sequenced and mapped to the reference genome. Integration of high-confidence SNPs with phenotypic data will identify significant loci associated with Al^{3+} and H^{+} resistance. The putative candidate genes will be functionally characterised for marker development in the chickpea breeding program and to understand the mechanism of acid resistance.

~~[2] https://excellenceinbreeding.org/toolbox/services/common-bean-mid-density-genotyping-services/~~**P109**

Insights into the role of silicon in improving the nodulation-related traits in lentil plants during drought stress.

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5. *Drought severely impacts lentil (Lens culinaris Medik.) yield worldwide. Our previous studies demonstrated that silicon supplementation improves lentils' ability to withstand drought, focusing mainly on aboveground traits. Building on this, this study explores whether Silicon can also affect below-ground traits and enhance symbiotic nitrogen fixation (SNF) during drought (Biju et al. 2023a, 2023b).*
6. *A controlled-environment study was conducted using two lentil cultivars, which were subjected to moderate (40-45% field capacity-FC) and severe (20-25% FC) drought at the anthesis stage, with or without Silicon. Results indicated that Silicon significantly increased nodulation-related traits in drought-stressed lentils with increased nodules number and biomass, alongside higher content of protein, leghemoglobin, total nitrogen, antioxidants, flavonoids, amino acids, and carbohydrates. Silicon enhanced the activity of enzymes linked to carbon-nitrogen metabolism and the rate of photosynthesis with improved aboveground biomass and seed yield compared to untreated plants ($p \leq 0.001$).*
7. *Principal component analysis revealed a total variance of 86.56% (PC1=70.89%; PC2=15.66%) with three distinct clusters, signifying the impact of Silicon across the studied cultivars and drought levels. The clustering relies on the positive correlations noted among amino acids, antioxidants, and flavonoids in PC2 (r=0.83-0.85) and nodulation traits, nitrogenase, and rate of photosynthesis in PC1 (r=0.91-0.98). These findings align with our previous research, demonstrating that Silicon modulates the gene expressions related to antioxidant defence and carbon-nitrogen metabolism, thereby assisting lentils in drought recovery (Biju et al., 2023b). This modulation likely contributes to improved nodulation, suggesting that Silicon supplementation could serve as a viable option to enhance SNF in drought-stressed lentils.*

References:

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9. *[2] Biju, S. et al., Novel insights into the mechanism (s) of silicon-induced drought stress tolerance in lentil plants revealed by RNA sequencing analysis. BMC Plant Biol, vol. 23, no. 1, 2023b, 498.*

P110

Olive tree (*Olea europaea*) shading effects on different varieties of faba bean (*Vicia faba*).

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Agroforestry basis on olive tree (*Olea europaea*) is a common practice by small farmers in Morocco to face climatic change and economic uncertainty. Under such system, different studies (Amassaghrou et al. (2021); Temani et al. (2021) have shown positive effects of legume crops comparatively to others, like cereals for example, on the productivity of the whole system. This even there is some negative interactions mainly competition for water and nutrients in addition to sun light intercept by intercrops. To evaluate adaptability of different faba bean (*Vicia faba*) genotypes under reduced sunlight a pot experiment, under open field conditions has been conducted. Six faba bean varieties were exposed to full sun light and reduced sun light intercepted under an olive tree canopy. At flowering stage, above-ground, root, nodule biomass and the ratio of root biomass / above ground biomass were determined. Results revealed a significant effect of variety, and exposition and their interaction on most studied parameters. We noticed significant differences between the studied varieties. Sun light reduction showed an increase of approximately 30.05% in above-ground biomass, 28.03% in nodular biomass and 15.27% in root biomass. Aguadulce showed high performance in above-ground (38.66 g/plant), root (63.33 g/plant) and nodule biomass (77,33 g/plant) under the olive tree canopy compared with plants exposed to full sun. In contrast, Zina was the lowest tolerant of shade in terms of nodule biomass (8,67 g/plant) und above-ground biomass (22,63 g/plant). The results suggest that incorporating Aguadulce variety into agroforestry systems can bring significant added value to local farmers, since it is the most tolerant to shade stress, which can improve the performance of the tree-plant association in terms of yield and productivity.

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11. *[2] Fida Temani, Ahmed Bouaziz, Khalid Daoui, Jacques Wery, Karim Barkaoui 2021. Olive agroforestry can improve land productivity even under low water availability in the South Mediterranean. Agriculture, Ecosystems and Environment 307 (2021) 107234*

P111

Phosphorous efficiency levels of different moroccan chickpea (*Cicer arietinum)* genotypes under controled conditions

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Phosphorus (P) is the second most important macro-nutrient required by the plants, next to Nitrogen and is considered among the main abiotic factors limiting chickpea productivity in Morocco. This study assessed P-efficiency levels by three genotypes different Moroccan chickpea genotypes (Bochra, Arifi et Taounate) and interactive effects between P fertilizer (Triple Super Phosphate; Rock Phosphate) and doses (i.e., 14.3, 28.6 and 42.9 mg P kg^{-1} soil) under controlled conditions for 110 days. Results showed that Taounate genotype presents the best results among three genotypes in pod yield, root morphophysiological characteristics and P uptake while the best results in shoot biomass and the lowest root characteristics are obtained with Bochra genotype without significant differences between Arifi. The highest pod dry weight was recorded under RP fertilizer with all doses 14.3, 28.6 and 42.9 mg without significant difference between rates 14.3 and 28.6, while plants grown under unfertilized conditions recorded the lowest pod dry weight without significant difference to fertilizer TSP in rates 28.6 and 42.9 mg.
The highest root phosphatase acticity was observed for RP fertilizer 28.6 mg without significant difference to TSP rates 28.6 and 42.9 mg and RP 14.3 mg. The highest leaf P concentration and Shoot P content at 120 days was noted significantly under TSP 42.9 mg. However, the worst results were noted under TSP 80 kg/ha for both leaf P concentration and shoot content without significant difference under all RP doses. In conclusion, P fertilizer increase the P uptake from the soil for the chickpea.

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Detection of genetic variation for heat and drought stress response in faba bean (*Vicia faba*)

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In 2022, faba beans were cultivated on about 71,000 hectares in Germany, being the second most legume crop after peas. Cultivation these beans enriches soil with nitrogen, diversifies crop rotations and bolsters domestic protein supply. Despite their benefits, faba beans are sensitive to heat and drought, especially during flowering. The anticipated increase in dry and hot growing seasons due to climate change highlights the necessity of developing adapted genotypes with effective tolerance strategies.

Here we present findings from phenotyping 80 genotypes for drought and heat tolerance. For heat stress, the genotypes were grown in two climate chambers with different temperatures (20°C vs. 28°C) during flowering. Preliminary results indicate that heat stress reduced seed yield of all tested lines but increased pod number of about 20 lines. In parallel, the same genotypes were cultivated in plantarray, a modern phenotyping platform designed for precise tracking of transpiration under tightly controlled conditions. Recording of transpiration profiles of the genotypes allowed the determination of their water uptake and transpiration efficiency. This parallel execution of experiments should disentangle heat and drought stress responses. Furthermore, we are evaluating the performance of these 80 genotypes in field trials spanning four locations. Employing diverse methodologies, we identified the genotypes demonstrating superior performance in the face of abiotic stress and selected specific genotypes for advanced screening. Currently, we are conducting a thorough evaluation of 300 RILs (F5 Population), utilizing a combination of methodologies and approaches to enhance our understanding. Afterwards, we'll map genetic regions to boost faba bean resilience.

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P113

Characterization of Kunitz Trypsin inhibitors in some Apulian accessions of Cicer arietinum L.: enhancing the value of local legumes.

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Legumes are rich in protease inhibitors (PIs) which are essential for various plant regulatory processes and are also active against pathogens and parasites. Collections carried out in the Apulia region during rural development programs yielded several local and still poorly explored landraces of chickpea (*Cicer arietinum* L.). This germplasm holds potential to improve the sustainability of regional modern farming systems. Chickpea, like other legumes, still requires comprehensive genomic annotation and characterization despite recent advances in genome sequencing. Our aim was to identify functional SNPs in genes encoding PIs and assess their behaviour by analysing their expression levels under conditions of water deficit. Comparative gene sequence analyses across eight landraces revealed lower polymorphic variations among the accessions. The Kunitz trypsin inhibitors, encoded by the CaTPI-1 and CAKTI genes, were analyzed at molecular level in more detail. The former is predominantly transcribed in vegetative organs and is influenced by light and growth stage, whereas CAKTI is known for an anti-metabolic effect on the feeding larvae of the pod borer, *Helicoverpa armigera*. Additionally, we analyzed the α-amylase inhibitor gene CASIL, active in pest control and in the prevention of certain human diseases. In summary, differential expression of the genes under study were observed across the local varieties subjected to water stress, in comparison to unstressed plants. Additionally, an unequivocal fingerprint of each landrace was obtained by the use of microsatellite markers. In conclusion, *C. arietinum* shows several defence genes actively involved in plant protection that appear suitable for further exploitation through breeding programs.

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Exploring the Effects of Soil Acidity on Root Nodulation in Chickpeas

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Chickpea (*Cicer arietinum*) is a major legume crop. The optimum soil pH range for chickpea growth is between 6.0 – 8.0. Soil acidity (pH<5.5) negatively impacts plant growth and causes nutrient deficiencies and toxicities. Previous studies hypothesize that, in legumes, soil acidity may disrupt the chemical signaling between host plants and rhizobia and reduce rhizobial colonisation, leading to reduced nodulation[1]. However, such studies are lacking in chickpeas. This project aims to evaluate the nodulation and nitrogen fixation of various cultivated chickpea varieties under acid soil conditions. Furthermore, it will identify the nodulation stages that are affected by soil acidity and the factors contributing to reduced nodulation. Around 75 cultivated chickpea varieties will be grown in river sand in glasshouses under two different pH ranges (4.5 - 5.0 and 6.0 - 6.5). Initially, a general screening will be performed in this setup to identify varieties that are nodulating better under acid soil. Two varieties with contrasting nodulation ability under low pH conditions would be used to perform detailed nodulation time-course experiments to identify the nodulation stages that are affected by soil acidity and to observe the changes in nodule anatomy, flavonoid production, and *nod* gene induction in rhizobial symbiont, under acid soil conditions. Overall, this study will identify the chickpea cultivars best suited to low pH conditions and provide breeders with options for improving chickpea nodulation and yields on acid soils.

References:

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P117

Functional gene analysis of priori acid soil tolerance genes in chickpea using hairy root transformation

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The limited genetic variation for key traits, important to the Australian chickpea industry such as tolerance to soil acidity poses a significant barrier to further genetic improvement and expansion of chickpeas to new production areas. Genetic transformation and gene editing methods offer the potential to improve the traits of interest in chickpeas. The stable genetic transformation of chickpeas is time-consuming and inefficient. To investigate the function of the priori genes involved in aluminium, and low pH tolerance, we used a hairy root transformation mediated by Agrobacterium rhizogenes K599 strain. The RUBY and BASTA herbicide resistance genes under the control of CaMV 35S promoter were used as selectable markers. Several transgenic lines expressing RUBY that convert tyrosine to vividly red betalain were developed. Further work is in progress to check the transcript levels of priori genes in the transgenic chickpea hairy roots.

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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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Evaluating Water Use Efficiency and Shoot-root Traits in Australian Mungbean Cultivars under Different Levels of Water Availability

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This study investigates the impact of varying water levels on the water use efficiency (WUE) of six Australian commercial mungbean cultivars (Jade-AU, Crystal, Berken, Opal-AU, Green Dragon, and King). The cultivars were subjected to three levels of water availability (75%, 50%, and 25% of field capacity) within a glasshouse setting, involving large 550mm x 150mm lysimeters filled with 8.5 kg air dried Grey Verosol soil. The water treatments commenced at 31 days after sowing (DAS) and maintained until the harvest. Key measurements included plant shoot traits like height, leaf area, shoot biomass, seed weight, water use, stomatal conductance, and root characterstics such as tap root length, diameter, root biomass, and root system architecture (number of root tips and forks). The study also measured the harvest index and water use and calculated the WUE for both seed yield (WUE_{seed}; ratio of seed weight to total water used) and shoot biomass (WUE_{shoot}; ratio of total shoot biomass to total water used). Results showed a reduction in the key traits and WUE at the lower water levels (50% and 25%) compared to the 75% water level for all six cultivars. However, the reduction in WUE varied among cultivars. Berken and Crystal showed a smaller decrease in WUE under reduced water availability (25% and 50%), while Opal-AU had the largest reduction. This study also identified correlations between WUE and the other measured traits, indicating the opportunities to improve WUE through selection of these traits.

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Deciphering the Faba Bean Genome: High-Quality Assemblies and Resolution of Haplotypes

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Faba bean (*Vicia faba)* is an agriculturally important crop plant with a large diploid genome. Its haploid size is estimated to comprise 13 billion base pairs (2n=12), with one chromosome larger than the entire human genome. Faba bean has also one of the highest percentage of repeats among crop plant genomes.
While assembling genomes representing completely homozygous lines is conceptually most straight forward, in real world breeding material a range of different levels of heterozygosity can be observed. This feature can make faba bean genomes more challenging to assemble, with haplotype resolved assemblies requiring further validation.
In our faba bean pangenome project we used Pac-Bio HiFi sequencing (30X) and assembled genomes from several diverse faba bean lines representing breeding material and spanning a range of heterozygosity.
By assessing heterozygosity and repeat percentage, we examine the challenge of haplotig purging in this large genome, ensuring the accurate separation of haplotypes in our assemblies with different approaches. We are optimizing our designed pipeline by testing it on a single individual serving as an example for hybrid synthetic variety fababean genome (Vertigo) to reveal the quality of haplotype resolution without the need for additional sequencing data.
Through this work, we anticipate achieving high-quality fababean genome assemblies and enhancing haplotype resolution in large repetitive diploid plant genomes.

P124

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 50 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.

P125

Evolution, development, and application of the DArTag common bean mid-density platform in a breeding program to develop rapid cooking beans in Africa

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The common bean mid-density single nucleotide polymorphism (SNP) panel was developed from whole genome re-sequencing (WGS) and genotyping by sequencing (GBS) of more than 1,700 breeding lines and landraces from Africa and America. These lines were characterised for various biotic and abiotic stresses, agronomic and commercial qualities, and inter-specific introgressions. A matrix of more than 40 million SNPs was generated making it suitable for drought and heat tolerance, pest and disease resistance, cooking time, and diversity studies. This matrix was later filtered using biological and technical replicates to remove defective markers resulting in a DArTag SNP array of 1,862 markers in the mid-density SNP genotyping panel (MDSG)~~(https://excellenceinbreeding.org/toolbox/services/common-bean-mid-density-genotyping-services)1,2~~.

Genomic selection with MDSG for rapid cooking biofortified common beans is underway 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. A genomic relationship matrix based on MDSG markers has been used to develop accurate genomic breeding values for cooking time~~, soaking time~~, seed iron and zinc, grain yield, and other traits[2~~3~~]. These accurate genomic breeding values have been incorporated into an optimised selection index for optimal contributions selection to optimise crossing designs, and results show significant genetic gains in these traits (see Saradadevi et al, abstract at this conference). Thus, MDSG is helping to overcome the biggest barrier to common bean consumption in Africa - the long soaking and cooking times of beans.

References:

[1] Ariza-Suarez, D., et al. (2023). Genetic analysis of resistance to bean leaf crumple virus identifies a candidate LRR-RLK gene. Plant J, 114: 23-38. https://doi.org/10.1111/tpj.15810

~~[2] https://excellenceinbreeding.org/toolbox/services/common-bean-mid-density-genotyping-services/~~

[~~1~~2~~3~~] 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.

P126

Characterization of seed storage protein diversity in Faba bean (Vicia faba)

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Faba bean (Vicia faba L., 2n = 12) is a cool-season legume with high average seed protein content and yield potential for temperate regions. It has an average seed protein content of 30% which is the highest among other cool-season legumes, but less than it's warm-season counterpart, soybean. Improving the seed storage protein quality and content is an important target for advancing faba bean as a sustainable source of plant protein. The most abundant class of seed storage proteins in faba bean are globulins, which are composed of vicilin/convicilin and legumin. The faba bean reference genome is annotated for 17 vicilin, 2 convicilin, and 18 legumin encoding genes, although previous storage protein characterization suggests that more are present. To begin characterizing the seed storage protein diversity in faba bean, PacBio Iso-Seq transcriptomes from seeds sampled at 25 DAP were generated for a diversity panel of ~200 accessions. Individuals from the same panel has also been evaluated for chemical amino acid composition and protein content and a seed proteome will be generated using LC/MS-MS. This comprehesive profile of seed protein diversity and content will be used in the indentification of markers for improved seed storage protein profiles in faba bean.

References:

17. *[1] Jayakodi, M. et al. "The Giant Diploid Faba Genome Unlocks Variation in a Global Protein Crop." Nature 615, no. 7953 (March 2023): 652–59*
18. *[2] Skovbjerg, C. et al. "Genetic Analysis of Global Faba Bean Diversity, Agronomic Traits and Selection Signatures." TAG. 136, no. 5 (April 19, 2023): 114.*

P127

MADis: Genomic Analysis Tool for the Revelation of Multiple Alleles Within a Single Gene

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Genome-to-phenome research in agriculture aims to improve and accelerate crop breeding. Genome-wide association study (GWAS) has been widely used to identify genomic loci underlying important traits. Numerous post-GWAS analyses were developed to narrow down the associated genomic regions. However, in many cases, they are still unable to identify candidate genes or causative mutations (CMs). Natural and artificial selection alter allele frequencies in genes controlling phenotypes. This raises the likelihood that numerous alleles with independent CMs would be present in a single gene, which poses an issue for GWAS analyses.

As of yet, there has been no association method addressing the issue of multiple alleles. Therefore, we developed a tool that computes a score for a combination of variant positions in a single candidate gene and based on the highest score identifies the best number and combination of CMs. The tool is available as a Python package on GitHub and as a web-based Multiple Alleles discovery (MADis) tool specifically designed for soybeans [1].

We demonstrated the utilization of MADis on an example of a recently cloned gene *L1* [2]. We successfully identified a candidate gene for the soybean pod color *L2* locus and predicted the existence of multiple alleles that potentially cause loss of pod pigmentation in soybeans. In this work, we demonstrated the use of genomic analysis in the exploration of the natural and artificial selection of multiple alleles. The MADis tool can be applied to other species to aid in the discovery of genes under selection for improved breeding.

References:

21. *[1] Biová et al., 2024. Front. Genet. 14:1320652.*
22. *[2] Lyu et al., 2023. Mol. Plant 16(7), 1178-1191.*

P128

Unlocking lablab potential: New genomic resources to accelerate breeding.

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Under-utilised orphan crops hold the key to diversified and climate-resilient food systems. However, lack of genomic and advanced breeding resources has made their improvement extremely slow leaving their potential largely untapped.

Here, we report on orphan crop genomics using the case of *Lablab purpureus* (lablab), a legume native to Africa and cultivated throughout the tropics for food and forage. An Africa-led plant genome collaboration produced a high-quality chromosome-scale assembly of the lablab genome and re-sequenced cultivated and wild lablab accessions. The assembly highlights lablab domestication, genomic organisation of important anti-nutritional factors, genetic and phenotypic diversity and genomic loci underlying variation of important agronomic traits.

The generated genomic data provide a valuable resource for lablab improvement. Our inclusive collaborative approach also presents an example that can be explored by other researchers sequencing indigenous crops, particularly from low and middle-income countries (LMIC).

References:
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P129

Exploring Gene Family Diversity with PANSCOPE and SLAC: Bridging Pan-Genomics and Manual Curation with Alignment Thumbnails

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Growing numbers of published plant genomes are fuelling the potential discovery of valuable intra-species gene variants. However, published gene-based pan-genomic data may inherit annotation assumptions that reduce their utility to gene family experts. This is particularly impactful for gene families that still involve human-aided sequence curation for sensitive detection, such as those encoding short peptides. Exceptional gene transcription patterns, such as narrow permissive conditions and/or low total expression may further obscure these genes from study in pan-genome gene sets guided by transcriptomic evidence.

Our bioinformatics toolset, PANSCOPE, is a generalised multi-genome, gene-centric search pipeline that aims to help bridge the gap between current pan-genome analysis methods, and manual curation workflows familiar to molecular geneticists. It demonstrates how a gene-structure-aware BLAST-based search pipeline can be integrated with a novel system for hit visualisation and interactive exploration. Combined, this facilitates sensitive, efficient and user-friendly exploration of a gene family query set's sequence diversity across an arbitrary set of related genomes.

Of broader utility, we introduce Single Line Alignment with Context (SLAC) encoding. This system enables three aligned DNA sequences to be represented in a single or even thumbnail-scale text preview. The system is designed to communicate alignments of a hit sequence against a query gene's genomic and coding sequences and holds potential to improve rapid human readability of gene DNA sequence variants and hit patterns.

P132

Proteomics assessment of conglutin seed storage protein diversity across six lupin species

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The demand for novel plant-based protein sources is on the rise. Lupins, important members of legume family, are one of the richest natural sources of protein and fibre and can positively contribute to global food and nutritional security. Despite their potential, lupins remain under-utilised as human food and are predominantly grown as feed for livestock and aquaculture.

The major protein fractions in lupin grain, known as conglutins, are classified into four major families: α, β, γ and δ-conglutins. Each of these protein families possesses distinct nutritional and functional properties. For instance, γ-conglutins exhibit strong effects on lowering blood glucose levels and superior foaming and gelling properties. In contrast, β-conglutin proteins are known as the major lupin allergens. Thus, variations in conglutin protein levels across lupin genotypes can impact the quality and functionality of food products derived from these varieties [1].

In this study, mass-spectrometry based protein measurements were utilised to explore the diversity of seed storage protein across 24 lupin genotypes from six lupin species. This comparative study revealed substantial differences in the β- and γ-conglutin protein levels among the lupin species analysed. The knowledge obtained from this evaluation enables the identification of lupin varieties with higher desirable and lower levels of detrimental proteins, which can be used for commercial production or be exploited in breeding strategies for developing customised lupin varieties.

References:

23. [1] Cabello-Hurtado F. et al, 2016, J Proteomics, 143, p. 57.

P133

The change of tannin content in faba bean seeds during long-term storage

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Tannins are among the several antinutritional factors present in beans and are located mainly in the seed hull. The tannin content of dry beans ranges from 0.0 to 2.0 %, depending on the bean species and color of the seed hull. The tannis content of faba bean seeds can be rather variable between species but also inside the same variety. Tannins reduce the protein digestibility, but they also help ruminants to improve their digestion and reduce the amount of gas in the intestines. In order to find out whether the content of tannins change over time, we analyzed the seeds of one faba bean variety 'Jõgeva' from seven harvesting years (2023, 2022, 2021, 2020, 2019, 2015 and 2014). In all years the fertilizing, plant protection the field trials and also storage conditions of seeds were similar. Since tannins and protein content in seeds are closely related, we determined the protein contenttwice, in the harvest year, and in April 2024. Protein content in seeds did not cange over time. The color of the seed hull darkened more over the years. Long-term storage was accompnied by a significant decrease in the content of tannins.

P135

Breeding faba bean for sub-tropical region of Australia

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Faba bean (*Vicia faba* L.) is an important rotational crop in Australia providing $50-$100/ha indirect benefit to growers through break in disease cycle, change of weed spectrum and the nitrogen fixation. It is primarily gown as an export commodity to the Middle eastern countries, mainly Egypt, Saudi Arabia and United Arab Emirates. Its production in sub-tropical region is constrained by biotic stresses, such as rust (*Uromyces viciae-fabae* (Pers.) Schroet.), chocolate spot (*Botrytis fabae* Sard.) and certain viral diseases [1], but sufficient resistance has been developed in new varieties to combat these constraints. Likewise, breeding is also targeted for better seed quality, tolerance to frost and certain herbicides, such as imidazolinone (Imi) (Group B) and metribuzin (Group C). Faba bean is a favoured grain for protein fractionation, but the grain contains vicine and convicine (vc) which can cause favism to people lacking glucose-6-phosphate dehydrogenase enzyme in their body system [2]. This can cause acute haemolysis causing death in the absence of a proper medical care. Breeding has commenced on avoiding the risk of favism by developing low vc breeding lines. Molecular markers linked to Imi tolerance and low vc content are being used regularly for selection. Tanin present in the seedcoat gives bitterness while feeding to livestock including monogastrics. This can be reduced by developing white seeded varieties as white seed is linked to a creamy white flower colour. The current status of breeding with respect to the above traits will be discussed in the presentation.

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P136

Unveiling New Insights into Faba Bean Sensitivity and Genetic Responses to the Mutagen Agent EMS (Ethyl Methanesulfonate)

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Mutagenesis breeding via Ethyl Methanesulfonate (EMS) has been successfully used in faba bean to improve some economically important traits. However, there is a knowledge gap of the factors/mechanisms related to its sensitivity/tolerance to EMS treatment toxicity. It was hypothesized that seed size could influence the response of the diverse botanical varieties of faba bean. Consequently, we conducted a comprehensive assessment of the sensitivity of six faba bean varieties: three major varieties (Aguadulce Superlonga, Reina Mora, Yasmine) and three minor varieties (Zina, Alfia05, and Alfia17), to three increasing concentrations (0.05%, 0.5%, and 1%, along with a control 0%) of EMS. Analyses included various germination parameters (germination percentage (GP), germination energy at 7 and 14 days (GE7, GE14), germination rate index (GRI) and vigor index (VI)) across different EMS concentrations. To further explore mechanisms involved in sensitivity to EMS, we measured coat thickness and assessed antioxidant activity. Our findings revealed the variation in seed size did not affect significantly the sensitivity to EMS, while different varieties displayed significant differences in their responses to increasing EMS concentrations ($p<0.05$) across all parameters, except for root length. These findings challenge the prevailing assumption that seed size influences EMS sensitivity in faba bean, as hypothesized and suggested in existing literature. Coat thickness exhibited consistent uniformity, indicating similar EMS absorption patterns. Finally, antioxidant activity assessed through the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay revealed significant variations between non-treated and EMS treated groups, highlighting adaptations in antioxidant defense mechanisms. Correlations between antioxidant activity and germination parameters under EMS treatment were significant, which emphasize the role of antioxidants in tolerance to EMS. The study suggests that factors beyond seed size contribute to responses to EMS, and detailed examination of antioxidant systems can elucidate the plant's ability to counteract EMS-induced oxidative stress.

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The variability of beta-amyrin synthase gene *PsBAS* in field pea varieties

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26.

Saponins are bitter compounds found in peas and many other edible plants. Saponins are plant secondary metabolites that consist of a hydrophilic sugar chain and a lipophilic aglycone. There is a wide diversity of saponins (Timilsena et al., 2023). Peas contain two saponins, saponin B and DDMP, which both contribute to pea bitterness, but DDMP saponin is more bitter and more abundant. The concentration of saponins can vary more than two-fold between varieties. The biosynthesis of both pea saponins, DDMP and saponin B, depends on a single gene: *PsBAS* (Beta-amyrin synthase) (Vernaud et al., 2021). PsBAS catalyzes the production of DDMP, which breaks down into slightly less bitter saponin B.

PsBAS consists of 15 exons, and has a long intron of over 3000 bp between exons 14 and 15. We selected several varieties known to be either bitter or not bitter and sequenced the exons of the PsBas gene. Our analysis shows considerable variability in the DNA sequence, and some mutations also result in a change in the the amino acid sequence. The bitterness of the selected varieties can also be affected by compounds other than saponins and the effect of the mutations on the concentration of saponins remains to be studied.

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P139

Hyper-recombinant faba bean for accelerated breeding

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Meiotic recombination is a critical process in plant reproduction which enables the creation of novel combinations of natural genetic variation. In plant breeding the rate of recombination is a limiting factor in genetic gain, and is required for many reasons, including stacking of positive loci, purging of deleterious mutations and efficient introgression.

While efforts have been made to use biotechnology to increase the rate of recombination in models and some crops, little research has been done in legumes. Faba bean is an important legume crop, with growing interest due to its plant protein qualities and use in rotations and intercrops. However, faba bean improvement is hampered by its extremely large genome (2n=12, 13Gb), and limited genetic variation. Increased recombination could be used to accelerate the breeding of new varieties.

We aim to assess the potential benefits of mutations to increase recombination in breeding program. This will be achieved by simulation of breeding programs, comparing normal rates of recombination (according to genetic maps) with hypothetical elevated recombination rates, including 2x, 4x, 6x and 10x. Simulation of breeding programs will also include a range in multiple other parameters including phenotypic vs genomic selection, marker density, and heritability and locus number of a hypothetic trait.

Current preliminary data indicates that even a moderate (2x) increase in recombination results in a significant improvement to genetic gain in breeding programs of varied parameters.

This supports the need to investigate these genetic modifications in faba bean, by translation of Arabidopsis molecular work into this important crop.

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Maximizing the agronomic potential and adaptation of white lupin (*Lupinus albus* L.) to short growing seasons through genetic improvement

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Lupinus albus is a promising alternative to soybean due to the high concentrations of essential amino acids and proteins in its seeds. However, the existing varieties need genetic improvement to maximize their agronomic potential. One of the major breeding limitations is the long period from sowing to flowering and maturity. Our previous studies revealed the complex regulation of flowering in white lupin, which is dispersed among numerous QTLs localized on several chromosomes.

Previously, we observed that the early flowering trait in the reference *L. angustifolius* is determined by a 1.4-kb deletion in the promoter region of *FT* homologue. Interestingly, despite their close relationship, the regulatory mechanisms controlling early flowering in *L. albus* are different. This discovery adds an intriguing aspect to comparative studies and may have implications for future research in this area.

We analyzed the genetic diversity and population structure of white lupin and confirmed a significant correlation between the phenotype and the distribution of the lines in the formed subpopulations. However, we also observed that the intrapopulation phenotypic variability did not follow the allelic phases of key genes from the previously identified flowering regulatory pathways. It suggests the presence of additional flowering-inducing mechanisms. Despite the identification of novel genetic sources of early flowering, additional regulation within the subpopulations was observed. Our GWAS analysis revealed several markers, correlated with flowering time, located in the promoter regions of *LalbFT* genes and intergenic regions, such as the hypothetical miRNA clusters, which indicates that the additional regulatory mechanisms may rely on miRNA.

Funding:
This research was funded by the National Science Centre, Poland (SONATINA3, 2019/32/C/NZ9/00055 and SONATA17, 2021/43/D/NZ9/00293).

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P141

Optimizing cross efficiency and seed multiplication in faba bean via insect pollination and retrospective genotyping

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As one of the highest-yielding cool-season legumes, faba bean is a promising crop in temperate climates to help meet growing demand for plant protein. However, faba bean suffers from low yield stability due to poor heat and drought adaptation. Since stress adaptation traits generally have complex inheritance, breeding programs must recombine and select genotypes carrying as many as possible beneficial genome-wide quantitative trait loci from different donors. However, achieving effective recombination for complex traits requires large segregating families, which are difficult to generate in faba bean due to poor crossing efficiency and a low seed multiplication rate. To overcome these bottlenecks, we used bumblebees in enclosed greenhouse chambers for mass crossing among genetically diverse F1 plants. Seeding rates were around seven times higher than hand crossing, enabling generation of large populations of offspring. Subsequently, plants were genotyped using an Illumina 10k SNP chip, and the marker data from parents and offspring allowed us to retrospectively identify the pollen donor of each seed from each mother plant. This allows assignment of every genotyped seed to a family of either F2 siblings from a self-pollinated maternal plant, or to a specific four-way cross combination with known grandparents. Subsequently, all families are advanced to recombinant inbred lines (RIL) via speed-breeding. This procedure enables us to rapidly generate a very large and diverse base population of fully genotyped biparental and multi-parental families as a basis for quantitative genetic analyses and breeding to increase genetic gain in faba bean.

P146

Development of a diagnostic assay for the rapid detection of different ascochyta blight pathotypes in lentil

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Pulses offer a great opportunity in creating a sustainable global food supply as they are an excellent source of protein, with low carbon footprint and have better environmental impact. Pulses however are not immune to fungal diseases, of which Ascochyta blight (AB) has the worst impact on yield and quality in Australia. Our group has identified and characterised the first effector protein, AlAvr1, for an ascochyta pathogen. This avirulence effector in *Ascochyta lentis,* causing AB in lentil, mediates resistance in certain lentil cultivars. Currently there are two known forms of the effector; AlAvr1-1 that was described for the PBA Hurricane XT-avirulent isolates (Pathotype 1) and AlAvr1-2 characterised in the PBA Hurricane XT-virulent isolates (Pathotype 2). Discovery of this effector gene enabled us to design a PCR-based diagnostic assay to classify isolates into the two pathotypes and predict virulence towards PBA Hurricane XT, PBA Hallmark XT and PBA Bolt. Here, we aim to extend this work by developing a high through-put diagnostic tool for the simultaneous detection and pathotype identification of *A. lentis* isolates using a qPCR-based assay. This method offers fast, sensitive and reliable approach for effective and correct disease diagnosis with the intention of deploying this technology in the field. This tool will help growers with disease management decisions, including cultivar choice and rotation, which in turn reduces the risk of AB infection and its impact on grain quality and yield.

P142

Characterisation of the Chickpea Germplasm for Vigour-related traits

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Chickpea (*Cicer arietinum* L.) is a valuable protein source and is currently ranked as the second-largest produced pulse worldwide. Often, chickpea cultivation experiences a range of biotic and abiotic stresses and thus increasing pressure has been put on breeders to develop high-yielding varieties that are resistant/tolerant to stress and resilient to climate change. The development of crop varieties with improved vigour is a strategy that promotes strong early establishment to overcome weed competition and also improves plant growth and performance under unfavourable conditions. But plant vigour is a genetically complex trait.

This research aimed to perform a SNP-Chip-based genomic characterization of advanced chickpea breeding lines for the presence of early vigour alleles using the genotypic data (from pulse multispecies 30K SNP-Chip) and published literature [1,2]. Based on the known early vigour QTL (Quantitative trait loci) regions, positions of the QTL-linked markers on chromosomes Ca1, 3 and 4 associated with vigour-related traits were used.

Results showed that of the 16,931 advanced chickpea breeding lines genotyped through 30K SNP-Chip, 113 have early vigour alleles present for all three QTL regions. Also, unique SNPs to accessions were identified from these locations. In the close vicinity of SNPs on chromosomes Ca3 and 4, 140 and 39 genes respectively, are found that may be associated with plant vigour. The results obtained may provide a roadmap for further research to support chickpea breeding for increased vigour.

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P147

Understanding insect resistant strategies in *Cajanus scarabaeoides* for improvement of insect resistance in cultivated Pigeonpea

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Pigeonpea (*Cajanus cajan*) is a multipurposes legume that plays an important role in arid and semid-arid tropics. However, it is very suceptible to insect damages, especially *Helicoverpa armigera,* which causes devastating yield losses. Meanwhile, their wild relative *Cajanus scarabaeoides* shows high level of resistance to *H. armigera*. This research investigated the insect resistant strategies in *Cajanus scarabaeoides* using comparative TMT (Tandem Mass Tag) proteomic and transcriptomic analyses. The posibility of integration of these insect resistant strategies to cultivated pigeonpea was also studied. Results showed that *Cajanus scarabaeoides* employed both antibiosis and antixenosis mechanisms for its high resistance to *H. armigera*. In addition, these insect resistant strategies can be transferred from wild relative to cultivated pigeonpea through breeding approach.

P148

Differential transcriptomic profiling of white lupin response to *Colletotrichum lupini*, the causal agent of anthracnose

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White lupin (*Lupinus albus* L.) is a cool season grain legume, valued for its high seed protein content and beneficial influence on soil fertility through phosphorus mobilization and symbiotic nitrogen fixation. However, it is very susceptible to anthracnose, a devastating disease caused by the fungus *Colletotrichum lupini* (Bondar) Damm, P.F. Cannon & Crous 2012. Increased quantitative resistance has been reported in a few Ethiopian landraces [1] and in two German cultivars, Frieda [2] and Celina. As a hemibiotrophic pathogen, *C. lupini* propagates and kills the host tissue if not hampered at the biotrophic stage.

To identify the molecular mechanisms involved in white lupin resistance to anthracnose we focused on transcriptomic profiling of early stages of plant response to inoculation with *C. lupini*. The plant material tested in a growth chamber consisted of six lines, including the resistant Ethiopian landrace P27174 and cultivar Celina, two breeding lines selected for improved resistance, and two susceptible cultivars (Start and Amiga). The plants were spray-inoculated at 4-6 leaf stage with *ca*. 10^6 spores/ml suspension. Upper leaves were sampled simultaneously from inoculated and control plants (5 replicates) at 10 time points after inoculation, densely covering the time span from 1 to 48 hours, and additionally at 5 and 9 days post inoculation. Following RNA isolation and sequencing (paired-ends, 2x150 bp, ~60 mln reads), differential gene expression profiling with Gene Ontology enrichment and weighted gene co-expression network analysis were performed.

Funding: National Science Centre, Poland, project OPUS 23 (ID: 2022/45/B/NZ9/01397) and EU LiveSeeding (ID: 101059872)

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P149

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

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

P150

Metabolite profiling of chickpea (*Cicer arietinum*) in response to necrotrophic fungus *Ascochyta rabiei*

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Ascochyta blight (AB) caused by a necrotrophic fungus, *A. rabiei* is one of the most damaging diseases of chickpeas worldwide. Resistance to AB is a highly complex and quantitative trait. The limited number of resistance sources and the erosion of resistance in commercial cultivars have posed a challenge for breeders to develop cultivars with durable resistance to aggressive pathogen populations. Despite this little or no research has been directed toward physiological and biochemical mechanisms to develop complementary crop protection strategies for the sustainable and profitable chickpea industry. Our study aims to identify the metabolites associated with resistance/susceptibility in chickpea in response to *A. rabiei* infection. Here, we present metabolite profiling of two chickpea accessions comprising a moderately resistant genotype (CICA1841) and a highly susceptible cultivar (Kyabra) in response to one of the most aggressive Australian *A. rabiei* isolates TR9571. Non-targeted metabolomics analysis using liquid chromatography-mass spectrometry (LC-MS) revealed constitutive or differentially altered metabolites in aerial tissue (leaf and stem) of CICA1841 and Kyabra. The host-pathogen interaction resulted in the accumulation and suppression of various metabolites, revealing a possible reason for susceptibility against *A. rabiei* in the highly susceptible chickpea cultivar. Several differential metabolites are the precursors for secondary metabolic pathways, including flavonoid biosynthesis, phenylalanine pathway, Aminoacyl-tRNA biosynthesis, pentose and glucuronate interconversions, arginine biosynthesis, valine, leucine, and isoleucine biosynthesis, and alanine, aspartate, and glutamate metabolism. This study has provided insight into how a necrotrophic fungus manipulates the host during infection to cause disease.

P151

Differential expression of defense related enzymes governing resistance against Ascochyta blight (*Ascochyta rabiei*) in *kabuli* genotypes of chickpea (*Cicer arietinum* L.)

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Ascochyta blight caused by necrotrophic fungus, *Ascochyta rabiei*, owing to its highly variable nature and rapid evolution, pose a substantial threat to chickpea production worldwide. Under warm and humid environments, the productivity of chickpeas is severely compromised; sometimes upto 100 per cent yield losses. The disease becomes more challenging due to lack of resistant sources and frequent episodes of resistance breakdown. In this study, we evaluated 238 *kabuli* chickpea genotypes for resistance against local pathotype of Ascochyta blight over the growing seasons from 2019-2022 at PAU, Ludhiana under artificial epiphytotic conditions. The evaluation resulted in identification of eighteen resistant to moderately resistant (disease scores of 3.0- 4.0) lines (GLK10-40, GLK2054, GLK20055, FLIP10-298C-IFC-S2, FLIP10-298C-IFC-S1, CS-3-E-24, FLIP 09-256C-55, FLIP07-314C-57, FLIP10-243C, FLIP09-194C, FLIP08-104C, FLIP-04-219C, ICCV55233, ICCV55215, ICCV55135, ICCV55108 and ICCV155141) with diverse genetic backgrounds. The activities of defense-related enzymes, phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), polyphenol oxidase (PPO) and peroxidase (POD) linked to phenol metabolism, as well as non-enzymatic contents, lignin and total phenol content were compared among six *kabuli* chickpea lines, five resistant (GLK 10-40, GLK 20055, FLIP-09-194C, FLIP-04-219C, ICCV 55215) and one susceptible (GLK 17301) at tested time intervals of 48, 96, 144, and 240 hours post-inoculation, respectively. Activities of PAL, TAL, PPO and POD enzymes increased till 96 hrs after inoculation, whereas, lignin and total phenols (non-enzymatic contents), showed the maximum increase till 144 hrs after inoculation in resistant lines compared to susceptible line, further validating the biochemical basis of resistance in resistant to moderately resistant test lines.

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P152

Genetic analysis of anthrancnose disease resistance gene in narrow-leafed lupin

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Lupinus L. is a large and diverse genus in the legume family (Fabaceae). Like other members in the legume family, lupins can convert atmospheric nitrogen to a usable form through rhizobium root nodule symbiosis to improve soil quality. Therefore, narrow-leafed lupin (*Lupinus angustifolius* L. 2n=40) has been used as a rotation crop in Western Australian agricultural system.

Lupin anthracnose disease is a serious and the most damaging disease in lupin growing areas. This disease has potential to cause complete crop losses in susceptible lupin varieties.

We identified one major QTL on chromosome 11 for anthracnose disease resistance gene from a RIL population derived from the cross between Unicrop (susceptible) and Tanjil (resistant). This QTL can explain 81.5% of phenotypic variations. We narrowed down this QTL to a 12.5 kb region through fine-mapping of ~5000 F2 lines. There were three annotated gene in this region. And one disease-like gene *AnthTjR* was identified as a candidate gene.

cDNA sequence suggested that there were six exons in this gene region. Eight amino acid changes exisited between Tanjil and Unicrop. Re-sequence analysis of 21 lupin accessions showed that two AA changes were highly associated with the disease resistance. The molecular markers have been developed for marker-assisted selectioin in breeding programs.

Functional analysis of resistance genes is beijing performed by virus-induced silencing (VIGS) method.

P156

Faba bean biochemical quality as affected by ascochyta blight incidence on grain

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Ascochyta blight caused by *Ascochyta fabae* (teleomorph: *Didymella fabae*) is a fungal disease with worldwide distribution. It affects faba bean yield and grain quality. A study of its impact on some biochemical parameters related to the nutritional value of faba bean such as, crude protein (CP), total soluble phenolics (TSP), total tanins (TT), condensed tannins (CT), soluble carbohydrates (TSC), starch (AMI), amylose (AMYL) and amylopectin (AMYPEC) was conducted on 2 grain lots of Badii cultivar representing disease incidences 0% (Healthy) and 100% (diseased). Statistical analysis showed significant effects of the disease incidence on all studied parameters (except for amylose content and TSP). With varying incidence from 0% to 100%, an increase in CP from 23 % to 26% was observed. Total tannins showed an increase from 4.8 to 20 g/kg and condensed tannins showed a decrease from 5 to 2.6 with increasing incidences from 0 to 100%. The soluble carbohydrates content decreased from 3.3 g / kg dry matter for healthy grains to 1.7 g / kg dry matter for diseased ones. Starch content has increased from 25.3% in healthy grains to 35.6% in diseased grains. Amylose content increased also from 15% in healthy grains to 20.3% in diseased ones. As Badii faba bean cultivar is used for animal feed, these results would be of interest for animal nutritionists.

Keywords: Ascochyta blight, *Ascochyta fabae,* faba bean quality, biochemical parameters

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P158

Improving cowpea development and physiology for growth in phosphorus-deficient African soils

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Cowpea is a staple crop in tropical and subtropical regions of the world. However, yields for subsistence farmers in Africa are often poor due to low soil phosphorus (P). We tested soils from Nigerian savannah and found that the soils are extremely depleted of P in all forms. An immediate approach for yield improvement will therefore require affordable external P inputs and cowpea varieties with high Phosphorus Uptake Efficiency and Phosphorus Use Efficiency (PupE and PUE). In the longer term, soil nutrients must be managed (particularly P). Whilst Sokoto rock phosphate is cheap and accessible in Nigeria, the P therein is not bioavailable. To address this limitation, we are identifying cowpea cultivars that can mobilise fixed P and exploring the potential of P-solubilising rhizobacteria (PSR). We have grown 52 cowpea genotypes under low and high P conditions and have identified high PupE and PUE genotypes. We have also isolated rhizobacteria and are screening for PSRs able to solubilise Sokoto rock phosphate. As field-grown cowpeas are nodulated, we have isolated N_2 fixing *Bradyrhizobia.* These resources will be used to identify combinations of cowpea genotypes and PSRs that can grow well, and maintain effective rhizobia N_2 fixation, in low P soils amended with Sokoto rock phosphate. Currently, we are applying transcriptomic and metabolomic analysis to understand the molecular pathways responsible for cowpea variation in the PupE and PUE genotypes and, we will assess the root system architecture to identify genotypes with deep tap roots for uptake of groundwater.

P164

Unravelling Seed Coat Development to Maximize Lupin Grain Value

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Lupin grain has a thick seed coat. In narrow-leafed lupin (also known as Australian sweet lupin or *L. angustifolius*) the percentage of seed coat is around 24% by weight compared with only 7% in soybean. The high proportion of seed coat in lupin reduces the economic value of the seed as after dehulling the seed coat is typically sold into low value uses. Reducing the seed coat thickness could lead to an increase in protein content therefore add significant value of this high protein pulse crop as a product or ingredient in food.

In model plants, such as *Arabidopsis*, *Lotus japonicus* and *Medicago truncatula*, research has shown that seed development is an intricate process which involves a complex interplay between seed coat, embryo, and endosperm. Genes, transcriptional regulation, and pathways involved in the programming of the interconnection are emerging. However, little is known about the genetic mechanism and metabolomic pathways underpinning seed coat development in lupins.

Seed coat typically composes of cuticule, macrosclereid layer, osteosclereid layer and parenchymal cells structures. Using a combination of imaging technologies, we have identified some characteristics of lupin seed coat structures. Analyses of seed coat structures during lupin grain development suggest some temporal and spatial events could attribute to the unusual thickness of the lupin seed coat. We have identified underlying molecular frameworks which regulate lupin seed coat development. The ultimate aim of this research is to reduce the lupin seed coat thickness through next-gen breeding technologies and help to transform lupin from feed to food crop.

P165

A critical suppression feedback loop determines soybean photoperiod sensitivity

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Photoperiod sensitivity is crucial for soybean flowering, adaptation and yield. In soybean, photoperiod sensitivity centers around the Evening Complex (EC) that regulates the transcriptional level of the core transcription factor E1, thereby regulating flowering. However, little is known about the regulation of the activity of EC. Our study reveals how E2/GI and its homologs modulate photoperiod sensitivity through interactions with the EC. Under long days, E2 interacts with the blue-light receptor FKF1, leading to the degradation of J/ELF3, an EC component. EC also suppresses *E2* expression by binding to its promoter. This interplay forms a photoperiod regulatory loop, maintaining sensitivity to photoperiod. Disruption of this loop leads to losing sensitivity, affecting soybean's adaptability and yield. Understanding this loop's dynamics is vital for molecular breeding to reduce soybean's photoperiod sensitivity and develop cultivars with better adaptability and higher yields, potentially leading to the creation of photoperiod-insensitive varieties for broader agricultural applications.

P166

Dissecting the genetics of canopy dynamics in mungbean using longitudinal modelling of UAV-derived traits

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Mungbean is a high-value export pulse crop grown in subtropical farming systems globally. Their indeterminate growth behaviour is a major physiological constraint negatively impacting yield, as it results in the accumulation of vegetative and reproductive components simultaneously. Understanding canopy dynamics can provide new insight to increase yield potential by improving resource accumulation, remobilisation, and light interception. Additionally, understanding the genetics underpinning these complex traits would allow breeders to strategically target improved phenotypes that could enhance mungbean productivity. However, due to the current phenotyping bottleneck that exists, screening these traits particularly at a large-scale remains a challenge, thereby limiting our understanding of their genetics. In this study, a diverse nested association mapping (NAM) population was evaluated across three environments in QLD, Australia and imaged using a drone fitted with a multi-spectral camera flown regularly throughout development. Several vegetative indices and geometric traits were extracted from each flight and used to establish biomass prediction models. Spline curve fitting was used to integrate the predicted values from single flights into a continuous time course to calculate canopy dynamic traits such as canopy accumulation rate. A multi-environment trial analyses was undertaken to understand the Genotype x Environment interactions influencing these traits to generate overall best linear unbiased estimates (BLUEs). Haploblocks associated with these dynamic traits were identified and can be explored to develop cultivars with optimised canopy development patterns. The identification of these haploblocks establishes a catalogue of chromosome segments that can be leveraged for the enhancement of mungbean breeding and crop performance.

P167

Proliferative arrest in *Pisum sativum*

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Flowering plants strategically adjust their reproductive timing to enhance the success of their progeny. Monocarpic plants, which undergo a single reproductive cycle before senescence and death, precisely regulate the initiation and termination of flowering. The end of flowering, referred to as proliferative arrest (PA), involves the cessation of inflorescence meristem activity, an evolutionary adaptation aimed at optimizing resource allocation for seed production and progeny viability [1,2]. Although factors influencing proliferative arrest were identified in several monocarpic species decades ago, recent comprehensive studies in Arabidopsis have examined its regulation through physiology, hormone dynamics, and genetic factors [1,2]. However, these studies are currently limited to Arabidopsis, highlighting the need to extend research to other monocarpic species to propose universal mechanisms.

This study investigates proliferative arrest in *Pisum sativum*, using available Arabidopsis studies as a comparative framework. We quantitatively assessed the influence of fruits/seeds on proliferative arrest, examined the positional effects of fruits/seeds on inflorescence meristem behavior, and analyzed the transcriptomic changes in the meristem associated with its arrested state. Our results demonstrate a high conservation of the factors inducing proliferative arrest in both pea and Arabidopsis. Nonetheless, the observed differences emphasize the necessity for similar investigations in other species to fully elucidate the general mechanisms governing this process.

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P168

Genetic control of flower number in pea

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Legumes typically have compound inflorescences, in which flowers are carried on secondary inflorescences (I_2) that arise from axillary meristems on the primary inflorescence. The number of flowers per I_2 is variable and characteristic of each species, ranging from one to over 30. Cultivated pea (Pisum sativum) typically produces one or two flowers per I_2, although several ”multi-pod” mutants have been reported with up to six flowers [1]. Despite the potential agricultural and evolutionary significance of this trait, few studies have explored its environmental and genetic control, with only two loci [2] and several other variants described to date.

Here, we examined the effect of photoperiod and temperature on I_2 flower number, and re-examined its genetic control using a selection of single-, double- and multi-flowered accessions. We found that low temperatures and short photoperiods promote the multi-flower character, with differing sensitivity among accessions suggesting different regulatory pathways. QTL analysis in three F_2 populations derived from crosses between NGB5839 (double-flowered, recurrent parent), Garfield (single-flowered), and two multi-flowered lines. showed that the single-/double-flowered difference is under polygenic control, while the multi-flowered character is controlled by two regions on chromosomes four and six that likely correspond with the classical loci Fn and Fna loci respectively [2]. Additional variation suggests the existence of at least one other photoperiod-responsive locus that remains undefined. This research provides greater understanding of the factors controlling the number of flowers/I_2 in pea, and refines the genomic position of known loci.

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P169

Identification of quantitative trait loci for flowering time in chickpea

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Chickpea (*Cicer arietinum*) is the second most important grain legume, produced in more than 50 countries globally, in diverse environments and production systems. As in most crops, matching the growth cycle to location-specific climatic conditions helps minimise exposure to environmental stresses and maximise yield. Phenology is therefore a key target for adaptation when considering expansion to new production areas or resilience to climate change. Previous studies have reported major loci controlling flowering time but their relationships and functions are not well understood, and only one gene has so far been identified [1,2].

This study aimed to clarify the genetic control of flowering in chickpea and its interaction with growth habit, through QTL analysis. Three F_2 populations derived from crosses between a common late flowering line and three distinct early flowering lines were grown under controlled short-day (8-h) conditions and phenotyped for a range of phenology- and architecture-related traits including time and node of flowering, podding and termination of main shoot growth. Most traits varied extensively in the F_2 and were found to be controlled by 3-4 QTLs in each cross, explaining up to 60% of the trait variation. Our results confirmed previously reported loci on chromosomes 1, 5, 6 and 8, and identified several novel loci with large effects. Better understanding of the genetic and environmental control of flowering time will be important for future chickpea improvement.

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P170

A new class of leaf morphology mutants in pea

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Many legumes have pinnately compound leaves, and show an increase in the number and size of leaflets during development. In pea, the first true leaf features a single leaflet pair and a simple tendril, while later-formed leaves possess a increasing number of leaflet pairs and tendrils. While several of the genes controlling leaf identity in pea have been characterized, the genetic control of leaflet number is largely uncharacterized. It has been suggested that the change in leaflet number with development could represent a juvenility gradient [1] and might reflect regulation of the conserved *miR156/SPL* aging (heterochrony) pathway, but so far there is no genetic evidence to support this.

To investigate this question, we identified mutants that accelerate and or/extend the developmental transition in leaflet number mutants and characterised four distinct loci (*AERO1 [2]*, *AERO2*, *APC1* and *APC2*). Double and triple mutant combinations showed additive and increasingly pleiotropic effects, suggesting the existence of distinct regulatory pathways or potential redundancy. Each locus was mapped to a narrow genomic region and sequencing of candidate genes revealed putative causal mutations for all four mutants. Surprisingly, these genes have no known association with the aging/heterochrony pathway, and may instead regulate cell proliferation in meristems and leaf primordia. These findings introduce a novel class of pea mutants offering fresh insight into the regulation of vegetative development and diversity in leaf form.

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Exploring the genetics of seed dormancy and pod dehiscence in pea

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During domestication, loss of seed dormancy and dispersal mechanisms was critical to successful farming of many crop species, including pea. Wild *Pisum* species show strong pod shattering and physical seed dormancy, and seeds have a thick, rough coat (testa) that is impermeable to water. In comparison, in domesticated pea, seed coats are are smoother, thinner and more permeable, and pods do not shatter [1,2]. Little is known about the genetic basis of these differences or how they may relate to each other.

To address this, we investigated variability of these traits in a panel of diverse genotypes representing the two wild species (*P.elatius/humile* and *P.fulvum)* and the two independendently domesticated genepools (*P.sativum* and *P. abyssinicum*), and performed QTL analysis in two *humile* x *sativum* bi-parental populations. In one population, segregation of Mendel's *A* pigmentation locus had a strong effect on both testa thickness and dormancy, whereas minor loci affected these traits independently. In this population, seed coat roughness was independent of both testa thickness and dormancy [3]. In a second population where *A* was not segregating, dormancy appeared to be under polygenic control, with seven distinct QTL detected on different chromosomes. We also detected 6 QTL for pod dehiscence, of which one co-located with a dormancy locus. Overall these results identify new loci and clarify relationships between these key domestication traits.

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P172

Quantitative trait locus (QTL) mapping of phenology in lentil

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Lentil (*Lens culinaris*) is an important pulse crop grown in more than 40 countries worldwide. Current domesticated lentil germplasm is generally considered to form three broad adaptation groups: Mediterranean, South Asian, and northern temperate, corresponding to the main global production regions. Adaptation of lentil from its Mediterranean origins to these other diverse environments involved changes in phenology, reflecting selective pressures that resulted in differential sensitivity to photoperiod and temperature [1,2].

In this study we used a biparental approach to characterize the genetic basis for major differences in phenology across the global lentil germplasm. A total of 940 F_2 individuals derived from the cross between the extremely early accession ILL5888 and the photoperiod-sensitive northern temperate accession Indianhead were evaluated for a range of reproductive development and plant architecture traits across two different photoperiods (16h long day and 8h short day). QTL analysis identified three prominent loci: *DFD6a*, *DFD1a* and *NFD5b*. ILL5888 alleles conferred dominant (*DFD6a*) and recessive (*DFD1a*) early flowering under LD and a small but consistent early effect on flowering node in short days (*NFD5b*). Investigation of candidate genes using cross-species synteny analysis indicated the presence of a cluster of florigen (*FTb*) gene orthologs among candidates for *DFD6a.* Relevant candidates for all loci are being further evaluated in F_3 and F_4 progenies. Ongoing mapping analysis and marker-assisted selection utilizing these resources will expand our understanding of the underlying genetic control of flowering time adaptation in cultivated lentil and inform breeding for diverse environments.

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Genomic Prediction in Faba bean for Heat and Herbicide Tolerance

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Genomic selection (GS) offers significant potential to enhance genetic gain. The present study aimed to evaluate the accuracy and potential of GS in faba bean (*Vicia faba* L.), and to identify areas for further improvement and better implementation in practical breeding programs.

125 diverse faba bean accessions were phenotyped for different agronomic traits under herbicide and heat stresses in 16 environments in Morocco, Lebanon, Sudan and the USA. These accessions were also genotyped. 170 SNPs highly associated with the target traits were identified. Subsequently, KASP markers were designed and validated across 4515 diverse breeding lines. Prediction accuracy (PA) was evaluated using the reproducing kernel Hilbert space model with and without considering genotype by environment interaction and considering two cross-validation strategies (CV1: predicting new lines; CV2: predicting complete records from unbalanced data). In addition, 75 KASP markers targeting heat tolerance traits were prioritized and used to estimate the PA of the models.

The findings indicated comparable PA between the two models. CV1 outperformed CV2, highlighting the challenge of predicting the performance of untested lines in tested environments compared to lines that were evaluated in some environments but not in others. Furthermore, the subset size and composition of SNPs significantly influenced PA, particularly under heat stress conditions. Notably, the highest accuracies were achieved for days to flowering and plant height, suggesting that these traits are suitable for use in training population selection. Optimizing the size and composition of the training population holds promise for successful application of GS in faba bean.

P174

Use of single step factor analytic linear mixed model to develop a pipeline for genomic selection of resistance to new aggressive *Ascochyta rabiei* isolates in chickpea breeding lines

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Ascochyta blight (AB), caused by the fungal pathogen *Ascochyta rabiei*, is a major chickpea disease in Australia and worldwide. Identification of new aggressive isolates and the breakdown in resistance of most Australian cultivars have put an enonrmous pressure on breeders to deliver new AB resistant varieties to growers. The latest techniques, such as genomic selection (GS) can be an ideal solution to develop new varieties with improved resistance in a shorter time frame. In this study, we phenotyped a diverse set of 14,836 Australian chickpea breeding lines in 11 environments, including controlled, semi controlled and field conditions, across three Australian states. Of the 14,836 phenotyped lines, 14,629 were genotyped using a Multispecies Pulse 30K SNP Array. This panel was then used to fit a factor analytic linear mixed model (FALMM), using a single step approach. The model incorporated all sources of variation relating to genetic and non-genetic effects, and the additive and non-additive genotype by environment interactions were modelled using factor models of order 2 and 1 respectively. The interaction classes (iClass) approach identified measurable, though small differences in resistance betweeen field and controlled/semi controlled environments. These results provide the basis for genomic selection of unphenotyped lines.

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Genetic advances in tackling emerging diseases of Faba bean (*Vicia faba* L.) in Ethiopia

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Faba bean (Vicia faba L.) is a crucial pulse crop for home consumption, income generation, and animal feed. It plays a key role in cereal-based rotation systems in the highlands of Ethiopia. However, sustainable production faces threats from an invasive disease called Faba bean Gall (FBG), caused by Physoderma viciae. The rapid spread of FBG in the cool highlands has significantly impacted production and biodiversity, also resulting in complete crop losses Collaborative efforts by EIAR, ICARDA, ARARI, UWA, and NSWDPI, through an ACIAR funded project, aims to manage the impact of the disease. Towards this outcome the epidemiology of Physoderma viciae is being defined. Importantly, the effective seed dressing chemical (Noble 25 WP), has been identified providing an immediate solution for farmers who had halted faba bean production. Largely, diverse faba bean genotypes from Ethiopia, ICARDA and Australia have been evaluated, leading to the identification of partially resistant genotypes for further evaluation. Nevertheless, breeding of resistant varieties for faba bean gall requires the support of molecular tools to maximize identification of resistant genes for FBG disease and aggregate in popular cultivars. These molecular techniques play a crucial role in enhancing crop resilience and combating diseases.

Key words: *Disease resistance, Faba bean; Molecular tools; Physoderma viciae, Resistance breeding*

Introduction

Faba bean (*Vicia faba* L.) is an important cool-season legume cultivated for human consumption worldwide, contributing to global food security and sustainable agriculture. Ethiopia is the secondary center of diversity for faba bean and ranks as the second-largest producer next to China, accounting for 18% of the world's dry faba bean production (FAOSTAT 2022). Approximately 4.3 million smallholders cultivate faba bean on an area of 520,551.7 hectares, yielding about one million tons of grain with a national average productivity of 2.1 tons per hectare (CSA 2022). This crop plays a vital role in providing plant-based protein for human consumption, generating cash income, and serving as animal feed within the highland crop-livestock farming system. Additionally, faba bean contributes to soil fertility when used as a rotational crop in wheat and barley-based cropping systems.

Despite its importance, faba bean production in Ethiopia faces various biotic and abiotic challenges. Since 2010, an unidentified and highly destructive disease called Faba Bean Gall (FBG) has been reported and rapidly spread through farmers' fields, resulting in significant yield losses. In some regions, this pathogen has caused up to 100% crop loss due to favorable environmental conditions, particularly in the highlands of Ethiopia (see Figure 1). Faba bean gall symptoms appear as sunken lesions on the upper side of leaves, which then protrude on the back side. These lesions gradually turn light brown and expand, eventually merging and causing necrosis of plant tissue. Affected plants become stunted and fail to produce seeds. The severity is more pronounced in higher-altitude growing areas with abundant precipitation and cool temperatures. Since 2018, Australian and Ethiopian scientists collaborated on an ACIAR-funded project with several key objectives: mapping the spread of Faba Bean Gall (FBG) disease in Ethiopia, identifying the true causal agent of FBG disease, studying the pathogen's epidemiology, and exploring management strategies for FBG, including resistance breeding. The research involved laboratory and field work, utilizing distinct methodologies for each project activity

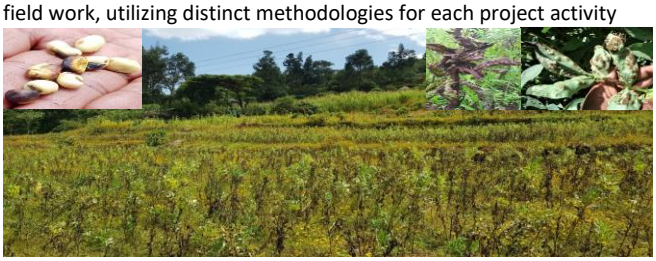

Figure 1: Faba bean field devastated by the faba bean gall disease in Northern part of Ethiopia;

Result

Morphological and molecular studies of the pathogen based on infected faba bean leaf samples from Ethiopian highlands, confirmed *Physoderma viciae* as the true FBG pathogen. Additionally, researchers identified primers for this pathogen, and ongoing studies focus on isolate diversity. Field pea, lentil, chickpea, Fenugreek, Trifolium Decorum, and Vetch (*Vicia sativa*, *V. dasycarpa, V. villosa*) have been found to be alternative hosts for the FBG pathogen. This pathogen spreads through infested soil, downhill run-off water, water splash, faba bean debris, and animal manure. Effective chemical treatments for controlling FBG have been confirmed in multiple trials; chemicals like Noble 25 WP as a seed dressing at a rate of 150 gm per 100 kg seed, Diprocon 33 EC, and Nativo SC 300 as spray applications. These practices are now being scaled out to end-users as an immediate short term solution allowing farmers to continue growing faba bean.

Numerous faba bean materials from various sources were assessed for resistance to faba bean gall disease under natural conditions, leading to the development of some tolerant materials. An extensive evaluation of ninety-five faba bean materials sourced from the International Center for Agricultural Research in the Dry Areas (ICARDA), Australia, and the Ethiopian Biodiversity Institute (EBI) was conducted to assess their resistance to faba bean gall, chocolate spot, and *Ascochyta blight* at Mush for faba bean gall and at Holetta for chocolate spot in central highlands and Northern part of Ethiopia. Among all tested three genotypes AF14062, PBA Amberley and Ac1524#14003 showed less faba bean gall disease susceptibility as compared to other materials including the checks. Additionally AF14062 was found to be promising with low faba bean gall disease scores and even better in grain yield and other traits of interest. Similarly, genotypes 126183, Numan, Gora, Dosha, 5247, 5123, 126181, 106733, 5199, 5248, 106703, and Gebelcho PBA Zahra, CHNQ04-28-51, AF14075, Farah, Fiesta, CHNQ04-5-11, Ac1241#21910, and 11NF001a-10, showed promising results with low chocolate spot disease scores and superior grain yield. Notably, genotype AF14075 and the improved variety Nunam exhibited both good grain yield and resistance to chocolate spot diseases. However, future resistant breeding needs molecular tools for the identification and accumulation of resistant gene to ensure faba bean diseases varieties with effective resistance.

Conclusion and recommendation

Although there has been progress in chemical control and the identification of tolerant materials, additional advancements in resistance breeding require

molecular tools to expedite the discovery and accumulation of resistance genes. This will aid in developing faba bean varieties that are resistant to faba bean gall disease.

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Presentation Title

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Mungbean is a highly versatile, multi-cultural crop used as a dry seed, sprouts, flour, forage and protein product. Furthermore, with its short duration, capacity for biological nitrogen fixation, and high stress tolerance, mungbean can fit into maize-soybean-winter wheat-mungbean rotations. Although still grown on a limited basis in North America, demand is rapidly growing. With growing genomic and phenomic tools, a variety of breeding approaches are increasingly not only possible but cost-effective. Public breeding efforts at Iowa State University and Tennessee State University are harnessing growing genomic information for mungbean. Participatory breeding efforts are also underway, under the umbrella of the Ujamaa Cooperative Farming Alliance. We highlight emerging genomic information on the domestication and human dispersal of mungbeans, limitations to genetic resources for mungbean, bottlenecks of domestication, benefits and drawbacks of different breeding approaches, and emerging challenges.

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Gene expression during parasitism interactions between faba bean and *Orobanche foetida*

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Faba beans cover about 72% of the area used for all food legumes in Tunisia. The main threat to this crop is the parasitic plant *O.foetida*, which may cause up to 90% yield loss in heavily infested fields. Identifying genes involved in the interaction between faba bean and *O. foetida* is crucial for breeding resistant varieties. However, there's no available transcriptome data on faba bean's response to *O. foetida* parasitism. In this study, we employed RNA sequencing technique to investigate gene expression changes in faba bean varieties at the root level during interactions with *O. foetida*. Our analysis focused on differential gene expression and Gene Ontology enrichment. We found changes in genes related to secondary metabolites like flavonoids, auxin, thiamine, and jasmonic acid. We also examined WRKY genes, important in plant-parasitic interactions. Given the crucial role of parasitic plant seed germination in this interaction, we investigated *V. faba* genes involved in the orobanchol biosynthesis pathway. This study clearly enhances our understanding of the *V. faba*/*O. foetida* interaction, highlighting the primary differences in gene expression between susceptible and resistant faba bean varieties during *O. foetida* infestation.

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Multi-Trait, Multi-Environment GBLUP Improves Genomic Prediction in Mungbean

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Mungbean is a culturally and economically important grain legume crop, and accelerating its genetic improvement is crucial. While genomic prediction has been shown to accelerate genetic gain in many other crops, there has been limited application in mungbean. This study reports the application of genomic unbiased best linear prediction (GBLUP) to enable genomic selection in mungbean. With the aim of maximising the accuracy of genomic predictions, we have investigated the use of a multi-trait, multi-environment GBLUP (MT-ME-GBLUP) approach, which exploits genetic correlations between traits within and across environments[1]. Here, yield, plant height and days-to-50%-flowering data from the Mini-Core, a set of diverse mungbean germplasm[2], collected from four trials in different environments in Australia, was used. MT-ME-GBLUP was compared with single-trait, single-environment GBLUP (ST-SE-GBLUP) under four cross-validation schemes which varied based on how the validation population was masked for traits (complete or yield only), and environments (balanced or sparse). Masking yield only represents a strategy wherein yield is predicted by a correlated trait which can be measured early or cheaply, while sparse testing across environment allows testing of more genotypes at lower cost. Models were validated on yield only. ST-SE-GBLUP had an average prediction accuracy of 49.2%. MT-ME-GBLUP didn't perform better under complete-balanced scheme. The genomic prediction accuracies from MT-ME-GBLUP did increase by 6.3%, 7%, and 8.1% under complete-sparse, yield-balanced, yield-sparse schemes, repectively. Overall, these results suggest the benefits of adopting genomic selection in mungbean breeding. Moreover, MT-ME-GBLUP, with an appropriate breeding strategy, could further increase efficiency of mungbean breeding programs.

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P182

Pan-genomics and -transcriptomics of symbiotic nitrogen fixation in mungbean

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Legumes can thrive in low nitrogen environments due to their ability to utilize atmospheric di-nitrogen via symbiotic nitrogen fixation (SNF) by rhizobia housed in legume root nodules. SNF is a complex plant trait with over 200 genes found to contribute substantially in a few model species. However, SNF varies between and within a species meaning the goal of improving SNF in legumes will require work on collections of diverse genotypes, to understand the genetic potential for improvement within each target species. To facilitate this for the tropical legume, mungbean, we have begun to characterize the pan-genome and transcriptome from the point of view of nodulation and SNF. Mungbean's short life cycle makes it an ideal, high value component of existing cropping systems that provides soil N enrichment amongst other benefits. To begin to explore the natural variation of SNF in mungbean, eight diverse genotypes were selected to compare SNF-related traits, including biomass, nodulation phenotypes, N derived from the atmosphere (Ndfa) and gene expression. ^{15}N isotope analysis allows us to determine %Ndfa and total Ndfa providing an estimate for SNF effectiveness. Comparative transcriptome analysis is being undertaken to investigate SNF gene expression in these different genotypes. Together, these results will provide a foundation to understand the SNF capabilities of diverse mungbean accessions. This, coupled with Genome Wide Association Studies in hundreds of diverse mungbean genotypes, will advance our understanding of the genetic basis of SNF and its improvement via predictive plant breeding and genome editing in mungbean.

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NLP2 regulation of Nitrite Reductase is required for vacuole integrity in N-fixing cells of Medicago truncatula under high nitrate

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NIN-Like Protein 2 (*NLP2*) is highly expressed in the N-fixation zone of mature nodules of *Medicago truncatula* where it directly activates the expression of *leghemoglobins* through "double" Nitrate Responsive Elements. Unlike NLP1/4, loss of NLP2 does not affect nitrate suppression of nodulation. However, we found that nodules that develop on *nlp2-1* under high nitrate exhibit abnormal vacuole morphology, which is not seen at lower nitrate concentrations. This phenotype is associated with starch accumulation, and higher expression of starch synthesis genes which specifically occurred at 5.0 mM KNO_3. Further investigation revealed decreased Nitrite Reductase (NiR) activity and increased levels of nitrite and nitric oxide (NO). Transcript profiling revealed that *Nitrite Reductase* expression was strongly reduced in *nlp2* nodules at all nitrate concentrations, reduced expression of genes important for hypoxia adaptation, such as *Alcohol Dehydrogenase*, *Pyruvate Decarboxylase (PDC),* and increased expression of *S-Nitrosoglutathione Reductase* (*GSNOR*) which is involved in NO metabolism. This was associated with lower levels of ATP and a higher NAD^+/NADH ratio, suggesting that energy metabolism is specifically compromised in *nlp2* nodules at high nitrate. Transgenic expression of *NiR* in nodules of *nlp2-1* mutants grown at 5.0 mM KNO_3 rescued the vacuole phenotype and restored the expression of *GSNOR* and *PDC* to normal levels. Overall, our data implicates NLP2 plays in energy maintenance under high nitrate through its regulation of *NiR*.

Key words: NIN-Like Protein, Hypoxia, Nitrite Reductase, Nitric Oxide

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Non-Nodulating Mutants to Quantify Nitrogen-Fixation of Legume Crops

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Legume plants recognise compatible rhizobia partners through the perception of signal molecules, called Nod factors. These molecules are recognised by cross membrane LysM receptor kinases called Nod factor receptors that are located on the host plants roots. The receptors form a complex comprised of NFR1 and NFR5. Perception of compatible Nod Factor signals initiates infection thread formation and nodule development, with knock-out mutations in either receptor resulting in a non-nodulation phenotype.

Non-nodulating legumes can be useful tools in crop breeding programs. They are only able to acquire nitrogen from the soil, compared with commercial varieties that source nitrogen from both soil and through nitrogen-fixation. These mutants can be used to measure the extent rhizobia nitrogen-fixation has on the nitrogen content of varieties that can form nodules.

We have identified NFR5 orthologs in mung bean (Vigna radiata) and cowpea (Vigna unguiculata). Using CRISPR/Cas9 and Agrobacterium tumefaciens mediated stable transformation, we are now generating knockout mutants in both species that can be used in future trials as a baseline for quantifying the extent nitrogen-fixation contributes to nodulating plants. Updates on current progress will be presented.

Nod factor receptors are a cross membrane LysM receptor kinase. They play a crucial role in recognizing rhizobial symbiotic signal molecules (nod factors) and initiate infection thread formation, leading to nodule development. The receptor complex comprises NFR5 and NFR51. Orthologs of NFR5 have been identified in various legumes, including L. japonicus, M. truncatula, P. sativum, G. max, A. hypogaea, and C. arietinum. Mutations in NFR5 cause significant disruption to nodulation.

The aim of this study is to identify the NFR5 ortholog in mung bean and generate CRISPR/Cas9 knockout plants, examining downstream impacts. We hypothesize that a CRISPR/Cas9-mediated knockout of NFR5 using an Agrobacterium tumefaciens mediated stable transformation system will produce a heritable nod- trait with no additional downstream effects.

Given the recalcitrance of many legumes to tissue culture techniques, optimizing current stable transformation methods is crucial. Agrobacterium-mediated transformation has emerged as the preferred option for legume stable transformation, although techniques vary among crops. In this study, we will explore and optimize Agrobacterium-mediated transformation for mung bean, focusing on factors such as explant type, co-cultivation conditions, and selection pressure. The identified NFR5 ortholog in mung bean will be targeted for knockout using CRISPR/Cas9, and the resulting mutants will be characterized phenotypically and genotypically to assess nodulation ability and any potential off-target effects. The resulting nod- mutant will provide a useful control to accurately quantify nitrogen fixation in similar crops.

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Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation

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Legumes form symbiosis with rhizobium leading to the development of nitrogen-fixing nodules. Nodule development begins with the differentiation of cortex of infected root and involves interplays of both nodules and rhizobia. However, our understanding of cellular heterogeneity and developmental lineage of nodule is still limited. By integrating single-nucleus and spatial transcriptomics, we established a cell atlas of soybean nodules and roots. In central infected zones of nodules, we found that uninfected cells specialize into functionally distinct subgroups during nodule development, and revealed a transitional subtype of infected cells with enriched nodulation-related genes. Further functional analysis revealed the important role of cell-type-specific genes in regulating nodule development and nitrogen fixation.

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Correlating Flavonoid Exudation and Microbiome of *Medicago truncatula*

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Symbiotic nitrogen fixation in legumes provides a sustainable nitrogen source for agriculture. However, the symbiosis is often not optimal. A potential method to improve the symbiosis is by modifying the exudation of flavonoids, a family of metabolites that signal the symbiotic infection of rhizobia. This poster reports on an experiment that utilised metabolomic and metagenomic methods to test for a correlation between the microbiome in three spatial compartments at different distances from the root of a null segregate *Medicago truncatula* (2HA) and an isoflavone synthase overexpression transgenic line (IFS:OE), which showed different flavonoid exudation when grown on media (Liu *et al.* 2017). Plants were grown in a mixture of sand and field soil, and nodulated with native soil rhizobia, with significant increases in total shoot N in the IFS:OE line compared to the control. The compartments harvested were bulk soil, rhizosphere soil and root tissue. The experiment successfully identified differences in the microbiome of the compartments, with the diversity of microbes decreasing with proximity to the root. However, the differences in flavonoid exudation observed when 2HA and IFS:OE were grown in media were not observed when grown in soil, with a difference in flavonoid accumulation only observed within the root. This result meant a correlation between flavonoid exudation and microbiome could not be made, as there was no difference in either flavonoid profile or microbiome outside of the root when comparing plant lines. These results suggest a greater understanding of the root exudation mechanism is necessary to manipulate nodulation through flavonoid exudation.

References:
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Identification of the SNP markers for drought tolerance and related agronomic traits in chickpea under multi-environments using GWAS analysis

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Drought tolerance is a complex trait that involves numerous genes. Identifying key causal genes or linked molecular markers can facilitate the fast development of drought-tolerant varieties. Using genome-wide association study (GWAS) is a powerful approach to identifying the genetic factors underlying the intraspecific phenotypic variations. To address this, we cultivated 185 chickpea accessions in two distinct locations in Lebanon over two years, subjecting them to both irrigated and rain-fed environments. We measured 11 traits, including morphological, yield, yield components and tolerance score. SNP genotyping revealed 1344 variable SNP markers distributed across the chickpea genome. A genome-wide association study (GWAS) revealed several marker-trait associations (MTAs) associated with the traits evaluated. Within the rainfed conditions, 11 significant markers were identified, each associated with distinct chickpea traits. Another set of 11 markers exhibited associations in both rainfed and irrigated environments, reflecting shared genetic determinants across these conditions for the same trait. We identified 28 genetic regions containing SNPs significantly associated with several different drought traits, which was an indication of pleiotropic effects. Among the identified genes are *CPN60-2, hsp70, GDSL(GELP), AHL16, NAT3, FAB1B, bZIP, and GL21*. These genes collectively contribute to the multifaceted response of chickpea plants to drought stress.

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Genomic selection for different agronomic traits in ICARDA chickpea breeding program

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Chickpea (Cicer arietinum) is a crucial legume crop for food security and agricultural sustainability. Genomic selection (GS), an emerging breeding approach, enables early selection by predicting the genetic value of individuals using genome-wide markers. This study investigated the prediction accuracy of key agronomic traits using ICARDA chickpea breeding germplasm. The training population for this study was comprised of 297 genotypes, where 48% were breeding lines, 22% recombinant inbred lines (RILs), 14% of varieties, landraces, and wild types. Approximately 8% of the training population represented materials imported From Australia and India. The germplasm was genotyped using 1,922 single nucleotide polymorphism (SNP) markers covering the eight chromosomes. Field evaluation was conducted over two years (2023 and 2024) at Terbol station, Lebanon, using a replicated Alpha Lattice design. Key agronomic traits assessed included 100 seed weight (100SW), flowering time (FLWR), maturity time (MAT), and plant height (PLHT). Grain and biological yield were also measured. The prediction accuracy was calculated with and without fitting the genotype by environment interaction in the prediction model with two cross-validation strategies (CV1: predicting new individuals; and CV2: predicting complete data across environments from unbalanced field trials). The results demonstrated moderate heritability values for 100SW, FLWR, MAT, and PLHT, and high prediction accuracy values ranging from 0.51 to 0.81 for CV1, and from 0.63 to 0.90 for CV2. However, yield and yield component traits exhibited relatively lower heritabilities and accuracies ranging from 0.38 to 0.48 for CV1, and from 0.37 to 0.51 for CV2. These findings underscore the potential of GS to enhance the efficiency and accuracy of chickpea breeding programs to achieve better yield stability and adaptability in chickpea across diverse growing conditions.

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Identification of QTLs associated with drought avoidance root traits in lentil: towards confirmation of constitutive and adaptive QTLs genomics-assisted breeding for drought tolerance

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Lentil (*Lens culinaris* Medik.) is an important crop and staple food in several developing countries. Its seeds are rich source of protein, carbohydrates, vitamins, and minerals. Drought stress is one of major constraints limiting lentil production worldwide. The development of drought-tolerant lentil varieties could help in improving lentil production in drought-prone regions. However, drought tolerance is a complex trait under quantitative control, making it difficult to improve drought-adapted genotypes using conventional breeding approaches. Drought avoidance root traits can be targeted to improve lentil production drought-prone region; however, the screening of these traits is challenging which limits their integration in breeding programs. Thus, genomics-assisted breeding could be a promising way for efficient development of superior drought lentil varieties. In this study, a lentil recombinant inbred line (RIL) population consisting of 126 F_7-derived RILs from the cross ILL6002 / ILL5688 was extensively phenotyped for root and shoot traits associated with drought tolerance using both conventional phenotyping methods and an advanced automated high-throughput phenotyping platform. In addition, Genotyping-by-Sequencing (GBS) derived high-density linkage map consisting of 1373 single nucleotide polymorphisms (SNPs) markers, distributed on 7 linkage groups and spanning total length of 2391.4 cM, was constructed for this population. Analysis of genotypic and phenotypic data allowed the identification of QTLs controlling drought avoidance root traits which could be used in lentil breeding programs targeting drought tolerance as an efficient alternative to labor-intensive and time-consuming conventional breeding methods. Keywords: lentil, drought tolerance, QTL, genomics-assisted breeding.