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Society Conference

Book of Abstracts



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IMPRESSUM

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5th International Legume Society Conference

8 – 12 June 2026, Dubrovnik, Croatia

Sessions

- Legume-based value chains – Panel Discussion
- Legume-based cropping systems: Performance, ecosystem services and profitability
- Biodiversity and beneficial plant-microbe interactions
- Legumes for human and animal nutrition and health
- Emerging technologies in omics-based crop improvement
- Crop breeding: challenges, tools, strategies and achievements
- Understanding and enhancing legume crop tolerance to abiotic stresses
- Understanding and enhancing legume crop tolerance to biotic stresses
- High-throughput phenotyping
- Crop modelling to predict yield, disease and other traits

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BOOK OF ABSTRACTS

Session2: Legume-based cropping systems: Performance, ecosystem services and profitability

INTEGRATING LEGUME ECOSYSTEM SERVICE MONITORING INTO GOOD AGRICULTURAL PRACTICE

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Integration of legumes may provide Ecosystem Service (ES) benefits to agricultural systems, including reduced greenhouse gas emissions associated with synthetic Nitrogen (N) fertiliser use-offset, and adaption via biological N fixation; increased cropping sequence N use efficiency; soil quality improvement; and reduced pest and disease pressure. The uptake of legumes is still lower than desired agroecologically due to a complex array of drivers and barriers to uptake. Moreover, there is a diverse array of how legumes may be introduced into production systems and trade-offs between cropped system types, and socioeconomic contexts of/for ES delivery. Measuring ES delivery provides a way for farmers, advisors and agri-environment scheme regulators to monitor success and optimise systems to deliver their priorities. Previous work highlights that measures and tools should be low cost and rapidly interpreted to be taken up widely. Therefore, characterising ES monitoring methods was informed by multi-actor workshops and policy scanning, as part of the www.legumESproject.eu, and a “logical sieve” of over 200 ES Indicator Methods (ESIMs) was carried out for a range of ES benefits and legume-use cases. ESIMs generally fell into three categories; methods to describe the management of the system (e.g., treatment frequency indices), methods to inform appropriate decision making on farm (e.g., available soil N), and methods to describe whether desired system state(s) are being achieved. Indicators of each type are required to ensure systems are designed and managed to deliver their desired outcomes. Considerations and progress on how these ESIMs can interact with current agricultural guidelines are discussed.

Keywords: Ecosystem Services, cropping systems, legume integration

Session 3: Biodiversity and beneficial plant-microbe interactions

FROM WILD TO CULTIVATED: HOW PLANTS AND MICROBES
EVOLVE TOGETHER

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Domestication has profoundly transformed crop plants, yet its consequences for ecological interactions within species remain largely unexplored. Plant performance depends strongly on both neighboring plants and rhizosphere microbes, thus understanding how domestication has reshaped these interaction networks is particularly important. We leveraged the almost unique evolutionary history of *Phaseolus vulgaris*, independently domesticated in Mesoamerica and the Andes, to investigate how evolutionary divergence and genetic relatedness influence plant–plant and plant–microbe interactions. A representative panel of wild accessions, landraces, and modern cultivars was grown in pure stands and all binary mixtures. By integrating transcriptomics, metabolomics, and rhizosphere metagenomics, we examined how neighbor identity modulates plant responses and microbiome assembly. Our results reveal a progressive shift in transcriptional plasticity along the domestication gradient. Wild genotypes maintain relatively stable gene expression across neighbor contexts, whereas modern cultivars show extensive transcriptional reprogramming, particularly when interacting with wild plants. Landraces display intermediate responses, consistent with their evolutionary position. Rhizosphere microbiomes mirror these patterns: microbial diversity and composition shift along the wild–landrace–cultivar gradient, and neighbor identity further reshapes microbial assemblages. Together, these findings indicate that domestication has reconfigured interaction networks within *P. vulgaris*, linking plant evolutionary history to microbiome assembly. Harnessing this evolutionary diversity may open new avenues to design more resilient and sustainable legume-based agroecosystems.

Keywords: *Phaseolus vulgaris*, crop domestication, plant interactions, rhizosphere microbiome, evolutionary diversity

Session 4: Legumes for human and animal nutrition and health

CAN WE HAVE IT ALL? NUTRITIONAL QUALITY IN RESILIENT LEGUMES AND THE TRADE-OFFS WE DIDN'T SEE COMING

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Legume seeds are central to global nutrition and sustainable agriculture. Yet the genetic architecture underlying seed metabolite composition often overlaps with loci controlling disease resistance and tolerance to drought or flood. Can we simultaneously enhance health-promoting metabolites while strengthening resistance to pathogens and climate extremes? Using metabolic GWAS, we uncovered both favourable pleiotropy and unexpected trade-offs between seed nutritional quality and resilience traits. This presentation will explore recent advances in common bean (*Phaseolus vulgaris*) and grass pea (*Lathyrus sativus*), highlighting forthcoming challenges in their quality improvement, particularly under a changing climate. Understanding this balance is key to breeding legumes that nourish both people and production systems.

Keywords: nutritional and health beneficial compounds, metabolic GWAS, quality-resilience trade-offs

*Session 5: Emerging technologies in omic-based crop improvement***DEVELOPMENT OF GENOMIC RESOURCES AND GENOTYPING TOOLS TO BOOST BREEDING IN LEGUME CROPS**

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Grain legumes are vital crops for ensuring food security and are also significant cornerstones for developing sustainable, agroecological cropping systems. As well as being a rich source of protein, growing them has many environmental benefits, such as reducing reliance on synthetic nitrogen fertilisers and associated greenhouse gas emissions, and enhancing soil biodiversity. However, climate change and regulations on reducing pesticide use present new challenges for these crops. There is therefore an urgent need to breed new varieties that can withstand existing and emerging biotic and abiotic stresses. This can be achieved by exploiting the diversity available in germplasm collections, in which -omic technologies can play a significant role. This presentation will focus particularly on genomics. Recent developments in providing high-quality reference sequences will be described, as will the importance of building on such resources and large polymorphism discovery panels. The latest genotyping solutions based on meticulous marker selection will be covered, paying particular attention to a multi-species tool for genotyping pea, faba bean, lentil, chickpea and white lupin. This tool offers good genome coverage, a high ratio of functional markers, and is cost-effective. We will discuss how these resources can be used efficiently in breeding programmes and list possible applications.

Keywords: grain legumes, genomics-assisted breeding, multi-species genotyping array, climate resilience, agroecology

*Session 6: Crop breeding: challenges, tools, strategies and achievements***LEGUME BREEDING SUPPORTED AND INSPIRED BY BLACK BOX AGROECOLOGICAL AND GENOMIC APPROACHES**

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Legume breeding programs usually face many possible target environments with limited budgets. Yield data from multi-environment trials or strategic experiments, and genome-enabled predictions, could inspire cost-efficient breeding strategies that limit the extent of field-based selection. Examples are provided with regard to genotype × environment interactions accounted for by artificial selection environments, breeding for intercropping, and outcomes of genomic selection. A pioneer application of AMMI analysis to model alfalfa cultivar yields across Northern Italy highlighted large genotype × location interaction due to extent of summer water and soil type. A set of managed environments allowed to exploit this interaction by selecting specifically for two subregions. For white lupin, featuring modest seed market, selection for wide adaptation to Italian environments is pursued by parallel selection for resistance to terminal drought and winter frost using artificial selection environments and genome-enabled predictions. Breeding for intercropping is complicated by many possible associated cultivars. A large white clover-grass experiment indicated that as the grass competition increases, the genetic correlation for clover genotype yield between pure stand and mixed stand decreases irrespective of the associated species. This result anticipated a general pattern in a meta-analysis of experiments relative to perennial legumes and cool-season or warm-season annual legumes, confirming the importance of breeding for intercropping when expecting severe competition. An advantage of genomic over phenotypic selection based on actual yield gains emerged from results for alfalfa and pea in different regions. Exploring opportunities and limitations of genomic prediction across different reference populations is important for genomic selection optimization.

Keywords: breeding strategy, genomic selection, genotype x environment interaction, intercropping, selection environment

*Session 7: Understanding and enhancing legume crop tolerance to abiotic stresses***OPTIMISING PLANT-MICROORGANISM INTERACTIONS TO
IMPROVE LEGUME RESILIENCE TO ABIOTIC STRESS**

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Legumes have a unique relationship with soil microorganisms, which can be either symbiotic, such as rhizobia or arbuscular mycorrhizal fungi, or non-symbiotic. Optimising these interactions is a promising strategy for improving the resilience of legumes to abiotic stress, as their productivity is often limited by adverse environmental conditions. A first approach involves identifying more efficient strains of rhizobia under abiotic stress to enhance N₂ fixation. Two independent experiments were conducted on pea and faba bean to determine whether the identity of the rhizobia strain could influence the plant's response to drought in terms of symbiotic nitrogen fixation, water flux management, or growth. We then sought to assess the role of soil microbial diversity in legumes' resilience to water stress by conducting diversity-dilution experiments on lentil and pea. For both species, an increase in microbial diversity did not lead to greater resistance to water stress; however, it did result in better plant recovery following the stress. Another important strategy is to consider the plant's role in modulating soil microorganism populations, which could be beneficial under abiotic stress conditions, particularly through exudation. To this end, we have investigated the 'black box' of interactions between soybean and soil microorganisms under water-stressed and high-temperature conditions. Using a multi-scale approach within the rhizosphere, we have identified potential key players in resilience to multiple stresses. Harnessing the natural synergy between plants and microorganisms would make agriculture more sustainable, productive and resilient, which are all valuable assets for tackling future environmental challenges.

Keywords: water deficit, rhizosphere, N₂-fixation, microbial diversity

*Session 8: Understanding and enhancing legume crop tolerance to biotic stresses***USE OF WILD RELATIVES IN BREEDING GRAIN LEGUMES FOR PEST AND DISEASE RESISTANCE**

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Diseases and pests are the main causes of yield reduction of legume impairing both yield and quality of the crops. Of the different control methods available, breeding for resistance is the most efficient, economical and environmentally friendly. Crop improvement is thus a crucial element for sustainable agriculture that can be performed by several approaches from classical breeding to genetic engineering. The success in developing varieties resistant to biotic stresses depends on the availability of good sources of resistance, the availability of an accurate method to identify the resistance and also on the inheritance of the resistance. Although development of genomic resources in legumes was relatively slow compared to other crops such as cereals, this is progressing impressively generating ample genomic resources in all the major pulse crops. Still, availability of sources of resistance and of affordable screening methods remains as major bottlenecks for resistance breeding. A number of sources of resistance to major stresses are available in some crops, but lacking in many instances. When a resistance is lacking it might be created by mutation or other means, but a sound strategy is to search for it in germplasm, as closely phylogenetically as possible to ease the breeding process. But at times, this is not feasible in the primary germ pool, so wild relatives should be explored. We present here and critically discuss the identification of sources of resistant to a number of pests and diseases in crop wild relatives and their effective deployment in crop breeding.

Keywords: pest, disease, resistance, breeding

Session 9: High throughput phenotyping

PLANT PHENOTYPING TECHNOLOGY ADOPTION – AND OUR
QUEST TO COPE WITH CLIMATE CHANGE

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Developing new plant varieties requires evaluating genotypic performance at scale - a process that remains slow and resource-intensive despite advances in novel breeding techniques. Digital phenotyping technologies, from robotic systems to smartphone-based imaging tools, are increasingly being adopted to automate this work. However, their full potential is far from realized, and accelerating their integration into breeding pipelines has become increasingly urgent given the pace of climate change and the growing demand for stress-tolerant crops. To address this challenge, Wageningen University & Research and Utrecht University established the Netherlands Plant Eco-phenotyping Centre (NPEC), a national infrastructure enabling high-throughput phenotypic screening across controlled, greenhouse, and field environments. Having supported over 300 indoor experiments and hundreds of UAV flights - contributing to more than 100 scientific publications - NPEC has reached a point where the main bottleneck is no longer data collection, but data interpretation. In response, NPEC has set a strategic ambition to become a cornerstone of AI research in plant sciences, committing to disseminate datasets according to FAIR principles: Findable, Accessible, Interoperable, and Reusable. Implementing FAIR data practices requires rigorous labelling, accurate reference data, and thorough metadata documentation covering equipment, software, and protocols. A notable early example is the Leaf Segmentation Challenge, launched by the International Plant Phenotyping Network, whose openly released dataset continues to attract growing interest - particularly from AI researchers outside plant science, demonstrating that well-curated open data can effectively bridge disciplinary boundaries. A more recent illustration is the TomatoWUR dataset, developed through the Virtual Tomato Crop project, which enabled 3D reconstruction and deep learning-based quantification of plant traits. Its release sparked an international collaboration with UC Berkeley, where researchers applied Gaussian Splatting to significantly improve 3D plant modeling. Adoption to new technology takes time and is a highly complex matter in all domains. Our food production system already suffers from the effects of climate change, therefore it's urgently needed to enable plant scientists in academia and industry to come up with solution to deal with the effect of climate change. Enabling these experts with efficient new screening technology will boost the quest to find more robust and tolerant crop varieties to cope with climate change effect. Large-scale research infrastructure is a driver for innovation in research and for achieving scientific breakthroughs globally. In the Netherlands NPEC was developed to enable large numbers of users to use high-tech, data driven screening technologies for the next big step forward towards more robust food system.

*Session 10: Crop modeling to predict yield, disease and other traits***ROLE OF CROP AND PLANT MODELLING IN DESIGNING FORAGE MIXTURES WITH ENHANCED POSITIVE INTERACTIONS AND YIELD**

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Multi-species grasslands hosting legumes are a major lever for strengthening the resilience and sustainability of forage systems. Their agronomic success is recognized, but the best way to adapt them to the challenges of climate change and reduced fertilizer inputs remains to be defined. Plant and crop models, such as Virtual Grassland, can simulate plant-plant interactions and grassland community dynamics, providing a virtual laboratory for testing community compositions, traits of interest for breeding, and cultural practices (e.g., variety mixtures, harvesting or fertilizer management). Through recent examples, I will show how mechanistic models have been used to seek for new traits of interests favouring overyielding and community stability over time in grass-legume mixtures. Models can also be used to identify agroclimatic zones and help choosing the most adapted species and varieties for present and future climate conditions. These approaches can be used together with field experiments and data-driven approaches, to pave the way for the design of legume-rich grasslands adapted to different soil and climate conditions.

Keywords: individual-based models, crop models, complementarity, facilitation, overyielding

STRENGTHENING FRAGILE FOOD SYSTEMS WITH CLIMATE-RESILIENT CROPS: LESSONS FROM GRASS PEA (*LATHYRUS SATIVUS*) IN CONFLICT-AFFECTED SMALLHOLDER FARMING IN NIGERIA

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Smallholder food systems in unstable Nigeria are under threat from climate change and violence. Recurrent displacement, instability, and market disruption in Plateau State have affected agricultural production, livelihoods, and food access for rural people. As a result, climate-resilient crops are pushed for adaptation and resilience. Agronomic performance, governance, institutional capability, and public health safeguards all affect food security. This study uses grass pea (*Lathyrus sativus*) to analyse how climate-resilient crops can improve food systems in conflict zones. The research examines how insecurity, displacement, and insufficient regulatory monitoring affect farmers' adoption decisions, market access, and risk exposure in Plateau State using qualitative field findings and policy and public health literature. While grass pea has benefits including drought tolerance and production stability under climatic stress, its cultivation and consumption increase neurotoxic exposure, seed regulation, extension services, and consumer awareness concerns. The analysis shows that unstable contexts require integrated, conflict-sensitive policy frameworks that go beyond production goals to promote climate-resilient crops. Effective implementation requires farmer education, institutional monitoring, and public health measures to reduce risk and build resilience. Without these conditions, resilience-oriented policies may increase vulnerabilities rather than food security. Grass pea is a key lens for studying climate adaptation, conflict, governance, and public health in smallholder food systems.

Keywords: climate change; violent conflict; food security; smallholder farming; *Lathyrus sativus*

PUBLICLY FINANCED MARKET INFORMATION SYSTEMS AS GOVERNANCE TOOLS TO STABILIZE FOOD-GRADE LEGUME SUPPLY FOR NUTRITION APPLICATIONS

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Legumes contribute to human and animal nutrition by providing plant protein, dietary fiber and micronutrients. Scaling food- and feed-grade legume ingredient chains is constrained by thin trading and high price volatility, combined with variable lot quality and safety like protein level and functionality, impurities, mycotoxins, residue compliance. This weakens processors' sourcing security and reduces farmers' incentives for quality management. We report results from developing a publicly financed, legume-specific market information system that consolidates official statistics and market signals and translates them into transparent reference indicators. Governance rationale is a neutral, shared information base that supports risk sharing and incentives for quality investments. The system integrates market, production and seed-sector indicators for pea, faba bean, lupin and soybean, linking price formation to production capacity and varietal availability. Using monthly signals from production, trade and proxy price series, we benchmark reference approaches used in practice: import-based unit values, feed-value proxies and cereal-linked rules. We then propose a composite indicator that combines global and regional signals to balance responsiveness and predictability. Using peas as an illustrative case, we discuss how reference pricing can improve cropping and sourcing decisions and increase the reliability of food-grade legume supply aligned with nutrition and health specifications. By combining market signals with crop production and seed sector data, the system creates an integrated information base that links price formation with production capacity and varietal availability, thereby improving supply reliability, production planning and coordination along nutrition-oriented legume value chains from breeding, seed production, farming to feed and food processing.

Keywords: legumes; nutrition and health; contract farming; reference pricing; market transparency; value chain governance

PROJECT INTEGRALE : GENETIC DIVERSITY AND FUNCTIONAL PROCESSING OF OF PULSE CROPS: AN INTEGRATED FARM-TO-FORK APPROACH FOR BREEDING AND MULTI-USE VALORIZATION

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Grain legumes offer a wide range of end-uses (whole seeds, functional ingredients, valorised co-products), but the genetic variability underpinning these uses remains poorly documented, particularly for faba bean and lentil. The INTEGRALE project aims to fill this gap by linking, from field to fork, genetic diversity, processing aptitude and end-use functionalities. We hypothesise that specific seed traits (cellular structure, mechanical properties, intrinsic composition) can serve as robust proxies for dehulling/grinding aptitude and technological performance in hydrated pasty food matrices. A European panel of ~50 varieties per species (mainly spring types, complemented by winter breeding lines) will be characterised phenotypically and genotypically. Seeds will be analysed for their physical and biochemical properties and their grinding behaviour, to build a multi-scale map integrating genetic polymorphism, seed traits and primary processing aptitude. From this map, 5–10 contrasted varieties per species will be selected and processed using a standardised dehulling/grinding scheme, by analogy with wheat milling, to relate fractionation yields, particle size distributions, cellular compartmentation and intra-cellular composition to aptitude for dehulling, fragmentation and extraction of biomolecules (polyphenols, sugars and other compounds) from seed coats using different extraction technologies. Finally, flours and derived fractions will be incorporated into model matrices (mashes, falafels,...) to link varietal diversity and fractionation processes to textural and sensory properties. By combining genetics, process engineering and sensory analysis within a multi-actor framework, INTEGRALE aims to define operational end-use quality criteria and tests for breeding schemes, and to deliver methodological resources directly usable by faba bean and lentil value chains.

Keywords: genetic diversity, faba bean and lentil, grinding efficiency, end-use quality criteria, functional food properties

LEGUME DILEMMAS: A NARRATIVE-BASED SERIOUS GAME TO EXPLORE VALUE CHAIN TRADE-OFFS AND SUPPORT MULTI-ACTOR POLICY DIALOGUE

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Scaling legume-based food systems requires navigating genuine trade-offs that cannot be resolved by technical information alone. Farmers, processors, consumers, and policymakers regularly face decisions in which legitimate values conflict: biodiversity versus market standardisation, fair producer prices versus consumer affordability, local food security versus integration into global value chains, and ecological infrastructure versus short-term profitability. These tensions are structurally embedded in current incentive systems and are not easily communicated through conventional research outputs. Drawing on findings from the LegumES expert Delphi panel - which surveyed 43 researchers and practitioners across 15 European countries - we developed six narrative vignettes, each grounded in an empirically identified trade-off. The vignettes present realistic decision scenarios for named characters: Elena, who must choose between a heritage bean variety and a subsidised commercial seed contract; Sarah, whose growers' collective must weigh a multinational export deal against its relationships with local schools; David, whose social enterprise cannot simultaneously honour fair prices for farmers and keep prices affordable for low-income urban consumers; and Maya, a working parent choosing between cheap, unfamiliar dried legumes and expensive, convenient ultra-processed alternatives, among others. These scenarios have been translated into a dual-format serious game - a conference tablecloth and a take-home card set - designed to make value chain dilemmas tangible, discussable, and experientially accessible to diverse stakeholder groups. In a cooperative mode, players collectively manage shared system indicators (soil health, biodiversity, farmers' income, consumer affordability). In a deliberative mode, players assume different actor roles and negotiate policy proposals in response to each dilemma. The game has been piloted at the COST Action conference in Prague (May 2026) and is presented here both as a research instrument and as an open invitation to collaboration. We invite legume researchers, practitioners, and policymakers to engage with the scenarios, refine the mechanics, and pilot the game within multi-actor innovation networks. The poster also serves as a game board: participants are encouraged to play during the session.

Keywords: legume value chains, serious games, food system trade-offs, stakeholder engagement, policy dialogue, Delphi method, narrative scenarios

CULTIVATION AND USE OF LEGUME SEEDS IN PIGS' FEEDING ON INDIVIDUAL FARMS IN POLAND – RESULTS FROM RE-LIVESTOCK PROJECT

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Livestock production is widely considered a major contributor to climate change within agriculture. The adoption of innovative practices may help address these challenges and improve the sustainability of the livestock sector compared with conventional systems. Data were collected from five Polish farms using the Public Goods Tool to assess their performance, sustainability, and profitability. In all selected farms, farmers cultivated and used legume seeds in pig feed. The farms were evaluated on a 1–5 scale across 13 categories representing environmental, social, and economic sustainability. Results were compared with national benchmarks from the Farm Accountancy Data Network (FADN) for the corresponding farm types. Greenhouse gas (GHG) emissions were estimated using the Agrecalc carbon footprint assessment software. The studied farms achieved the highest scores in economic performance, nutrient management, food safety, and animal health and welfare. They were more than twice as profitable as the average Polish pig farms, mainly due to lower feed production costs, which significantly increased net income. These farms also exhibited lower GHG emission indicators than sector benchmarks, primarily due to reduced fertilization of legume crops and the avoidance of imported soybean meal. However, particularly high emissions were associated with the use of inorganic fertilizers in cereal production. A significant weakness was identified in water resource management. The use of legume seeds in crop and livestock production can significantly enhance the environmental and economic sustainability of pig farming systems by reducing feed costs and lowering greenhouse gas emissions.

Keywords: legumes, pig production, PGtools, benefits

LEGUME VALUE-CHAINS IN PORTUGAL: A PRODUCTION-LEVEL PERSPECTIVE

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Legume production plays a strategic role in sustainable agriculture by improving soil fertility, reducing reliance on synthetic nitrogen inputs, and enhancing crop diversification. Despite these benefits, adoption in Portugal, remains limited due to economic, technical and market-related barriers. Understanding these factors is essential for strengthen the national legume value chain and supporting more resilient production systems. This study presents insights from a quantitative survey of 175 legume farmers with small- and large-scale production. Descriptive statistics characterized production profiles, limitations, and drivers. Results indicate that production systems were predominantly organic (45,6%), followed by conventional (35,7%) and integrated systems (13,5%). Regarding end uses, 52,4% of farmers produced legumes for human consumption and 12,4% for forage. Commercialization relied mostly in short supply chains, particularly direct sales (22%), local markets (11%), and processing industries (8,8%). The relatively high share of organic legume farmers using short supply chains likely reflects sampling within a sector where organic systems are closely associated to legume cultivation for their agronomic benefits. Moreover, organic farmers tend to rely more on direct sales and local markets capture added value, explaining their stronger presence compared to predominantly conventional agricultural. Motivations included legumes into crop rotations (54,5%), soil fertility (37,2%), biodiversity (39%) and household consumption (42,4%). Main constraints included limited material resources (28,1%), lack of market access (20%), low profitability (18,5%), insufficient technical knowledge (20%), and unfavourable edaphoclimatic conditions (11,6%). Overall, strengthening technical support, improving resource access, and reinforcing value chains are crucial to expand legume production and enhancing sustainability outcomes in Portugal.

Keywords: farmer perceptions, production barriers, supply chain resilience

PULSE REPLICATED ON-FARM INDEPENDENT TRIALS (PROFIT) PROGRAM IN SASKATCHEWAN, CANADA

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Agronomic research is critical for addressing production constraints to narrow the gap between genetic yield potential and realized yields on-farm. Results from research projects need to be truth tested across environments and implemented at field-scale to validate benefits. Saskatchewan Pulse Growers has developed Pulse Replicated On-Farm Independent Trials (PROFIT) program to help evaluate research results across environments as well as help with adoption on-farm. Agronomists work with growers to ensure protocols are followed and the appropriate data collection is completed. Western Applied Research Corporation manages the program including statistical analysis and play a role in extension of results. The program was refined in 2023 and has evolved to where it is today with standardized replicated protocols that growers have the ability to adapt to their farm practices. Over the past three field seasons there have been 70 field-scale trials completed across 11 protocols including fertility rates, fungicide applications, product evaluations, variety comparisons, and fine-tuning seeding rates across peas, lentils, chickpeas, and fenugreek. Overview of the farm-scale PROFIT program, standard protocol development and select results from the Saskatchewan trials will be presented.

Keywords: extension, peas, lentils, chickpeas, faba beans

CO DESIGNING A SERIOUS GAME TO EXPLORE LEGUME SYSTEM TRADE OFFS: USING NARRATIVE DILEMMAS TO SUPPORT INTEGRATED DECISION MAKING

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Legume-based farming systems are central to sustainable food futures, yet farmers, processors, consumers, and policymakers frequently face complex trade offs when integrating legumes into real-world decision-making. These trade offs balancing biodiversity, profitability, labour demands, environmental benefits, market volatility, and social equity are well recognised in research, but remain difficult to communicate effectively to wider stakeholder groups. To address this gap, we present the early-stage development of a serious board game designed to make legume system dilemmas tangible, discussable, and experientially understandable. Building on narrative case studies developed from European and global legume research such as seed choice conflicts, mechanisation pressures, export market dependencies, ecological infrastructure loss, affordability challenges, and consumer knowledge barriers the game translates real dilemmas into playable decision scenarios. Drawing inspiration from existing food-systems games (e.g., Food Systems Activities, Trading Zones, Bartertown), we propose two complementary gameplay frameworks: (1) a cooperative systems-thinking game in which players collectively manage shared indicators such as soil health, biodiversity, farmer income, and consumer affordability; and (2) a competitive economic simulation in which individual actors pursue different strategic pathways within a constrained legume food system. Both formats are designed to illuminate how individual decisions cascade across supply chains, how risks accumulate or disperse, and how legume integration is shaped by structural incentives. The game aims to support stakeholder dialogue, teaching, and policy engagement by offering a low risk environment to explore high stakes legume system choices. We invite collaboration from researchers and practitioners to refine mechanics, validate scenarios, and pilot the game within multi-actor legume innovation networks.

Keywords: legume systems, serious games, food system trade offs, stakeholder engagement, sustainability

BEYOND NITROGEN: THE MULTIFUNCTIONAL PROVISIONS OF PULSE NON-NUTRITIONALS FOR 'ONE HEALTH'

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Grain legumes are central to the One Health framework due to their capacity to support sustainable crop production, soil function and human health. This study integrates agronomic, soil and nutritional data to assess the systemic value of pea (*Pisum sativum* L.) and faba bean (field beans; *Vicia faba* L. var. *minor*) within a circular bioeconomy perspective. Plot-scale field trials included diverse genotypes of field bean and pea - grain yield and above-ground residues at harvest were quantified. Residues were incorporated into soil mesocosms to evaluate their effects on functional diversity using the MicroResp™ technique; while grains were characterised for their nutritional and bioactive composition in view of their potential role in gut health. Chemical characterisation of residues (total phenolics, tannins and lignin) guided the selection of phytochemically diverse materials that markedly influenced soil microbial respiration, with basal respiration ranging from 0.81 to 4.79 $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil h}^{-1}$. Both suppressive and stimulatory responses were observed, indicating heterogeneous functional profiles and shifts in the microbial capacity to metabolise structurally complex aromatic substrates. Using the same genotypes, the grain fraction delivered a wide range of bioactive compounds that promoted short-chain fatty acid production comparable to known prebiotic effects, particularly regarding butyrate, highlighting relevance for human gut function. By bringing together agronomic performance, soil responses and nutritional outcomes, this work shows how pulses can deliver benefits across plant, soil and human health. The compositional profile of residues also indicates potential for future feed applications, supporting full biomass valorisation within a One Health approach.

Keywords: pulses, one health, soil, gut, function

HARNESSING THE POWER OF PULSES: BREEDING FOR A FARMING SYSTEM TO IMPROVE SUSTAINABILITY

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Legume crops provide protein-rich food, critical disease breaks in cereal rotations, and contribute to soil fertility through symbiotic nitrogen fixation. However, crop improvement programs typically focus on individual crop performance rather than system-level benefits. We hypothesise that legacy effects (the influence of one crop's genotype on subsequent crop performance) are under genetic control and could be leveraged in breeding programs. To test this, we evaluated how 309 genetically diverse mungbean genotypes influence subsequent wheat performance. The mungbean panel was grown, followed by a single wheat cultivar sown in the same plot locations. Remarkably, wheat yield varied by nearly 1 t ha⁻¹ (2.52-3.49 t ha⁻¹) depending solely on the preceding mungbean genotype, with legacy traits displaying moderate heritability (H^2 : 0.43-0.65), demonstrating untapped genetic potential for breeding. Integrated analyses of traits, soil properties, and volatile organic compounds identified potential biological drivers including root architecture, symbiotic nitrogen fixation and soil microbiome. Haplotype mapping identified genomic regions in mungbean associated with wheat yield and protein, revealing trade-offs between direct and legacy performance. Genetic simulations using empirically derived marker effects compared genomic selection strategies targeting mungbean yield, wheat yield, or both simultaneously. Balanced selection (50:50 weighting) achieved simultaneous gains in both crops (19.5% and 7.6%), highlighting the opportunity to breed for system-level productivity with reduced input requirements.

Keywords: genetic legacy, sustainability, crop rotation, haplotype breeding

LEGUMES IN MEDITERRANEAN AGROECOSYSTEMS: LESSONS FROM FOUR DECADES OF RESEARCH IN CHILE

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Mediterranean agroecosystems are characterized by strong seasonal water limitation, high interannual climatic variability, and increasing pressure from climate change. In these environments, legumes play a critical role in sustaining productivity, improving soil fertility, and enhancing the resilience of farming systems. This presentation synthesizes four decades of research on the agronomic, ecological, and productive roles of forage and grain legumes in central Chile. Legume–cereal rotations significantly improve soil nitrogen availability through biological nitrogen fixation, enhance soil structure, and increase subsequent cereal yields while reducing the need for synthetic fertilizers. Grain legumes such as lupin and pea have been evaluated as rotation crops with cereals, contributing to the diversification of production systems and improving resource-use efficiency. Forage legumes have also played a central role in Mediterranean livestock systems. Research on annual legumes and perennial species, particularly alfalfa (*Medicago sativa*), has shown their high potential to provide quality forage while improving soil carbon and nitrogen dynamics. Alfalfa-based systems have proven particularly effective in irrigated and semi-arid environments due to their deep-rooting systems and capacity to maintain productivity under water-limited conditions. Recent research has also explored legume–cereal intercropping systems, which can increase system productivity, improve nitrogen use efficiency, and enhance resilience to drought and climatic variability. Advances in remote sensing and high-throughput phenotyping are opening new opportunities to better understand plant interactions and optimize intercrop management. Overall, legumes are a cornerstone in the development of more sustainable and climate-resilient Mediterranean agroecosystems, offering opportunities to improve soil health, productivity, and environmental sustainability.

Keywords: annual legumes, climate change, crop rotation, intercropping, nitrogen transfer, wheat

AGRONOMIC PERFORMANCE OF NON-TRADITIONAL GRAIN LEGUMES IN DENMARK

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The increasing demand for locally produced plant-based protein highlights the need to diversify grain legume production in northern Europe. In Denmark, peas and faba beans are well established, whereas soybean (*Glycine max* L.) and lupin (*Lupinus* spp.) remain minor crops. Previous attempts to cultivate soybean under Danish conditions have had limited success. This study evaluates the agronomic performance of early-maturing soybean and lupin varieties under Danish field conditions as part of a broader effort to strengthen local protein value chains. Field trials were conducted in the 2025 growing season at AU Viborg (central Jutland) with nine varieties of each crop. Soybean genotypes represented diverse European breeding backgrounds, while the lupin material included white (*L. albus*) and narrow-leaved (*L. angustifolius*) types, including one variety bred under Danish conditions. Agronomic performance, yield and yield components were assessed. Soybean yields ranged from 1.8 to 3.6 t ha⁻¹. Narrow-leaved lupins yielded 1.8–3.1 t ha⁻¹, while white lupins reached 3.5–4.2 t ha⁻¹. Major agronomic challenges included downy mildew in narrow-leaved lupins and lodging in white lupins under high rainfall, which affected grain yield and quality. These preliminary results indicate that both soybean and lupin can achieve promising yields under Danish conditions. The experiment will be repeated in the 2026 growing season to confirm varietal performance and stability. Overall, the findings support the potential of these crops to contribute to crop diversification and locally produced protein. This work was supported by the Lupin&SoyFoods project, funded by the Plant-Based Foods Foundation.

Keywords: *Glycine max* L., *Lupinus* spp., plant-based food

TRANSFORMING SOUTH AFRICAN AGRICULTURE: THE RISE OF SOYBEANS AS A CATALYST FOR SUSTAINABLE PRODUCTION SYSTEMS

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The transformation of South African agriculture through soybean agronomy is a remarkable success. Once dominated by maize monocultures, farming systems have increasingly adopted soybeans, driving sustainable intensification and diversification. Since the early 1990s, soybean area has expanded twentyfold, reaching over 1.1 million hectares by 2024/25, with production exceeding 2.7 million tons. Yield improvements stem from better cultivars, agronomic practices, and input use. Soybeans have enhanced soil health via biological nitrogen fixation, diversified rotations, and disruption of pest and disease cycles. Conservation agriculture like no-tillage, complements soybean production. This progress is underpinned by a coordinated value chain involving research, breeding, seed systems, and processing. Industry bodies such as the Protein Research Foundation, Oilseeds Advisory Committee, and others, have driven investment in crushing capacity (i.e., nearly 3 million tons annually), reducing import dependence and supporting the livestock feed sector. Technological advances include region-specific cultivar selection, improved inoculation, and integrated pest and disease management, drawing on global leaders like Brazil. However, challenges remain such as seed system inefficiencies, export infrastructure gaps, and emerging threats like sclerotinia. Significant future growth lies in expanding into underutilized regions aligned with modern seed technologies and agricultural practices such as conservation agriculture. These “greenfield” areas offer potential for sustainable intensification, but require targeted agronomic packages, infrastructure, and policy alignment. This contribution will show how soybean agronomy has reshaped South African agricultural landscapes, underpinning economic viability and sustainability goals.

Keywords: biological nitrogen fixation; greenfield expansion; soybean agronomy; sustainable intensification; value chain coordination

HOW DOES INTRODUCING LEGUMES TO EUROPEAN CROP SEQUENCES AFFECT ENVIRONMENTAL IMPACTS AND ECOSYSTEM SERVICES: RESULTS FROM A LIFE CYCLE ASSESSMENT STUDY

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Life Cycle Assessment (LCA) is a well-established tool to quantify environmental impacts of products or services, including legumes. Ecosystem service (ES) are included in the LCA methodology to quantify agricultural system impacts. Central ES provided by legumes include e. g. provisioning of protein yield for nutritional purposes, regulation of soil quality by biological nitrogen fixation (BNF) and providing habitats and food for pollinating insects. In this study, we assessed the impacts of introducing legumes in crop sequences on environmental impacts and ES in pilot studies carried out in four European countries using LCA-compatible assessment methods. The results of pilot studies located in two countries, Germany (Brandenburg) and Italy (Umbria), are presented here. The reference crop sequences contained wheat, barley and rapeseed in Germany and wheat and sunflower in Italy. The legumes introduced to the crop sequences included pea or soybean in Germany and faba bean or pea and chickpea in Italy. The results show reduction in several environmental impact categories, such as climate change impact, eutrophication and acidification potential. The protein yield of the crop sequence increased or decreased (total variation of results from -10 to +20 %) depending on which legume were added. BNF was greatest when introducing pea and chickpea in the crop sequences in Italy and soybean in Germany. The negative impact of land use on pollinator abundance decreased slightly when introducing pea or soybean in the crop sequences in Germany, but did not differ between the studied crop sequences in Italy.

Keywords: crop rotation, cropping system, sustainability

CLIMATE-SMART NITROGEN MANAGEMENT: SENSOR-BASED INSIGHTS INTO LEGUME-BASED CROP ROTATIONS

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Maximising the climate mitigation potential of legume-based rotations requires better understanding of the quantity and timing of biologically fixed nitrogen available to crops following legumes, enabling reductions in synthetic nitrogen fertiliser applications and associated greenhouse gas emissions. This presentation reports initial findings from a glasshouse experiment using large soil modules to investigate soil-plant-atmosphere interactions in legume-based rotations in greater detail than possible under complimentary on-farm trials within the wider Nitrogen Climate Smart project. On average, bean crops increased soil available nitrogen by 40 kg N/ha in the upper 30cm soil profile alone, suggesting fixed nitrogen contributions may exceed those suggested in current nutrient management guidelines. Although greater soil nitrogen availability did not increase subsequent wheat yields, both grain and straw nitrogen concentration increased significantly, translating into greater total nitrogen offtakes and supporting the role of beans in achieving protein specification for milling quality wheat. Below-ground imaging evidenced that a bean previous crop supported deeper and more prolific wheat rooting. Ongoing experiments are exploring whether these changes arise from legacy soil structural improvements caused by beans, and whether this could enhance resilience against climate change-induced drought stress. Combining above-canopy imagery with ¹⁵N isotope analysis is determining whether plant physiological and canopy indicators (e.g., yield, biomass, vegetation indices) can act as low-cost proxies for better estimation of biological nitrogen fixation, potentially enabling more precise fertiliser recommendations. Collectively, this research provides actionable evidence for optimising legume-based rotations to improve nitrogen management and deliver greenhouse gas mitigation whilst improving resilience to climate change pressures.

Keywords: greenhouse gas emissions (GHG), biological nitrogen fixation (BNF), resilience, roots, agronomy

DOES MIXED CROPPING OF LUPINS WITH CEREALS IMPROVE YIELD STABILITY AND CROP PERFORMANCE BY FOSTERING ECOSYSTEM SERVICES SUCH AS WEED AND PEST SUPPRESSION?

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Incorporating legumes such as lupins into crop rotations enhances system sustainability by supporting ecosystem services and supplying plant based protein alternatives in human nutrition. Nevertheless, growing legumes is frequently associated with a high risk for farmers due to volatile year-to-year crop performance. Furthermore, overall accessibility of arable land is decreasing. Mixed cropping could buffer risks, increase land-use efficiency and improve crop stands by diversification, resulting in high-quality yields. The aim of this study is to investigate pre- and postharvest crop performance of lupin-cereal mixtures and their pure stands. Two-year field experiments were conducted in 2024 and 2025. All cropping systems included two narrow-leafed lupin varieties and consisted of pure lupin stands, lupin mixtures with oats or wheat, and pure respective cereal stands fertilized according to Swiss national standards or without fertilization. Depending on the companion crop and variety, mixed cropping reduced weed biomass substantially under high weed pressure compared to pure stands. In cereals cereal leaf beetles and aphids dominated in 2024 and 2025, respectively, while in 2025, the aphid pressure was also high in lupins. Under high pressure, the number of aphid colonies in lupins in mixed stands was less than 50% of that in pure stands. The land equivalent ratio (LER) values were below one. The protein yield and protein content could be increased depending on year and pure crop stand performance. This study demonstrates the overall performance and risk-buffer-effect of lupin-cereal mixed cropping systems, supporting decision making for farmers interested in cultivating lupins.

Keywords: narrow-leafed lupins, mixed cropping, crop performance, weed suppression, pest suppression

KICKCHICK: KICKSTART OF CHICKPEA (*CICER ARIETINUM*) GROWING IN FLANDERS

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Chickpea (*Cicer arietinum*) is the main ingredient of falafel and humus, and is adapted to warm, dry climates. Recently, warmer and drier weather has made it possible to grow chickpea in NW Europe. Consumers and food processors in Flanders (Belgium) show increased interest in locally produced plant-based proteins, and chickpea in particular. Although farmers obtain satisfactory yields in dry years, yield stability could be greatly enhanced with better adapted varieties, optimized growing techniques and suitable *Rhizobium* inoculants. In the VLAIO 'KickChick' project, we investigate the effect of sowing time, sowing density, varieties and commercially available inoculants on chickpea yield in Flanders. In spring 2025 mini-plot trials were established at two locations including six sowing dates between March 20th and June 5th, two relevant varieties 'Flamenco' and 'Kira', and four sowing densities (40, 50, 60 and 70 germinating seeds m⁻²), with four repetitions per combination. Average yields were between 2.0 and 4.4 tons/ha for the six sowing dates. 'Flamenco' and 'Kira' yielded on average 3.6 and 3.9 tons/ha, respectively. Higher sowing densities resulted in higher yields: 3.5, 3.6, 3.8 and 3.9 tons/ha for the four sowing densities, respectively. Our results suggested that the optimal sowing period for Flanders is the second half of April against a sowing density of 50 to 60 seeds m⁻². Repetition of the trial in 2026 will consolidate these results. Further tasks within KickChick will focus on improving nodulation, optimizing control strategies for *Ascochyta* disease and *Helicoverpa*. Matchmaking between local producers and processors will be explored.

Keywords: chickpea, growing techniques, NW Europe

TOWARDS LUCERNE VARIETIES FOR USE AS A LIVING MULCH IN CEREAL PRODUCTION

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Perennial, legume-rich grasslands are widely recognized for their ecosystem services such as weed suppression and nitrogen enrichment in crop rotations. However, the decoupling of plant and animal production has led to a decline in these systems. Use of perennial legumes like lucerne as living mulch for cereal production thus gains interest. Yet, lucerne vigorous growth often creates excessive competition with cereals, limiting its adoption. This study focused on defining a lucerne ideotype suited for living mulch and explored the genetic control of the traits involved, aiming to develop dedicated varieties. The research evaluated 30 lucerne accessions representing a wide range of diversity, under both spaced plant nursery and living mulch conditions. Wheat–lucerne interactions were established from the earliest stages of wheat crop cycle. Dormancy, growth habit, height, and lodging of lucerne determined the trade-off between reducing competition with wheat and maintaining the ecosystem services. An intermediate dormancy, a moderate height and a low lodging susceptibility determined the ideotype. Genetic correlations between traits measured in spaced plants and living mulch conditions highlighted varying trait responses: some traits, such as height, remain stable across conditions, whereas others were highly context-dependent. This finding supports a two-step breeding strategy, with firstly an early indirect selection in spaced plant nurseries and secondly an indirect selection under living mulch conditions. Additionally, the use of molecular markers for genomic prediction could accelerate the identification of genotypes best suited for living mulch, ultimately facilitating the development of lucerne varieties that optimize both cereal production and ecosystem benefits.

Keywords: ecosystem service, *Medicago*, cover crop, diversity, breeding

ANALYZING THE GENETIC TOLERANCE OF CHICKPEA GERMPLASM FOR DIFFERENT MODES OF ACTIONS OF HERBICIDES

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Chickpeas are an important source of plant protein, nutrients and minerals for human consumption. However, chickpeas face challenges from weed pressures throughout the growing season on Canadian farms. Chickpea is a poor competitor with weeds, with yield loss ranging from 24% to 88%. Therefore, it is important to find ways to alleviate weed pressures in chickpea through the incorporation of greater and broader herbicide tolerance. The aim of this research is to identify tolerance to different modes of action of herbicides in chickpea to improve weed control, especially for broadleaf weeds. Breeding for improved tolerance to imidazolinone, sulfonylurea, metribuzin, bentazone and saflufenacil/trifludamoxazin chemistries permits the use of these herbicides for broader in-crop weed control. Three groups of chickpea populations, a mutated, interspecific, and cultivated populations were screened in controlled growth chambers for tolerance. At the 6 to 10 node stage, plants were sprayed separately with metribuzin, imidazolinon, sulfonylurea, bentazone, or saflufenacil/trifludimoxazin. Plants were evaluated for injury using a 0 to 10 scale developed at the University of Saskatchewan at 7-, 14- and 21-days post-herbicide application. Varying degrees of tolerance have been noted across all herbicide groups within screenings. The mutated population showed tolerance to imidazolinone with 8 selections rating 1 to 3 and the interspecific population demonstrated tolerance to bentazone in 10 lines with ratings of 1. These selections were increased and rescreened to verify the tolerance. The best three selections from the imidazolinone, sulfonylurea, and bentazone screenings will be sequenced to determine the genetic component associated with the tolerance.

Keywords: herbicides, weeds, biotic stress

BIOSTIMULATION BY GERMANCE IMPROVES SOYBEAN PERFORMANCE THROUGH ENHANCED NITROGEN FIXATION AND SEED PROTEIN QUALITY

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The effects of Germance (a biostimulant highly concentrated plants extracts and applied as seed treatment) were investigated on soybean nodulation, nitrogen acquisition and seed proteome using controlled and field conditions. Preliminary tests in phytotron confirmed that Germance® seed coating did not impair germination (95% success for treated and control seeds). Germance-treated plants tend to have a faster emergence and an increased nodule number ($p = 0.099$), suggesting potential benefits for nitrogen acquisition. Proteomic changes in greenhouse-grown seeds indicated that GERMANCE® led to (i) increase the abundance of sulfur-rich 11S glycinin subunits and (ii) decrease the accumulation of sulfur-poor 7S β -conglycinin subunits. In field trial, Germance significantly boosted plant density at 5 weeks after sowing (45 vs. 36 plants/m²). Shoot biomass remained unchanged at flowering (R1 stage) and early pod formation (R3 stage), but shoot nitrogen (N) content was significantly higher at R3 (+25%). At maturity (R8 stage), Germance did not affect yield components but tended to increase pod density per m² (+40%, $p = 0.092$). Seed N content rose significantly (+10%), and biologically fixed N₂ per m² more than doubled (+119%). Proteomic analysis of field-grown seeds revealed Germance® increased sulfur-rich 11S glycinin subunits (+5.9% for 33 kDa A2 subunit) and decreased sulfur-poor 7S β -conglycinin subunits (−13% for 48 kDa β subunit), confirming data observed under controlled conditions and suggesting positive influence of Germance on sulfur metabolism and protein quality. Overall, these studies have demonstrated that Germance enhances early plant establishment, nitrogen acquisition and seed protein composition of soybean.

Keywords: biostimulation, N₂ fixation, soybean seed protein, quality

BOOSTING CLIMATE RESILIENCE IN MEDITERRANEAN AGROFORESTRY SYSTEMS USING ORPHAN LEGUMES: THE FORECAST APPROACH

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The transition of agroforestry (AF) systems towards climate-smart solutions requires the input of climate-resilient germplasm to minimize farmers' vulnerability. Mediterranean AF systems include olive trees, oaks, chestnut trees, carobs, almonds and date trees, associated with legume cultivation that improves soil fertility and provides high-quality fodder/forage. The FORECAST (Fostering Resilient Agroforestry Systems with climate-ready Tree and Herbaceous Germplasm) project (<https://www.forecast-project.eu/>) is focused on the development of orphan legumes (OLs)-based AF systems, as a crucial step in climate change mitigation. OLs are neglected, underutilised species that represent a unique biodiversity niche adapted to extreme soil/climate conditions, grown by local communities, providing balanced nutrition and added values in rotation systems, and intercropping-based sustainable agriculture. OLs-based value chains are emerging, although most of these climate-ready species are still unexploited (<https://www.benefit-med.eu/>; <https://www.prosper-horizon.eu/>). FORECAST will potentiate the existing AF systems by designing smart combinations of OLs with local tree crops. Highly resilient, locally-adapted OLs and dedicated seed technology will be applied to the existing AF systems, under a range of different socioeconomic/pedoclimatic conditions across the Mediterranean Area. AF/OLs systems will be tested for resilience/adaptation through, working with comparative demo sites located in Italy, Portugal, France, Tunisia, Algeria to evaluate growth performance, abiotic/biotic stress tolerance, and product quality traits. FORECAST will both reinforce the existing value chains and establish new chains, facilitating market access for AF/OLs products, contributing to the economic viability and sustainability of AF/OLs systems, as well as to food security of local population.

Keywords: agroforestry, orphan legumes, intercropping, climate resilience, food security

INTERCROPPING GRASS PEA (*LATHYRUS SATIVUS* L.) WITH SPRING CEREALS UNDER CENTRAL EUROPEAN CONDITIONS: EFFECTS ON PROTEIN AND β -ODAP ACCUMULATION

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Grass pea (*Lathyrus sativus* L.) is a promising climate-resilient protein crop due to its tolerance to both drought and waterlogging. In Central Europe, however, cultivation remains limited because its prostrate growth habit and thin stems increase lodging risk and hinder mechanical harvesting. Intercropping with a supportive companion cereal may reduce lodging and improve harvestability. Another constraint is the neurotoxin β -N-oxalyl-L- α,β -diaminopropionic acid (β -ODAP) present in the seeds, which can cause lathyrism if consumed in excess as part of an unbalanced diet. We evaluated the suitability of grass pea–spring cereal intercropping under Central European conditions in a multi-location trial. Two grass pea cultivars were grown in mixture with five spring cereal species at five locations in Germany and Switzerland over two years (2023–2024). Grass pea protein concentration ranged from 23.1 to 28.8 % and varied mainly with environment, not with cropping system. β -ODAP concentration ranged from 0.08 to 0.46 % and was likewise not affected by intercropping. In contrast, cereal protein concentration increased significantly in mixtures with grass pea across locations. Highest total grain yields were achieved in mixtures with oat and triticale. Overall, intercropping grass pea with spring cereals improved cereal protein concentration while maintaining grass pea protein and β -ODAP concentrations, supporting grass pea cereal mixtures as a viable approach to enhance production feasibility in Central Europe.

Keywords: grass pea, intercropping, protein

CAN WE INTRODUCE COVER CROPS INTO CANOLA AND WHEAT PRODUCTION IN THE CANADIAN PRAIRIES WITHOUT REDUCING YIELDS?

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There is increasing interest in incorporating cover crops into grain cropping systems to help restore soil health, increase biodiversity and increase soil carbon sequestration. However, in semi-arid environments such as the Canadian Prairies, there are concerns that cover crops might compete for soil moisture and reduce grain yields. The current project aimed to find appropriate ways to introduce cover crops and tested their establishment in both wheat and canola, and their residual impact on the subsequent crop in 2-year sequences of canola-wheat and wheat-canola crops at three sites: at the University of Alberta, at the University of Saskatchewan, and at the University of Manitoba, and repeated in time in 2022-2023 and 2023-2024. While not very common, farmers who do plant cover crops tend to do so in the shoulder season, i.e., in September after the harvest of the cash crop. This often fails, so we tried to establish cover crops earlier i.e., mid-season by broadcasting cover crops, or even earlier, by intercropping cover crops as alternate rows with canola and wheat crops or by broadcasting cover crops just prior to planting the main crop. We also tested four different species of cover crops (annual clovers, overwintering clovers, alfalfa and Italian ryegrass (in canola) or phacelia (in wheat)). Results were inconsistent between site-years, establishment methods and cover crop species, so that cover crops sometimes reduced grain yields, either in the first year, in the second year, or both. There may be potential with annual or biennial clovers which will be discussed.

Keywords: cover crops; productivity; cropping systems

PERFORMANCE OF GRAIN LEGUME/CEREAL INTERCROPS IN AGROFORESTRY IN SOUTHERN SWEDEN

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Silvo-arable agroforestry, the combined cultivation of trees and crops on arable land, facilitates the provision of a range of ecosystem services. Until present, little research has been performed on grain legume performance in agroforestry in northern Europe. The SITES (Swedish Infrastructure for Ecosystem Science) Agroecological Field Experiment; SAFE, is a long-term cropping system experiment located in southern Sweden. Grain legumes are included in two of the four systems in SAFE: an organic eight-year crop rotation and an agroforestry system where apple trees and multi-species hedges are planted in rows 12 m apart and the same eight crops as in the organic system are rotated on the land between the tree rows. Since the establishment in 2016, grain legumes have been included three times simultaneously in the organic and agroforestry systems: as intercrops of narrowleaf lupin/spring barley in 2016 and 2024, and faba bean/spring wheat in 2020. Except from a higher yield of lupin grains and above-ground biomass in the organic system in 2016, no significant differences in crop yield or weed biomass has been observed between the two systems. In 2024, eight years after planting the trees, the agroforestry system produced slightly higher (although not statistically significant) grain and biomass yields of both lupin and spring barley. These results show that silvo-arable agroforestry can be implemented in south-Swedish agriculture without losing productivity of grain legume/cereal intercrops, and even indicate a possible advantage over time in organic agroforestry compared to growing same arable crops without trees. More information about SAFE: <https://doi.org/10.23700/4ASX-8870>

Keywords: cropping systems, *Lupinus angustifolius*, organic agriculture, long-term experiment, *Vicia faba*

SEED BIOPRIMING AND HYDROPRIMING ENHANCE COMPETITIVE ABILITY OF GRAIN LEGUMES AGAINST WEEDS

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This study aimed to evaluate the effects of seed priming—hydropriming (HP), biopriming with *Bacillus subtilis* (BP), and their combination (Hydro-Bio priming, HBP)—on the competitive ability of grain legumes (*Lathyrus* sp., *Pisum* sp., *Trigonella* sp.) under field conditions. Field trials used a split-split plot design with three replicates. Main plots: species; subplots: varieties; sub-subplots: seed treatments (T1: unprimed, T2: HP, T3: BP, T4: HBP). Crop performance was assessed via biomass, seed yield, thousand-seed weight (TSW), NDVI, and the weed biomass was measured at multiple stages. All priming treatments reduced weed biomass relative to unprimed controls. In *Lathyrus*, T2 and T3 most effectively reduced weed biomass and enhanced TSW, especially in the Sofades variety. In *Pisum*, T3 and T4 increased TSW, with varietal differences in response. *Trigonella* showed minimal weed pressure; T3 enhanced TSW across both varieties. Hydro-Bio priming (T4) generally provided the strongest improvement in crop competitive ability, particularly for *Lathyrus* and *Pisum*. Seed priming with hydration, biopriming, or their combination enhances competitive ability against weeds in legumes, with effects dependent on species and variety. Hydro-Bio priming shows promise as a sustainable approach to improve establishment, biomass, and yield, contributing to greener cropping systems and improved crop–soil microbiome interactions.

Keywords: biotic stress, seed germination, seedling emergence

LEGUME-DIVERSIFIED CROPPING SYSTEMS FOR POLLINATION SERVICE SUPPORT: AN LCA PERSPECTIVE BASED ON METHODOLOGICAL DEVELOPMENT

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Pollination service plays a pivotal role in maintaining the sustainability of our food systems. Legume-intensive agroecological practices are considered a promising solution to support pollinator abundance by providing suitable habitats and foraging resources. Life cycle assessment (LCA) is a widely accepted methodology for addressing the environmental burdens of a productive system. However, it still lacks standardized methodologies for assessing certain ecosystem services (ES), such as pollination support. Pollinator abundance relies on the availability of habitats and feeding grounds within and beyond the field, which makes mechanistic models for the estimation of pollinator abundance spatially explicit. The only LCA existing methods that attempt to overcome these barriers are based on expert knowledge, which makes the results less accurate, especially at the crop species level. Based on a review of ES assessment methods for legume-based cropping system, we identified the nectariferous value as a promising proxy for estimating pollinator abundance. The conceptual basis for this method is that pollinators are primarily attracted by olfactory and visual stimuli, with nectar producing flowers being among the most important sources of stimuli perceived by these insects. A preliminary screening of nectar production among agricultural crops showed that legumes are the most represented, accounting for more than 50% of the crops evaluated in studies on nectar production and composition. This preliminary screening highlights both the relevance of legumes for pollination support, and the urge to develop new crop-specific methods for LCA.

Keywords: ecosystem services, LCA, pollination

AGRONOMIC PERFORMANCE AND CULTIVATION RISKS OF YELLOW PEA (*PISUM SATIVUM*) UNDER BELGIAN CONDITIONS

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The demand for locally produced protein crops is increasing, driven by interest from consumers, the food and feed industries, agriculture, and research. Yellow pea (*Pisum sativum*) is a promising crop for food applications, while its derivatives and by-products also offer potential for animal feed. Between 2021 and 2025, a series of field trials was conducted at two sites in Flanders, Belgium, to evaluate the agronomic feasibility of winter and spring yellow pea varieties under Flemish conditions. The trials further assessed the effects of fertilisation and biostimulants on yield and quality, and examined pea–cereal mixed cropping systems, which are associated with benefits such as risk diversification and enhanced nitrogen availability for companion crops. Across all trials, several cultivation risks were identified. Bird damage at sowing and harvest emerged as a major challenge, highlighting the necessity of effective bird repellent strategies and appropriate post-harvest processing. To reduce harvest losses caused by lodging, seed shattering, and bird damage, earlier harvesting combined with post-harvest drying proved to be a promising approach. Disease susceptibility was particularly critical in winter pea varieties, with anthracnose causing substantial plant losses, underscoring the importance of distinguishing between winter and spring genotypes. Fertilisation and biostimulant treatments did not result in significant improvements in yield or quality. In mixed cropping systems, successful performance depended on careful matching of pea and cereal varieties, especially with respect to traits such as maturation timing.

Keywords: yellow pea, cultivation risks, feasibility

EFFECTS OF RHIZOBIAL INOCULATION AND NITROGEN FERTILIZATION ON NITROGEN FIXATION IN SOLE AND INTERCROPPED FABA BEAN-WHEAT SYSTEMS

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Sustainable nitrogen (N) management is critical for maintaining crop productivity while reducing environmental impacts. Intercropping legumes with cereals offers a promising strategy to enhance nitrogen use efficiency. However, the effectiveness of N fixation depends on mineral N supply and the performance of rhizobial strains. We examined whether inoculation with specifically selected rhizobia strains increased the N fixation when faba bean was grown alone or intercropped with spring wheat. A mesocosm experiment (1 m height, 20 cm diameter) was conducted using field soil with the upper 30 cm consisting of topsoil. Cropping systems included faba bean sole cropped (cv Stella) and faba bean–wheat (cv Thorus) intercropping. Each system was grown under two N fertilization levels and with and without rhizobial inoculation. The rhizobacterial strain (*Rhizobium leguminosarum* bv. *viciae* iSM597) was specifically chosen for the soil properties. In the treatment without inoculation indigenous rhizobial strains were present. Plant biomass, height and chlorophyll index (SPAD), as well as root biomass and nodule number were determined. Rhizobial strains occupying the nodules were identified to assess inoculation success and competitiveness. Finally, soil and plant N was determined and N fixation based on the ¹⁵N natural abundance method. Preliminary results show that inoculation increased N content in leaves under low fertilization reflected by a higher chlorophyll index and that more nodules were found on the tap root. This did not seem to affect biomass production during the experimental period. Results on soil N dynamics, N fixation and rhizobia strains in nodules will be presented.

Keywords: intercropping, inoculation, nitrogen dynamics

ASSESSING HOW LEGUME CROPS AFFECT SOIL EROSION

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Soil erosion remains a major threat to agro-ecosystem sustainability, particularly in regions where high rainfall erosivity coincides with vulnerable land use systems. Legume based cropping systems have been widely assessed for the delivery of ecosystems services such as soil erosion control. Here current evidence on the role of legumes in controlling erosion-related processes was reviewed, considering both legume fodder and grain crops. Annual legumes such as faba bean and common vetch, as well as mixtures including subterranean clover, show rapid canopy development and high soil surface cover, reducing C-factor values (of the Universal Soil Loss Equation, USLE) from approximately 0.3-0.4 under conventional management to below 0.1. Perennial legumes such as alfalfa and sainfoin further enhance erosion control by maintaining continuous cover and developing extensive root systems. Belowground, legume roots improve soil resistance to erosion by increasing infiltration capacity and aggregate stability, with infiltration rates increasing several-fold relative to bare soil and soil cohesion gains commonly ranging from 1.5 to 8 kPa, depending on species and rooting strategy. Although legume residues decompose faster than grass residues, their integration into diversified cropping systems contributes to sustained soil structural resilience. Overall, this review provides a process-based and quantitative synthesis supporting the strategic use of herbaceous legumes in erosion-control and soil conservation systems. The gathered evidence is mostly based on a series of field experiments in Mediterranean, Temperate and semiarid climate which could be further expanded with new sites and longer monitoring to improve the robustness of the conclusions.

Keywords: legume, soil erosion, ecosystem services

A SYSTEMATIC MULTI-SITE EVALUATION OF NITROGEN FIXATION IN EUROPEAN PURE STAND AND MIXED INTERCROPPING SYSTEMS

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The use of grain legumes in European cropping systems reduces the reliance on mineral nitrogen (N) fertilizer, diversifies crop rotations, and contributes to European plant protein self-sufficiency. Mixed intercropping of annual legumes with cereals may enhance resource-use efficiency through niche complementarity and facilitation. Compared with pure stands, intercropped legumes increase their percentage of fixed atmospheric N₂ (Ndfa%), thereby sparing mineral N for the accompanying cereal. However, biological nitrogen fixation (BNF) and N uptake in European mixed intercropping systems have not been systematically researched. Over two years, as part of the Horizon Europe LEGENDARY project, we established identical field experiments across eight European countries. Four annual legume species – faba bean (*Vicia faba*), narrow-leaved lupin (*Lupinus angustifolius*), lentil (*Lens culinaris*) and field pea (*Pisum sativum*) – were grown in pure stands and in mixtures with oat (*Avena sativa*) or barley (*Hordeum vulgare*). Ndfa% and BNF were determined using the 15N natural abundance method. Results showed significant effects of year, cropping system and an interaction between lupin and its pure stand on Ndfa%. Mixed intercropping significantly increased Ndfa% for faba bean, field pea, and lentil by 14–17% ($p < 0.001$) compared to pure stands. Although BNF decreased in mixed intercropping due to significantly reduced legume biomass, total N uptake – representing the sum of cereal and legume N – remained comparable to pure legume stands. This demonstrates complementary N resource use and confirms legume-cereal mixed intercropping systems as a robust strategy to enhance N-use efficiency across diverse European agroecological conditions.

Keywords: nitrogen, biological nitrogen fixation, mixed intercropping, field experiments

LEGUME PERFORMANCE IN VALERECO'S (EU-HORIZON) FIELD TRIALS IN GREECE

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VALERECO's scope is to identify and assess the environmental and economic value of the Ecosystem Services (ES) of legumes through the establishment of nine Living Labs (LLs) in 11 European countries. An objective of this underway EU Horizon Project is to optimize legume cultivation through implementing sustainable cultural practices. A two-factor field trial (split-plot arrangement) was established in the LL in Central Greece during the 2024–2025 growing season. Crop rotation was the main plot factor with seven levels: lentil (cv. 1)-wheat-sulla, lentil (cv. 2)-wheat-sulla, wheat-wheat-wheat, sulla-lentil (cv. 1)-wheat, sulla-maize-sulla, sulla-wheat-sulla. Weed management was the sub-plot factor with five treatments: sole crops with mechanical weeding (T1), sole crops untreated (T2), intercropping without weed control (T3), cover cropping (oats + white mustard) without weed control (T4) and cover cropping followed by additional weed control (T5). Treatment T5 enabled lentils to produce the highest grain yields ($> 800 \text{ kg ha}^{-1}$). The performance of T5 treatment may be attributed to the weed suppressive potential of the cover crop mixture combined with the follow-up mechanical weeding. However, sulla produced high amounts of biomass that were close to $2,000 \text{ g m}^{-2}$ even in T1 plots where weed biomass was quite high ($> 600 \text{ g m}^{-2}$) indicating its high competitiveness in contrast to lentils. In all plots, NDVI was a reliable indicator of crop yield ($R^2 > 0.70$). Our results underline that integrating cultural practices as cover cropping and mechanical weeding can lead to the optimization of legume cultivation in the Mediterranean region.

Keywords: legumes, ecosystem services, sulla, wheat, lentil

IMPACT OF INTRODUCING LEGUME CROPS TO AGRICULTURAL CROPPING SYSTEMS ON THE CLIMATE IMPACT AND SOIL ORGANIC CARBON LEVELS: A GLOBAL-SCALE META-ANALYSIS

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Increasing the share of legume crops in agricultural cropping systems has been identified as a way to improve nutrient management and decrease environmental impacts of agriculture. Especially the climate impact mitigation and related soil organic carbon (SOC) sequestration potential have been of particular interest to date. Legumes can be introduced to cropping systems in various ways, including as a main crop (as part of crop rotation), intercrop, cover crop, green manure and ley mixtures with grasses. In our review, we analysed 114 Life Cycle Assessment studies comparing the climate impact and/or SOC change of cropping systems with and without legume crops. In total, 256 cropping system comparisons were selected for analysis. Legumes were most frequently introduced in cropping systems containing cereal crops, such as wheat, maize or rice. In total, 30 different legume crops were included in the studies, the most studied ones being soybean, pea, peanut and faba bean. On average, the introduction of legumes reduced the climate impact both per field area and product mass in all types of cropping systems (except for cover cropping) on all continents and climatic regions regardless the cropping system length or legume crop species added. There was a statistically significant positive correlation between the difference in crop N fertilization level and difference in climate impact, meaning that reduced N fertilization resulted in reduced greenhouse gas emissions. SOC was increased in all types of cropping systems after introducing legumes, but there was large variation in the magnitude of the increase.

Keywords: environmental impacts, LCA, sustainability

DOES MOLYBDENUM NUTRI-PRIMING ENHANCE BIOLOGICAL NITROGEN FIXATION IN FABA BEAN (*VICIA FABA* L.)?

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Molybdenum (Mo) is an essential micronutrient for biological nitrogen fixation (BNF) due to its structural role in the nitrogenase enzyme. Seed priming with Mo has been reported to enhance nodulation and BNF in several grain legumes, yet evidence remains limited and highly variable, and data for faba bean are scarce. This study investigated whether Mo seed priming influences early nodulation and BNF in faba bean, and tracked the fate of seed-derived Mo during early plant growth. Seeds were primed with water or sodium molybdate under contrasting conditions designed to generate low and high seed Mo contents, alongside unprimed controls. Preliminary experiments confirmed that Mo priming substantially increased seed Mo concentration, with Mo accumulating predominantly in the seed coat. Germination and early seedling growth were not adversely affected by Mo priming, although longer priming duration enhanced germination and seedling vigour irrespective of Mo addition. Two independent glasshouse experiments were conducted under N- and Mo-free conditions using an inert sand-perlite growth medium and inoculation with *Rhizobium leguminosarum* bv. *viciae*. Nodulation was assessed 30 days after sowing and BNF-related parameters at 60 days. Contrary to expectations based on the literature, Mo seed priming did not significantly affect nodule number, nodule biomass, or plant N accumulation at either priming level. Mo redistribution during growth was also assessed. The results highlight the importance of Mo localisation and timing, and caution against assuming seed priming as an effective Mo delivery strategy for enhancing BNF in this species.

Keywords: faba bean, molybdenum, priming, nitrogen fixation

INFLUENCE OF GREEN MANURING WITH LEGUMINOUS AND GRAMINEOUS SPECIES, AS AN AGROECOLOGICAL ALTERNATIVE, ON THE PROPERTIES AND FUNCTIONS OF AN AGRICULTURAL SOIL IN THE CAMPO DE CARTAGENA (SE SPAIN)

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This study was conducted on an agricultural farm located in the Campo de Cartagena (Murcia, Spain). The results demonstrated that green manuring, had a positive impact on the soil's physical and chemical properties, particularly under saline conditions. Following the incorporation of green manure, soil organic carbon content increased by 12.5%, enhancing soil structure, moisture retention, and cation exchange capacity. This increase appeared to stimulate microbial activity and improve the biogeochemical balance of the soil, thereby promoting its natural regeneration. The cultivation of oat and vetch helped reduce the concentration of soluble salts (Na^+ and Cl^-) and nitrates (NO_3^-) in the soil, owing to plant phytoextraction and subsequent leaching facilitated by irrigation. This process contributed to the desalination of the surface horizon and improved overall soil quality. As a leguminous species, vetch (*Vicia sativa* L.) fixed atmospheric nitrogen through symbiosis with *Rhizobium* bacteria, thus reducing the dependence on synthetic fertilizers. Oat (*Avena sativa* L.), in turn, contributed biomass and carbon, balancing the soil C/N ratio and optimizing nutrient availability for the subsequent crop of coriander (*Coriandrum sativum* L.). The use of green manures not only improved soil fertility and structure but also enhanced its role as a carbon sink, with a positive carbon balance exceeding 4.5 tonnes of carbon per hectare. This reinforces the function of soil as a climatic regulator and a protective factor against desertification in semi-arid regions such as the Campo de Cartagena. This practice represents a viable and agroecological alternative to the intensive use of mineral fertilizers.

Keywords: green manures, legumes, soil fertility, agroecological alternative, ecosystem services

HAIRY VETCH MANAGEMENT STRATEGIES TO ENHANCE ECOSYSTEM SERVICES AND RESOURCE-USE EFFICIENCY IN SORGHUM GROWN UNDER RAINFED CONDITION

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In Mediterranean rainfed systems facing heat and water constraints, diversification strategies such as cover crops and intercropping can enhance ecosystem services (ES) while reducing dependence on external inputs. In this context, legumes are central because they enhance soil functioning and nutrient availability, supporting sustainable production under low-input conditions. The EU Horizon Europe VALERECO project aims to quantify and increase the value of ES delivered by legumes, supporting their adoption. Within VALERECO, a field trial evaluates hairy vetch (*Vicia villosa* Roth cv. Villana) as a legume cover crop preceding sorghum (*Sorghum bicolor* (L.) Moench cv. PR88Y47), focusing on ESs delivery and system resource-use efficiency. The experiment follows a randomized complete block design with three treatments: (i) no-cover, (ii) vetch managed as a relay intercrop and maintained as a living cover until strip-till sorghum seeding, and (iii) vetch managed as green manure before sowing. We expect different ES outcomes, with relay intercropping enhancing soil cover and weed suppression, and green manure increasing N supply to sorghum. To quantify the multifunctional value of vetch, we measure vetch biomass and N content, sorghum N uptake, and indicators of N-use efficiency. We also assess soil cover, weed density and biomass, and sorghum yield and quality. Results will support management recommendations for hairy vetch to optimize N contributions, weed regulation and resource-use efficiency, improving subsequent sorghum performance and quality in Mediterranean rainfed cropping systems.

Keywords: cover crops, crop diversification, strip tillage, green manure, agroecology

POST-BURN SOIL RECOVERY AND LEGUME (*ONOBRYCHIS VICIIFOLIA*) FORAGE PERFORMANCE USING ORGANIC AMENDMENTS

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Slash-pile burning of accumulated biomass during re-cultivation of abandoned agricultural land can alter soil chemical properties, making post-burn recovery dependent on targeted organic amendments and soil-improving forage legumes. This study aimed to evaluate how farmyard manure (40 t ha⁻¹), biochar (40 t ha⁻¹) with NPK 15:15:15 (450 kg ha⁻¹), and NPK fertilization alone (450 kg ha⁻¹) influence the recovery of soil organic matter, nutrient availability and the forage productivity of *Onobrychis viciifolia* one year after slash-pile burning. The study was conducted in the coastal part of Croatia (Baćina location) on an abandoned agricultural land affected by slash-pile burning. Soil sampling was conducted in three replications two days after slash-pile burning in November 2023 and repeated one year later in November 2024. In summer 2024, *Onobrychis viciifolia* was sown in a randomized design with three replications according to the designated amendment treatments. The forage yield of *Onobrychis viciifolia* was determined during the third cut in November 2024. In total, 18 composite soil samples and nine representative forage samples were collected and analysed. Despite the slash-pile burning event, the soil exhibited remarkably favourable initial chemical properties, with high organic matter (28.4 ± 7.22%), abundant P₂O₅ (559.4 ± 150.8 mg kg⁻¹) and K₂O (1896 ± 623 mg kg⁻¹), and elevated total nitrogen (0.568 ± 0.09%), indicating a nutrient-rich starting point for subsequent recovery. In light of the high initial nutrient and organic matter levels, the analysis of variance revealed no significant treatment effects on most soil chemical properties one year after slash-pile burning. Only total nitrogen statistically differed among treatments (0.438% in the biochar treatment, 0.548% under farmyard manure, and 0.492% with mineral fertilizer). Similarly, forage yield did not differ significantly among treatments, amounting to 1.41 ± 0.14 t ha⁻¹ DM in the biochar treatment, 1.60 ± 0.26 t ha⁻¹ DM under mineral fertilization, and 1.54 ± 0.26 t ha⁻¹ DM with farmyard manure. Overall, the study indicates that high initial fertility overshadowed amendment effects, leading to minimal soil and yield responses one year after burning. These findings imply that in highly fertile post-burn soils, amendment application may not be required during the early recovery phase. This work was supported by the PRIMA Foundation through the SHARInG-MeD project ("Soil Health and Agriculture Resilience through an Integrated Geographical information systems of Mediterranean Drylands", GA number 2211).

Keywords: slash-pile burning, biochar, farmyard manure, leguminous, total nitrogen

DESCRIBE EXISTING CROPPING SYSTEMS IN FRANCE TO ASSESS THE IMPACT OF LEGUMES ON THEIR ECONOMIC AND ENVIRONMENTAL PERFORMANCE

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Assessing the economic and environmental performance of cropping systems is crucial in order to advise the various stakeholders in their strategical decisions. This may involve creating a reference framework of situations that are easily identifiable by farmers and their advisers. It may also be useful to carry out simulations requiring a realistic description of cropping systems to provide information at regional level. In both cases, a typological method can be useful for condensing large datasets into a limited number of scenarios that are easy for users to simulate and understand. The Land Parcel Identification System provides a comprehensive description of crop sequences since 2015 at the field level. Based on Ballot et al. 2023, a processing chain has been adapted by Terres Inovia to produce “crop sequences” types in France in two steps: 1) unsupervised classification according to the frequency of return of each crop; 2) identification of representative individuals (i.e. real fields) for each type. The method is applicable to any spatial resolution. We implemented it to produce assessments of economic performance of cropping systems with and without grain legumes in the former French administrative regions. It will also be implemented to assess differences in agronomic and environmental performance between types of cropping systems on a scale that allows for similar soil and climatic conditions.

Keywords: cropping system, typology, LPIS, grain legumes, economic performance

SEED DORMANCY, SOIL PROPERTIES AND PH SHAPE EARLY ESTABLISHMENT IN LUPINUS COVER CROPS

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Early establishment is a key bottleneck for using *Lupinus* spp. as winter cover crops in Mediterranean perennial systems, where heterogeneous soils and variable pH constrain emergence and seedling growth. We assessed how physical seed dormancy (PD), soil properties and substrate pH act as complementary filters shaping early performance. Fifty accessions of *L. angustifolius*, *L. luteus* and *L. albus* (wild materials, landraces and cultivars) were evaluated in three assays: (i) PD and germination dynamics under scarified vs non-scarified conditions; (ii) emergence and establishment across five contrasting orchard soils (pH 5.6–8.4; organic matter 0.8–2.5%); and (iii) controlled substrate pH treatments (5.5, 7.0, 8.5) measuring germination, emergence and early growth. Scarification accelerated and synchronized germination, confirming PD as a genotype-dependent trait, yet PD alone did not predict establishment across soils. Soil properties were a dominant filter, with significant species×soil effects: acidic and moderately alkaline soils supported high establishment, especially in *L. albus* and *L. luteus*, whereas highly alkaline, low-organic soils restricted performance. Substrate pH had limited effects on final germination but delayed emergence and reduced early growth under alkaline conditions, particularly in *L. luteus*. Weak correlations among PD, soil response and pH tolerance indicate largely independent filters, supporting integrative selection and targeted deployment of resilient lupin cover-crop ideotypes matched to local soils. This work was carried out within the MAXI-CoherLEG Operational Group and funded by the CAP Strategic Plan 2023–2027 (80% EU EAFRD co-financing; 20% MAPA, Spain). Miriam Gallardo is funded by the PIF2024 doctoral thesis completion grant (Ref. 38031).

Keywords: physical dormancy, *Lupinus*, genotype × environment interaction, soil performance, pH tolerance

INSERTING SUCCESSFULLY GRAIN LEGUMES IN FOOD AND CROPPING SYSTEMS

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Despite their potential for sustainable agriculture, grain legumes occupy only 2–5% of arable land in Europe, compared to ~15% globally. This reflects long standing path dependencies that have locked farming systems into cereal dominated rotations aimed at reducing costs and supporting exports. The French consortium INSEREZ LES operates on the principle that overcoming these bottlenecks requires coordinated innovation across the entire value chain, from production to processing. To help farmers successfully integrate pea, faba bean, lupin, soya, chickpea, and lentil, the project adopts a farmer centric approach addressing agronomic and economic dimensions. Climate change adaptation remains a major challenge. Through modelling and experiments, the partners identify limiting factors for six species and assess current and future yield potentials and production areas. Two strategies are investigated to improve yield levels and stability: co innovation with farmers to design adaptive crop management, and risk mitigation through optimized varietal choice based on tolerance and complementarity, supported by extensive genotypic and phenotypic datasets. To enhance value creation, the project explores low capital processing methods (dehulling, fermentation) as well as ecosystem service remuneration schemes. Research examines variability in processing efficiency and sensory properties of prototype products (on farm dehulling, couscous or biscuit applications of legume flours and fermented grains). Ecosystem service characterization targets preceding crop effects, greenhouse gas mitigation, and entomofauna support. Multi actor case studies will translate this into guidelines for valuing positive externalities. Project outcomes will feed tools supporting French farmers' decisions to promote value creation and climate resilient legume based systems.

Keywords: coordinated innovations, added value, yield gap, seed transformation, decision-making

AGROECOLOGICAL EFFECTS OF MAIZE–BEAN MIXED CROPPING: IMPLICATIONS FOR YIELD, WEEDS, PESTS AND BENEFICIALS

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The European feed sector is expected to reduce reliance on imported protein sources. Simultaneously, the European Green Deal aims to reduce fertiliser and pesticide use. Mixed cropping of cereals with legumes is frequently proposed as a strategy to maintain productivity with reduced inputs. However, existing research focuses on specific legume species and environmental challenges, with limited attention to their potential to reduce pesticides. This study evaluates the effects of cropping system and fertiliser level on silage yield, as well as incidences of weeds, pests and beneficials. Field experiments were conducted in 2024 and 2025 with maize, bean and maize–bean mixed cropping at 100% and 75% of the Swiss recommended nitrogen fertiliser rate. In 2024, the dry biomass yield of the mixed crop was significantly lower than maize, whereas no effects of cropping system or fertiliser level were observed in 2025. Nevertheless, the Land Equivalent Ratio exceeded 1 across all fertiliser levels, indicating a higher land-use efficiency in mixed cropping compared with sole cropping. The weed pressure was low in both years and was unaffected by cropping system and fertiliser level. Aphids were the most prevalent pest insects in both years. Regarding natural enemies, hoverfly eggs dominated in 2024 and lacewing eggs in 2025. Bean sole crops had most floral visitors, followed by beans in the mixed crop and maize in the mixed crop. By combining agronomic and agroecological parameters, this study evaluates the potential of maize–bean mixed cropping as a strategy to reduce environmental impacts while maintaining productivity.

Keywords: intercropping, biodiversity, pollinators, silage

CHICKPEA (*CICER ARIETINUM* L.) VARIETY RESPONSES TO SEEDING DENSITY: PRELIMINARY RESULTS FROM A MULTI-YEAR TRIAL IN SWITZERLAND

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Grain legume consumption in Switzerland remains low (2 kg/year/capita) and largely import-dependent, with imports exceeding domestic production by a factor of three. Among them, chickpeas (*Cicer arietinum* L.) show strong import growth: from 1280 t (2017) to 1900 t (2022; +48%), reflecting rising demand. While chickpeas are considered climate-resilient under projected warming, agronomic data under Swiss conditions remain limited. A multi-year field trial (2022–2025) at two sites evaluated three varieties (Cicerone, Flamenco, Olga) differing in plant habit (erect and semi erect) and branching intensity (low, middle and strong), across five seeding densities. Yield components, thousand-kernel weight (TKW), and maturity were analysed using linear mixed models to account for spatial and temporal variation. Plant density was the dominant factor influencing yield, with distinct optima at 53, 61.5, and 76 plants m⁻² for Olga, Flamenco, and Cicerone, respectively. Strong effects of year and site, and a significant interaction between site and variety, were detected. Mean yield across site-years was 2.1 t/ha (range: 0.3–4.2 t/ha). Among yield components, number of pods and TKW explained the largest proportion of yield variance, followed by the number of seeds per pods. Higher densities significantly reduced pods per plant and side branching, and lowered water content at maturity. Seeds per pod varied significantly among investigated varieties, independent of density. These results highlight density-dependent compensation mechanisms and emphasize the importance of variety-specific management strategies for sustainable chickpea production in Switzerland. Despite strong year-site effects, choosing variety-specific densities seems essential for successful chickpea growing.

Keywords: chickpea, seeding density, grain legume, varieties

AGRONOMIC AND ECONOMIC COMPLEMENTARITY PROMOTES SUBSTANTIAL GAINS IN A PEA-LENTIL INTERCROP

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Crop mixtures can improve land-use efficiency and yield stability, yet their adoption is often constrained by agronomic and economic challenges. Most successful intercrops combine cereals with legumes, relying on ecological complementarity, specifically complementary nitrogen-acquisition strategies. Here, we demonstrate that substantial and stable benefits can also arise from a legume–legume intercrop. We evaluated mixtures of lentil (*Lens culinaris*) and pea (*Pisum sativum*) across three years and two farms with contrasting soil pathogen pressures in Switzerland, testing seeding ratios and varietal combinations. Following an initial optimization year, optimal intercrops, consisting of 25% pea and 75% lentil, consistently outperformed monocultures. Specifically, semi-leafless pea varieties sown at such low densities interconnected and formed a supportive canopy that improved lentil standability and harvestability. This was most pronounced under extreme weather conditions that otherwise increased lodging and disease pressure. Since peas and lentils differed strongly in their susceptibility to soil-borne foot diseases, the mixture reduces risks of legume soil fatigue and allows for more flexible crop rotation strategies. In summary, the intercrop achieved land equivalent ratios of 1.38 and generated 24% higher economic returns than expected based on monoculture yields, or 10% more than the higher-valued crop. Our results show that agronomic and economic complementarity—rather than classical ecological niche differentiation—can underpin productive, stable, and market-oriented intercrops, offering a viable pathway to increase gluten-free plant protein production.

Keywords: intercropping, *lens culinaris*, *pisum sativum*, land-use efficiency

TRACING THE FATE OF ^{13}C AND ^{15}N IN NEW CHICKPEA CULTIVARS TO SOIL ORGANIC MATTER

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With concerns of greenhouse gas (GHG) emissions from agricultural production, it is important to evaluate the role of chickpeas in the mitigation of GHGs to lower agriculture's carbon (C) footprint. Isotopic labelling is a powerful tool to trace the C and nitrogen (N) fate in plant-soil systems. However, there is a knowledge gap regarding the fate of ^{13}C and ^{15}N in chickpeas, especially the new chickpea cultivars in soil organic matter (SOM) formation. Three kabuli chickpea cultivars were selected for the research: one new (CDC Hardy), one popular (CDC Leader), and one older (CDC Frontier). We evaluate the C and N inputs derived from a new chickpea cultivar to SOM, the extent of C decomposition and sequestration, and subsequent N credit from above and below-ground biomass inputs to soil. The $^{13}\text{CO}_2$ labelling was conducted weekly until plant maturity in growth chamber, with a one-time application of ^{15}N Urea fertilizer in rate proportionate to the desired plant enrichment levels. This research will determine whether CDC Hardy will have greater biomass C and N inputs compared to older and most popular chickpea cultivars and, therefore, increased C sequestration and N credit for subsequent crops. A decomposition study swaps ^{13}C and ^{15}N enriched materials with control unenriched materials to trace the decomposition of the above-and below-ground biomass to the soil through a six-month incubation period. This research will generate information on the impact of chickpea cultivars on C and N inputs to soil, therefore increasing soil C sequestration and increasing Canadian Prairie soil quality.

Keywords: isotope labelling, ^{15}N , ^{13}C , chickpea

SUMMER LEGUME COVER CROPS ENHANCE NITROGEN SUPPLY AND WINTER WHEAT YIELD

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Cover crops play a key role in maintaining soil health and supporting sustainable crop production. In addition to winter cover crops, summer-sown species can rapidly produce high biomass and improve nutrient availability for subsequent winter crops. A field experiment was conducted in Estonia in 2024–2025 to assess summer cover crop performance under reduced tillage prior to establishing *Triticum aestivum* L. Cover crops were sown in mid May 2024, and aboveground biomass was sampled in early August. The species included *Crotalaria juncea*, *Vicia villosa*, *Lupinus angustifolius*, and a mixture of *Vicia sativa* with *Avena sativa*. *Hordeum vulgare* served as the control. *Crotalaria juncea*, despite its high biomass and nitrogen accumulation potential in warmer regions, failed to establish under northern conditions and became weed infested, indicating limited suitability for cooler climates. The *Vicia sativa*–*Avena sativa* mixture accumulated the highest biomass (11.6 t ha⁻¹) and nitrogen (275 kg N ha⁻¹), with *Vicia sativa* accounting for the majority of both biomass and N accumulation. *Vicia villosa* grown alone also performed strongly (9.3 t ha⁻¹; 197 kg N ha⁻¹), whereas *Lupinus angustifolius* produced the lowest biomass (2.7 t ha⁻¹). *Triticum aestivum* exhibited higher grain yield following cover crops compared with *Hordeum vulgare*, indicating that continuous cereal rotations may constrain yield potential, whereas summer cover crops can improve conditions for subsequent winter cereals.

Keywords: biomass; nitrogen fixation; sustainable agriculture; northern climate

BIO-TEST FOR POWDERY MILDEW (*ERYSIPHE TRIFOLII*) IN RED CLOVER (*TRIFOLIUM PRATENSE*)

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The biotrophic pathogen *Erysiphe trifolii* causes powdery mildew which reduces productivity in European red clover (*Trifolium pratense*). Breeding for resistance is impeded by the outcrossing nature of red clover and the variation in disease intensity between years and locations, which renders selection after field infection inefficient. A bio-test to identify resistant plants before planting in the field is needed. In the BELIS project (www.belisproject.eu), we optimized a bio-test to screen young red clover plants for mildew resistance. Six-week old plants were placed on a greenhouse table covered with a transparent plastic hood. Infected plants of the susceptible variety 'Violetta' were placed between the young plants to ensure an even spread of the inoculum. After two to three weeks, young plants were scored and the disease index was calculated. Repeatability was assessed by screening 6 varieties (192 per variety) with varying resistance levels on three different moments. Average disease indices ranged between 77% (Tandy) and 22% (Violetta). Ranking of variety averages was highly similar in the three reps and correlations between variety averages were $R = 0.94$ to $R = 0.98$. Cutting plants and allowing re-infection resulted in similar disease levels ($R = 0.99$), allowing breeders to cut plants and postpone scoring when necessary. Our bio-test may speed-up breeding efforts for mildew-resistant varieties. Future research will explore the correlation between disease scores obtained in our bio-test and in a naturally infected field. In addition, we are optimizing a method to maintain powdery mildew on red clover plantlets in vitro.

Keywords: *Trifolium pratense*; powdery mildew; resistance; bio-test; breeding

CROPDIVA: ENHANCING EUROPEAN AGROBIODIVERSITY

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Within the European project CROPDIVA, we focused on two legumes, lentils (*Lens culinaris*) and narrow-leaved lupin (*Lupinus angustifolius*), and assessed their performance in mixed cropping with cereals (hull-less barley (*Hordeum vulgare* var. *nudum*) or oats (*Avena sativa*)) compared with pure stands. Mixed cropping followed a replacement design with three varieties per species. Field experiments were conducted over two years at two sites. Overall, mixtures enhanced productivity and yield stability. Compared with pure lentil stands, mixtures with hull-less barley increased the yield by 13% and the land use efficiency by 15%, while reducing weed biomass by 42% and lodging by 36%. The yield gains were mainly driven by improved hull-less barley performance in mixture. Furthermore, barley protein content rose by 1.8 to 2.6% in mixed stands, whereas lentil protein and barley beta-glucan contents were unaffected. Relative to pure lupin stands, the weed pressure in lupin-oat mixtures declined by up to 83%, and the cereal leaf beetle infestation in oats decreased by 41 to 46%, depending on the variety combination. The oat protein content increased from 11 to 14%, and hectolitre weight improved by 2 kg/hl, while the lupin protein content remained unchanged. A Europe-wide field experiment (five locations, three years) further showed that lupin-oat mixtures achieved yields comparable to pure stands while stabilising yields across years and locations. Future research will focus on practical implementation, particularly assessing economic viability under farm conditions, optimising plant protection strategies for species mixtures, and improving post-harvest processing to ensure grain quality and marketability.

Keywords: lentil, lupin, mixed cropping, LER

INTERCROPPING OF TRADITIONAL PEA VARIETIES WITH FABA BEAN IN ORGANIC AGRICULTURE

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Cultivation of pea together with faba bean is a fairly unexplored legume-legume intercrop combination that used to be a common traditional farming practise in parts of Sweden. This study evaluated the grain yield potential of faba bean-pea intercropping with a replacement design in organic farming, utilising a wide range of pea varieties including both modern and local traditional varieties (represented by old varieties and landraces) in various seeding ratios. The experiments were conducted over the course of three years in the south of Sweden. Many of the intercrop combinations that included traditional pea varieties produced similar or higher total grain yields compared to pure stands of the modern reference varieties of either one or both species. The different pea varieties showed different competitive impact on the faba bean companion crop. Traditional pea varieties that suffered from severe lodging in pure stands was provided standing support when intercropped with faba bean. In many cases there was a difference in the maturity time between the two species, but some pea landraces had later maturation similar to that of the faba bean. If this intercropping method is to be applied by farmers, it should be considered that many varieties of both species have overlapping grain size and shape, which may complicate post-harvest separation. This study has provided key knowledge to understand relevant design parameters for faba bean-pea intercropping – highlighting the importance of utilising the right combination of variety choice and seeding ratio.

Keywords: traditional crop varieties, legume-legume intercropping, crop diversification, *Vicia faba*, *Pisum sativum*

FIELD EVALUATION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES UNDER SLOVAK CONDITIONS WITHIN THE EVA NETWORK

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Within the framework of the European Evaluation Network (EVA) for Legumes project, a field evaluation of selected genotypes of common bean (*Phaseolus vulgaris* L.) was carried out in 2025 with the aim of assessing their agronomic performance, yield potential, and variability of morphological traits under Slovak environmental conditions. The experiment was established in a lowland area at an altitude of 165 m above sea level, on predominantly black soil, with a long-term average precipitation of 563.6 mm and an average temperature of 6–8 °C. A total of 16 genotypes were included in the evaluation, comprising 10 Slovak varieties and 6 varieties originating from Serbia. The field trial was conducted in two replicates, with a sowing density of 13 seeds per meter and managed using standard agronomic practices, including protection against weeds, diseases, and pests. Evaluation was performed according to the *Phaseolus vulgaris* descriptors (IBPGR, 1982), and a total of 19 traits related to growth, development, and the morphology of plants, pods, and seeds were recorded. Full maturity of the evaluated accessions was reached within 122–128 days, while flowering began 44–59 days after sowing. The number of pods per plant ranged from 9 to 47, and the height of the first pod varied between 8 and 16 cm. Considerable variability in seed size and color was observed among genotypes. The highest grain yield was achieved by the Kreola accession, whereas Lilana, Marta, and Nugetka were characterized by large to very large seeds, indicating promising breeding potential and adaptability. This work was supported by the project Implementation of the ECPGR European Evaluation Network on legumes and preparatory actions for the creation of a new network on perennial plants (berries and fruit trees) – EVA Boost, funded by the German Federal Office for Agriculture and Food. The study was carried out under Grant Agreement No. L26ROM112. This work was supported by the Agency for the promotion of research and development within the project APVV-24-0226 “Uniqueness of genetic diversity of Slovak bean: Innovative approaches for increasing resistance to phytopathogenic fungi and viruses”.

Keywords: common bean, genetic resources, agronomic evaluation, grain yield, morphological variability

CONVERGENT AND PARALLEL EVOLUTION SHAPE SEED DORMANCY AND POD DEHISCENCE DURING LEGUME DOMESTICATION

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Domestication is characterized by phenotypic modification under sustained human selection, often followed by diversification to accommodate diverse agricultural practices and environments. Similar domestication-related traits may evolve independently through parallel evolution in closely related taxa or via convergent evolution in more distantly related lineages. These phenotypic changes commonly arise from alterations in conserved regulatory networks or biosynthetic pathways, such that analogous traits may be controlled by homologous loci (genetic parallelism) or by distinct genetic determinants (genetic convergence). Domestication therefore represents a selection-mediated process, largely imposed by humans, that promotes the fixation of traits advantageous under cultivation, collectively termed the domestication syndrome. Here, we present a comparative analysis of independently domesticated legume crops from two major centers of domestication: chickpea, lentil, and pea from the Fertile Crescent, and common bean from Mesoamerica. Focusing on two key domestication traits, loss of seed dormancy and altered pod dehiscence. We integrate phenotypic characterization with comparative transcriptomic and metabolomic profiling across species and domestication status. Cross-species differential gene expression analyses, orthology inference, and network-based approaches are combined with metabolite profiling, including lignin content and monolignol composition in pods. This integrative framework enables the identification of conserved and lineage-specific molecular signatures underlying domestication-related phenotypes and permits direct assessment of the relative contributions of parallel and convergent genetic mechanisms. Elucidating the molecular and biochemical bases of these traits advances our understanding of crop evolutionary history and provides actionable targets for modern breeding strategies aimed at improving resilience and sustainability.

Keywords: convergent evolution, domestication, legumes, pod dehiscence, seed dormancy

SEEDS AS ECOSYSTEM ENGINEERS: METABOLOMIC PROGRAMMING OF SPERMOSPHERE MICROBIAL ASSEMBLY AND EARLY-LIFE RESILIENCE IN COMMON BEAN

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Germinating seeds actively shape their surrounding microenvironment, the spermosphere, through the release of a complex mixture of primary and specialized metabolites that modulate microbial assembly and diversity, creating conditions that support successful establishment of the autotrophic seedling. In common bean (*Phaseolus vulgaris*), we characterized the spermosphere of eight genotypes grown in two contrasting environments. We demonstrated that both genotype and production environment determined the composition of seed exudates and associated microbial communities, revealing genotype-specific chemical signatures correlated with distinct microbial assemblages. The activity of selected metabolites on microbial growth was experimentally validated, confirming their functional role in shaping these communities. We further investigated how seed physiological status, particularly aging and vigour loss, alters spermosphere chemistry and microbial interactions. Using integrative multi-omics approaches, including untargeted metabolomics and microbiome profiling, we characterized how genotype, environment, and seed aging collectively influence metabolite–microbe associations, including interactions with diverse or opportunistic microorganisms. This framework positions the seed as an ecosystem engineer, orchestrating its microbial niche through biochemical signalling, with early-life implications for resilience, establishment success, and buffering against environmental and biotic stress. These findings provide a comprehensive view of how genetic, environmental, and physiological factors converge to shape spermosphere biodiversity and highlight the ecological significance of seed exudates in plant–microbe interactions and early plant establishment.

Keywords: *Phaseolus vulgaris*, seeds, spermosphere, metabolome, microbiome

SEARCHING FOR THE NEXT *RHIZOBIUM* SUPERSTAR USING HIGH-THROUGHPUT SCREENING APPROACHES

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Legumes form symbiotic associations with nitrogen-fixing rhizobia housed in root nodules, providing an environmentally sustainable alternative to synthetic nitrogen fertilizers with high ecological costs. The effectiveness of rhizobial symbiosis is determined by two key traits: the ability to fix nitrogen efficiently and competitive fitness, defined as the capacity to successfully colonize the host and occupy nodules in the presence of other strains. Dry beans (*Phaseolus vulgaris*) engage in symbiosis with rhizobia but generally exhibit lower rates of symbiotic nitrogen fixation compared to other legume-rhizobia systems. One possible explanation is the promiscuous nature of dry beans, which associate with a broad range of rhizobial strains, many of which are suboptimal nitrogen fixers. To address this challenge, we conducted a high-throughput competition experiment using a library of approximately 300 rhizobial strains isolated from dry bean nodules collected in North Dakota. Our objectives were to (i) identify rhizobial strains with elite competitive abilities for nodule occupancy and subsequently characterize their nitrogen-fixation efficiency with dry beans, and (ii) determine whether the most competitive and effective strains vary depending on host genetic background, represented by different dry bean market classes. Rhizobial strains were competed for nodule occupancy on pinto, black, and kidney bean cultivars, and competitiveness was quantified using next-generation sequencing of unique DNA barcodes. The nitrogen-fixation effectiveness of highly competitive strains was further evaluated. Overall, this study aims to improve nitrogen fixation and productivity in dry beans by identifying rhizobial strains that combine strong competitive ability with high symbiotic effectiveness.

Keywords: elite Rhizobial isolates, symbiotic nitrogen fixation, dry bean (*Phaseolus vulgaris*), common bean

PHENOTYPIC PLASTICITY FOR THE RECRUITMENT OF SOIL MICROBIOMES IN THE CHICKPEA RHIZOSPHERE AND NODULE

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The interaction of plant and soil microbiomes is a source of ecosystem complexity with implications for plant adaptation to environmental stress. However, interactions between plant genotype, soil microbiome, and environment remain largely unexplored. This study investigated the rhizosphere soil and legume nodule microbiome in a factorial combining four contrasting chickpea (*Cicer arietinum* L.) genotypes and five rainfed field environments spanning a 10-fold range of plant productivity primarily associated with drought stress. We quantified the rhizosphere effect (RE) as the normalised difference in diversity of microbial communities between bulk soil and rhizosphere, and its plasticity in response to environment. Rhizosphere effect was genotype-dependent and varied with bacterial and fungal communities. Two genotypes maintained a stable RE regardless of conditions, and two genotypes enhanced recruitment of bacterial communities under increasing stress. Genotypic ability to modulate the rhizosphere bacteriome was negatively correlated with their N fixation under harsh conditions ($r = -0.7$). Fungal RE showed a non-linear relationship with environmental stress, peaking at moderate stress in all chickpea genotypes. In nodules, symbiotic rhizobia consistently dominated (~95% abundance) across all environments and genotypes, resulting in a low plasticity of nodulation effect on microbial recruitment. We identified and isolated four non-rhizobial bacterial strains (*Burkholderia* spp. and *Pseudomonas* spp.) that stably colonised nodules. Three of these strains promoted nodulation and N fixation through production of indole-3-acetic acid. In an agronomically relevant, large-scale field setting, we provide novel insights into the role of plant microbiome plasticity with implications for legume productivity in drylands.

Keywords: chickpea, rhizobia, microbiome, environmental stress

COMPARATIVE METABOLOMIC PROFILING OF *LUPINUS ALBUS* AND *L. ANGUSTIFOLIUS* HARVEST RESIDUES: EXPLORING CHEMICAL DIVERSITY AND VALORIZATION POTENTIAL

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The increasing demand for *Lupinus* species as sources of food and animal feed has led to a parallel rise in the generation of post-harvest residues. These residues are commonly left in the field for burning or soil incorporation, representing an underutilized biomass resource with potential environmental impacts. This study investigates the metabolomic composition of harvest residues from different *Lupinus albus* and *Lupinus angustifolius* varieties to identify species-specific biochemical signatures and evaluate their potential for value-added applications. Methanolic extracts of the residues were analyzed using UHPLC–MS/MS, leading to the tentative identification of 181 metabolites. Among these, saponins and flavonoids were identified as the predominant compound classes. The two species exhibited distinct metabolomic profiles. *L. albus* residues were characterized by higher levels of isoflavonoids such as luteone, as well as flavonols and flavanones occurring as aglycones, glycosylated, and malonylated derivatives. In contrast, *L. angustifolius* extracts contained higher concentrations of saponins, particularly soyasaponins, together with flavones including chrysoeriol derivatives and isoflavones such as genistein and wighteone derivatives. Correlation analysis revealed a positive relationship between flavonoid abundance and antioxidant activity, whereas saponins showed a negative correlation with antioxidant potential. These findings highlight complementary valorization opportunities for *Lupinus* residues. The antioxidant-rich profile of *L. albus* suggests potential applications in nutraceutical, pharmaceutical, and functional food products. Meanwhile, the high saponin content of *L. angustifolius* indicates promising potential for the development of biopesticides and antimicrobial agents, supporting circular bioeconomy approaches through agricultural waste valorization.

Keywords: *Lupinus* residues, metabolomics, saponins, flavonoids, agricultural waste valorization

HARNESSING GENOMIC DIVERSITY AND HOST-RHIZOBIA SYMBIOSIS TO ENHANCE SYMBIOTIC NITROGEN FIXATION IN CHICKPEA

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Symbiotic nitrogen fixation (SNF) results from metabolic interactions between legumes and rhizobia whereby the host plant provides carbon and a low-oxygen environment to rhizobia, which in turn reduce atmospheric nitrogen to ammonia to support host protein synthesis. This symbiosis may be exploited to improve grain protein content and benefit soil fertility. However, the host genetic potential for SNF and the relationships between SNF traits, soil nitrogen concentration, and yield remain poorly understood. We evaluated 289 diverse chickpea accessions in replicated glasshouse trials to measure the proportion of nitrogen derived from atmosphere (NDFA) via mass spectrometry, alongside biomass and nodulation-related traits. Applied nitrogen concentrations of 0.5 mM and 7 mM N enabled selection under permissive and repressive soil-N conditions, with plants harvested at 8 weeks. Results indicated clear repression of nodulation and NDFA under high N. Trait narrow-sense heritabilities were generally lower under high N conditions. Biomass traits were consistently highly heritable, whereas nitrogen fixation and nodule traits were moderate to highly heritable under low N and low to moderately heritable under high N. The magnitude of genetic correlations between traits across conditions was generally high, with biomass traits positively related to NDFA. The correlations suggest that selection on NDFA would result in strong, correlated responses under alternate conditions. Thus, SNF appears to be positively associated with biomass, which may serve as a proxy for yield. To further exploit this host-symbiont interaction while preserving genetic diversity, we plan to utilize FastStack technology to improve SNF across diverse chickpea germplasm.

Keywords: symbiotic nitrogen fixation, genomic selection, nitrogen derived from atmosphere, Rhizobia, biodiversity

OPTIMIZING BEAN INOCULANTS FOR CANADIAN VARIETIES: ENHANCING BIOLOGICAL NITROGEN FIXATION IN COMMON BEANS

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Common beans (*Phaseolus vulgaris*) are generally less efficient in biological nitrogen fixation (BNF) compared to other legumes. In Canada, variable performance of bean inoculants has reduced confidence and limited adoption by growers, increasing reliance on synthetic nitrogen fertilizers. Identifying effective, crop-specific *Rhizobium* strains and responsive bean genotypes is essential for improving nitrogen use efficiency and supporting sustainable soil and crop management. This study evaluated the BNF potential of a 177-genotype Northern Bean Diversity Panel inoculated with two *Rhizobium* strains isolated in Canada (Rh258 and Rh71). Plants were grown in a sterilized vermiculite–sand medium under controlled conditions and assessed for chlorophyll status using normalized difference vegetation index (NDVI), nodulation, and biomass traits at flowering. Both inoculants significantly improved plant performance relative to uninoculated controls, with clear treatment effects across NDVI, nodulation, and shoot and root biomass. Inoculation enhanced canopy vigor and nodulation capacity, while nitrogen-fertilized controls promoted biomass accumulation but suppressed nodulation traits. Trait-specific differences between the two inoculants were observed, indicating functional specialization rather than uniform responses across phenotypes. Substantial genotypic variation and genotype-specific responsiveness to inoculation were detected, highlighting the importance of host–microbe interactions in BNF efficiency. Notably, older released varieties and landraces exhibited stronger and more consistent responsiveness to inoculation than many modern lines, suggesting that BNF responsiveness may have been unintentionally reduced during historical bean breeding under high-input systems. Findings support re-integrating BNF responsiveness into breeding programs, and developing genotype-specific inoculant strategies is key for sustainable, low-input production systems.

Keywords: BNF, common bean, *Rhizobium*

QUANTIFYING NITROGEN FIXATION IN LEGUMES USING CRISPR-DERIVED NON-NODULATING MUTANTS

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Legumes can form beneficial symbiotic relationships with rhizobia, which convert atmospheric nitrogen into ammonia that can be used by the plant, reducing reliance on synthetic fertilisers. However, quantifying the contribution of biological nitrogen fixation (BNF) remains challenging, as plants draw nitrogen from both soil and symbiotic sources. Stable non-nodulating mutants can provide a useful baseline to quantify BNF. Nodulation is initiated by host perception of rhizobial signalling molecules, Nod Factors (NFs), by two NF receptors called NFR1 and NFR5, located on the root epidermis. Disruption of NFR5 inhibits NF perception and results in a non-nodulating phenotype. This makes it an ideal target for generating legumes unable to form a symbiotic relationship even in the presence of compatible rhizobia. We have identified NFR5 encoding gene orthologs in mung bean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and common bean (*Phaseolus vulgaris*). Using CRISPR/Cas9, novel vectors have been designed to target conserved functional domains. We are now generating knockout mutants in these species that can be used in future trials as a baseline for quantifying to what extent nitrogen is derived from BNF in these species. This research will provide a translational framework for improving BNF and nitrogen use efficiency in legume crops. Updates on current progress will be presented.

Keywords: biological nitrogen fixation, CRISPR/Cas9

DUAL ROLES OF PEA CCD7 IN STRIGOLACTONE-DEPENDENT AND -INDEPENDENT PROCESSES AS REVEALED BY TRANSCRIPTOMIC PROFILING AND MYCORRHIZATION ASSAYS

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Strigolactones (SLs) are carotenoid-derived molecules that act as both rhizosphere signals and plant hormones. Although they play pivotal roles in plant development and signaling, our understanding of their functions, particularly in response to environmental stresses, remains limited. Moreover, the SL biosynthetic enzyme CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7) has been proposed to exert SL-independent biological roles, but experimental evidence is lacking. To address this, we analyzed root transcriptomic profiles of the wild type (WT) pea cultivar 'Térèse' and the two near-isogenic mutants *rms5*, defective in CCD7, and *rms1*, defective in CCD8. Gene Ontology enrichment analysis revealed that SL deficiency suppresses stress-related processes, including responses to salt, heat, and hydrogen peroxide, as well as cellular functions related to cell cycle progression, cell division, and protein and amino acid metabolism. LC-MS/MS profiling revealed significant cross-talk with stress-related hormones, with SL levels positively correlated with abscisic acid and negatively correlated with jasmonic acid. Analysis of mutant-specific transcriptomic changes indicated that CCD7 contributes to several biological processes beyond its role in SL biosynthesis, including light-related functions, apocarotenoid metabolism, and mycorrhization. Colonization assays further demonstrated a SL-independent positive role of CCD7 in mycorrhization, as *rms5* mutants exhibited reduced fungal colonization compared to *rms1* and WT. Overall, our findings reveal a broad involvement of SLs in plant stress adaptation and uncover additional CCD7-mediated pathways beyond canonical SL biosynthesis, which are potentially linked to the accumulation of 9-cis- β -carotene-derived apocarotenoids or alternative CCD7 substrates.

Keywords: strigolactones, carotenoid cleavage dioxygenases, *Pisum sativum*, transcriptomics, mycorrhization

MATCHMAKING: UNVEILING OPTIMAL SOYBEAN-RHIZOBIA MATCHES IN NORTH-WESTERN

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Bringing soybean to colder North-western European climates is important to sustain the demand for high protein food without compromising the environment in doing so. Using nitrogen-fixing rhizobia that are already adapted to the colder conditions is one way we hope to achieve this goal in this collaborative project between VIB, ILVO and Ghent University. After the success of “Soy in 1000 Gardens” (Méndez et al., 2025), we have taken interesting rhizobial candidates from this previous work forward to identify the most efficient matches between them and different soybean genotypes. This will help us achieve our goal in bringing soybean agriculture to new regions by allowing soybean to achieve the high protein content required for human consumption. We are also unravelling the questions on: What makes a good or bad match between rhizobia and legumes? What are the underlying mechanisms driving this? Are there any other helpful (or “cheater”) bacteria hiding inside the nodules? We are combining this with testing our combinations under different environmental conditions (e.g. drought) in the lab and the field. This will allow us to construct a matchmaking matrix to find the best soy-rhizobia partnerships, no matter the weather. This a large project with many interesting aspects to it, from using AI to help us count and measure thousands of nodules to using GWAS analysis to uncover the genetic mechanisms behind efficient nodulation. This project celebrates the amazing partnership between nitrogen-fixing rhizobia and legumes.

Keywords: rhizobia, nodulation, efficiency, soybean

VARIATION IN SEED COBALT CONCENTRATION IN FIELD PEA (*LATHYRUS OLERACEUS* LAM.) AND FABA BEAN (*VICIA FABA* L.) GENOTYPES: IMPLICATIONS FOR BIOLOGICAL NITROGEN FIXATION

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Legumes such as field pea (*Lathyrus oleraceus* Lam., formerly *Pisum sativum* L.) and faba bean (*Vicia faba* L.) are important protein sources for both animal feed and human consumption. Enhancing their agronomic performance and nutritional value requires improved understanding of key factors regulating biological nitrogen fixation (BNF), a central process that drives nitrogen supply and seed protein accumulation in legumes. Cobalt is essential for nitrogen-fixing bacteria due to its role in the coenzyme cobalamin. Adequate cobalt levels during early symbiotic establishment, either through inherent seed cobalt content or external application, may improve nodulation and nitrogen fixation efficiency. However, natural variation in legume seed cobalt concentrations across species and genotypes and the implications for BNF remains poorly characterised. We examined a panel of faba bean and pea genotypes including both older, local varieties and modern cultivars (14 in total). Each genotype was grown organically at three to five locations in Denmark, representing different soil types. Seed mineral composition was quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and seed cobalt concentrations were used as a proxy for relative cobalt uptake within genotypes. Protein concentrations and nitrogen isotope ratios were determined by Isotope Ratio Mass Spectrometry (IR-MS), and the relative contribution of BNF was estimated from the isotopic composition. The potential correlations with cobalt uptake, protein concentrations and BNF capacity are presented at the conference. In addition, within-genotype responses to contrasting soil types are assessed, providing insights for optimising cobalt fertilisation strategies aimed at improving BNF efficiency.

Keywords: cobalt, biological nitrogen fixation, plant nutrition, pea, faba bean

SCREENING OF DIVERSE MUNGBEAN GERMPLASM UNDER LOW AND HIGH NITROGEN REVEALS DIVERSITY ACROSS SYMBIOTIC NITROGEN FIXATION AND RELATED TRAITS

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Mungbean (*Vigna radiata*) is a highly nutritious summer pulse with a growing global market. One of its key benefits is its ability to carry out symbiotic nitrogen fixation (SNF), allowing it to thrive in low-N soils without fertiliser-N inputs. There is natural variation of SNF in mungbean, with genotypes showing diversity in SNF traits such as percentage of nitrogen derived from the atmosphere (%Ndfa). Therefore, there is potential to enhance SNF mungbean by harnessing the genetic diversity already present within the species. A Grains Research and Development Corporation-funded initiative intends to do this by first characterising the genetic diversity of SNF in mungbean. In glasshouse conditions, over 300 mungbean genotypes were screened for SNF activity. Each genotype was evaluated firstly under low nitrogen favourable to SNF and again under high nitrogen inhibitive of SNF. Genotypes were ranked for SNF effectiveness by assessing percent and total Ndfa. In a low-N trial, the current Australian industry check, Crystal, exhibited 71% Ndfa, while some genotypes in the diversity panel showed up to 78% Ndfa, demonstrating useful variation of this trait. Additional traits including harvest index and phenology were also recorded. Phenotypic data analysis revealed strong broad-sense heritability for key traits including shoot biomass with relatively little variation due to the spatial or blocking effects. With this robust phenotypic data genome-wide association studies aim to pinpoint genomic regions associated with SNF effectiveness. Identified SNPs and haplotype data can then be employed by breeders to develop improved varieties through marker-assisted selection and predictive breeding strategies.

Keywords: symbiotic nitrogen fixation, GWAS, mungbean

SYMBIOSIS-RECRUITED GENETIC TRAITS FOR SUSTAINABLE FABA BEAN PRODUCTION

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The legume crop faba bean (*Vicia faba*) is a rich source of protein for food and feed. In addition to its high nutritional value, it can be invaluable in sustainable crop rotations leaving surplus nitrogen in the soil demonstrating its high biological N₂ fixation ability. However, the low biotic and abiotic stress resilience of present faba bean cultivars results in low yield stability. For these reasons, faba bean grows on only 0.7% of the arable land in Germany (FAOSTAT). The development of more resilient faba bean cultivars is therefore most important. Drought stress is a major constraint in yield stability of faba beans by affecting flower development. It can further reduce the size and activity of the nitrogen fixing nodules in grain legumes. In addition to enhancing nutrient supply, beneficial microbes can protect plants against stress. This phenomenon resembles plant systemic immunity in response to a prior stress and is defined as priming, representing a genetically determined plant process that induces higher tolerance against stresses. To access this still untapped beneficial potential of microbes requires the analysis of the interactions of below-ground plant-microbe interactions under stress. Priming can be induced by the endophytic fungus *Serendipita indica* in plants. Upon root colonisation, *S. indica* provides systemic (leaf) and local (root) resistance against diseases and tolerance against abiotic stresses, including drought stress tolerance in a broad range of legumes. As a clearly determined genetic trait, we aim to identify gene candidates that mediate priming-induced protection against drought stress in faba bean.

Keywords: *Vicia faba*, drought stress, priming, *Serendipita indica*

PEPTIDE-BASED SEED TREATMENT ENHANCES NODULATION IN SOYBEAN

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Biological nitrogen fixation in legumes offers a major opportunity to reduce dependency on synthetic nitrogen fertilisers, yet nodulation efficiency and symbiotic performance remain highly sensitive to environmental conditions. We report on the development and mechanistic evaluation of a peptide-based seed-coating derived from protein side-streams from the food industry, developed by the company Stamagro. The seed treatment is designed to enhance the interaction between soybean and nitrogen-fixing rhizobia. Application of the product has been shown to increase soybean yield in field conditions and nodule number in open-air pot trials. We hypothesise that specific peptide fractions can act as plant signalling modulators, strengthening early symbiotic establishment and improving nodule functionality under both optimal and stress conditions. Using controlled nodulation assays, we evaluate effects on nodule number, nodulation timing, and markers of nitrogen fixation activity. In parallel, in vitro bacterial assays assess potential impacts on rhizobial survival and transcriptional responsiveness to host-derived signals. Layered screening across soybean genotypes, rhizobial strains and environmental variables will assess robustness and context dependency. Complementary molecular analyses will investigate whether the biostimulant influences signalling pathways associated with flavonoid biosynthesis, Nod factor perception and nodule development. This work illustrates how protein side-stream valorisation can be translated into targeted biostimulant innovation, contributing to higher legume productivity with reduced nitrogen input.

Keywords: soybean, rhizobia, peptide seed-coating, nitrogen-fixation

HOST GENETIC BACKGROUND MODULATES ROOMIE1- ASSOCIATED MICROBIAL RECRUITMENT AND TRANSCRIPTOMIC RESPONSES IN *LOTUS JAPONICUS*

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Root microbiome establishment is a critical process for plant health. It is under the control of host genetics which allows plants to select beneficial microbes while excluding pathogens. We previously identified ROOMIE1, a key regulator involved in rhizospheric microbiome assembly in *Lotus japonicus* through genome-wide association analysis. Here, we investigate the mechanistic basis of this control using transcriptomic profiling of roomie1 mutants alongside natural accessions from Iwadeyama and Utobara that carry allelic variation in ROOMIE1. We identified the microbial taxa *Magnetospirillum*, *Novosphingobium*, *Geobacter*, and *Pelobacter* were significantly enriched in roomie1 mutants grown in Utobara soil. The same genera were enriched in most of the natural Iwadeyama and Utobara genotypes both grown in Utobara soil, though the recruitment was markedly more pronounced in Iwadeyama genotypes. Interestingly, the largest overlap with the roomie1 mutant transcriptomic profile was observed in a distinct subset of Iwadeyama genotypes, which conversely showed lower enrichment of these ROOMIE1-associated microbes compared to other Iwadeyama individuals. Furthermore, we observed strong correlations between the expression of specific host genes and the abundance of these bacteria in Iwadeyama genotypes, whereas Utobara individuals did not exhibit these transcriptomic-microbial interactions. This standing variation within Iwadeyama's population, combined with the ROOMIE1 allelic variation, suggests a tightly regulated, genotype-dependent microbial recruitment strategy when plants are subjected to non-native soil. Together, our findings provide a foundation for understanding ROOMIE1-orchestrated microbiome establishment, which we aim to further elucidate through transcriptomic variant calling, untargeted metabolomics, microbial isolation, and targeted mutagenesis.

Keywords: *Lotus japonicus*, root microbiome, transcriptomics, host genetics, plant-microbe interactions

HILUM, THE ENIGMATIC REGION OF THE SEED COAT

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The seed coat (SC) forms a protective barrier shielding the developing embryo from biotic and abiotic stresses. The hilum, a scar marking the former attachment site of the funiculus, represents a distinct anatomical region of the SC that is specified early during seed development. The activity of a seed coat-specific pea polyphenol oxidase (PPO) differs between black and pale hila and determines their pigmentation. PPO-catalyzed oxidation of phenolics likely reinforces this mechanically vulnerable region (the “Achilles’ heel”) and provides precursors for melanin biosynthesis. To elucidate PPO function *in vivo*, we produced recombinant PPO and characterized its substrate specificity, providing insights into its role in seed development and melanin formation. In addition, PPO activity and hilum pigmentation were associated with differential accumulation of reactive oxygen and nitrogen species in the hilum region. Pyrolysis–gas chromatography–mass spectrometry revealed significant differences in condensed tannin composition between black and pale hila, with enrichment of galocatechin units in the former. Beyond pigmentation, the hilum also functions as an abscission zone between the seed and the funiculus. Transcriptomic analyses identified differential expression of ethylene- and auxin-related genes between a hilum-deficient mutant and the wild type, which forms a normal hilum. Consistently, phytohormone quantification confirmed their distinct accumulation patterns between these genotypes. Together, our results highlight the hilum as a multifunctional and developmentally specialized region that integrates mechanical protection, pigmentation, redox regulation, and hormone-mediated control of seed development. Acknowledgement: This work is supported by Grant Agency of Czech Republic (24-10730S) and TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK.

Keywords: abscission, funiculus, melanin, *Pisum*, polyphenol oxidase

REINTRODUCTION OF GRASS PEA (*LATHYRUS SATIVUS*) INTO ALGERIAN AGRICULTURE: GENETIC DIVERSITY, YIELD POTENTIAL, AND INCORPORATION INTO ANIMAL FEED

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Although grass pea (*Lathyrus sativus*) has demonstrated strong tolerance to harsh environmental conditions, particularly under climate change, its cultivation in Algeria remains marginal, despite its historical presence in local farming systems. This study evaluates the agronomic performance and feed value of local and foreign accessions to support the reintroduction of this species into Algerian agriculture. The field assessment of 52 local and foreign grass pea accessions demonstrated substantial genetic diversity. Seed yield exhibited the highest variability (Genetic Coefficient of Variation (GCV) = 41.67%), followed by biomass production (GCV = 21.10%), whereas protein content showed limited variation (GCV = 10.56%). Accessions originating from eastern Algeria produced higher biomass and seed yield, suggesting strong local adaptation and valuable genetic resources. Biomass of all studied accessions averaged between 16.2 and 100 q/ha while seed yield ranged from 1 to 36 q/ha. The potential use of grass pea in animal feeding (rabbit case) was evaluated by comparing a conventional diet based on imported maize and soybean meal with three experimental diets in which soybean meal was partially replaced by grass pea at 20%, 30% and 40%. Animals fed diets containing 20% and 30% grass pea exhibited higher weight gains (587.22 g and 365.86 g, respectively) than those receiving the conventional diet (265.2 g), indicating efficient utilization of grass pea proteins at moderate inclusion levels. Overall, these findings highlight grass pea as a well-adapted crop for semi-arid regions, with significant potential to improve protein self-sufficiency in animal feed and promote sustainable livestock farming.

Keywords: *Lathyrus sativus*; grass pea; alternative plant protein; genetic variability; sustainable animal feeding systems

COATING BEAN SEED WITH VEGETAL RESIDUES IMPROVES GERMINATION AND TRICHODERMA DEVELOPMENT

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Seed treatment using a coating is a common practice in agriculture production. The coating is applied to the surface of the seed to protect the seed and improve germination. Biocontrol is a pest management strategy making use of living natural enemies, antagonists, or competitors, and other self-replicating biotic entities. The use of biocontrol agents (BCAs) is a sustainable way to control phytopathogens. BCAs need a carbon source to develop properly and thus be able to protect plants. However, the biological resources used as carbon sources, such as peat are finite and extracting them directly from our planet's ecosystems maybe unsustainable. The aims of this study are to evaluate the development of Trichoderma isolates in hop waste using the qPCR technique, to check the effect of pelleting with hop waste on the germination of bean seeds. The results demonstrated the potential of hop cone residues as an effective substrate for the development and application of Trichoderma suggesting that the cone material serves as a suitable carbon source. When hop powder was combined with Trichoderma isolates in bean seed coatings, the performance was improved, particularly under field conditions. In bean field trials, hop-based coatings consistently outperformed peat-based formulations, particularly in integrated production systems. These results highlight the agronomic potential of hop waste as a sustainable carrier for biocontrol inoculants in legume seed coating applications.

Keywords: circular bioeconomy; qPCR technique; biocontrol agents; by-products; sustainable waste management; microbial inoculants

NON-MODEL SPECIES: MISSING PIECES IN THE LEGUME EVOLUTION PUZZLE

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Legumes (Fabaceae) represent one of the most diverse and ecologically important plant families, playing a crucial role in nitrogen fixation and sustainable agriculture. While several model species have been extensively studied, genomic information on non-model legumes remains limited, constraining our understanding of genome evolution and diversification within the family. Here, we report genome draft assemblies of ten non-model legume species: *Gymnocladus dioica*, *Adenolobus pechuelii*, *Ceratonia siliqua*, *Faidherbia albida*, *Albizia chevalierii*, *Dichrostachys cinerea*, *Senna obtusifolia*, *Chamaecrista mimosoides*, *Cassia sturtii*, and *Desmanthus velutinus*. In this study, we explore their genomes to investigate patterns of gene content variation, duplication, and loss across lineages. Using high-throughput sequencing and comparative genomics, we examine the genomic basis of adaptive traits and evolutionary transitions related to symbiotic nitrogen fixation, stress tolerance, and ecological specialization. Our analyses reveal lineage-specific genomic rearrangements and gene family dynamics that have shaped the evolution of key functional traits in legumes. By integrating genomic and evolutionary perspectives, this work provides new insights into the mechanisms driving legume diversification and establishes foundational genomic resources to support sustainable agriculture and enhance crop resilience under changing climate conditions. Acknowledgments: This work was supported by National Science Centre project number: 2016/21/D/NZ8/01300.

Keywords: genome draft assemblies, non-model legumes, symbiosis

INTERACT: UNDERSTANDING EVOLUTION OF COMMON BEAN PLANT-PLANT INTERACTIONS TO DEVELOP A MORE SUSTAINABLE AGRICULTURE

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Agro-ecosystems have been strongly shaped by crop domestication and modern breeding, which have simplified biotic and abiotic interactions and reduced genetic diversity in cultivated systems. Climate change increases the challenges faced by agriculture, highlighting the importance of understanding plant–plant interactions as a strategy to improve sustainability. Despite their relevance, studies on intraspecific plant interactions remain limited. The INTERACT project aims to identify key regulatory pathways involved in intraspecific plant–plant interactions in *Phaseolus vulgaris* by integrating transcriptomics, metabolomics, and microbial community analyses. The evolutionary history of common bean, characterized by two independent domestication events in the Mesoamerican and Andean gene pools, provides a unique replicated system to investigate these mechanisms. Eighteen genotypes (nine per gene pool), representing wild types, landraces, and modern cultivars, were selected using materials and data from previous projects (INCREASE, ...). Controlled experiments evaluate 90 genotype combinations, including 18 pure stands and 72 mixed pairs (wild–landrace, wild–cultivar, and landrace–cultivar). The experiments follow a complete randomized block design with three replicates per genepool and are conducted in 276 pots containing a uniform soil mixture. Two parallel experiments are performed. In the destructive experiment, root, leaf, and soil samples are collected for RNA extraction, metabolomic profiling, and microbial community analysis. The non-destructive experiment focuses on seed production, phenotypic trait assessment, and seed quality evaluation using multispectral imaging. This integrative approach will identify genes, metabolites, and microbial dynamics associated with plant–plant interactions, supporting breeding strategies to improve crop resilience, productivity, and sustainability.

Keywords: plant–plant interactions; metagenomics; transcriptomics; metabolomics

NUE-LEG: 'ELITE' RHIZOBIA FOR NOVEL FORAGE LEGUMES

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For NUE-Leg (Nitrogen Use Efficiency Legume) which aims to minimize the reliance of UK grasslands farms on synthetic nitrogen fertiliser, we are characterising rhizobial symbionts for a hybrid white clover [*Trifolium repens* L. x *T. ambiguum* M.Bieb.], 'DoubleRoot', a novel red clover [*T. pratense* L.], 'RedRunner' that forms stolons, and a grazing tolerant birdsfoot trefoil (*Lotus corniculatus*; capable of reducing methane production by ruminants). We started by identifying ~235 potential rhizobial strains from culture collections as well as newly 'trapped' symbionts isolated from soils in Scotland, collected at a lowland arable farm and an upland mixed farm. No 'Lotus-compatible' rhizobia could be trapped from the upland farm. 16S rRNA blastn similarity to type material indicated that the majority of the clover-associated strains share high sequence identity with *Rhizobium brockwellii* (previously *R. leguminosarum* genospecies A), while the Lotus-associated strains were more diverse, with strains forming part of several rhizobial genera including *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium*. A BOX PCR-fingerprinting approach was followed to demarcate the clover-associated strains to distinct 'BOX profiles', from which a representative was selected to include in ex-situ pot trials measuring biomass and confirming nodulation. Multi-locus sequence analysis of the clover *Rhizobium* strains has indicated that there could be possible novel species within this group while the pot trials have shown that there are different 'elite' strains between 'DoubleRoot' and a traditional white clover, 'AberSwan'. Future analyses will include in-field testing of the 'best performing' strains from the pot trials, as well as whole genome sequencing of promising strains.

Keywords: clover, *Lotus corniculatus*, *Rhizobium*, *Mesorhizobium*

TRANSPOSABLE ELEMENT DIVERSITY AND ITS POTENTIAL ROLE IN SYMBIOSIS REGULATION EXPLORED WITH NEW CHROMOSOME-SCALE ASSEMBLIES OF THE MODEL LEGUME *MEDICAGO TRUNCATULA* ECOTYPES

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Legumes play a central ecological and agricultural role due to their ability to establish symbiotic nitrogen fixation with rhizobia. *Medicago truncatula* is a widely used model for studying legume biology, including nodulation and symbiotic genome regulation. To expand genomic resources for this model legume, we generated chromosome-scale assemblies for three additional geographically distinct *M. truncatula* ecotypes using long-read sequencing technologies. By literally doubling the number of available high-quality assemblies for this species, we establish a comparative genomic resource for investigating its structural variation and repetitive sequence diversity. Using a multi-stage de novo transposable element (TE) annotation pipeline we curated accession-specific TE libraries and characterized TE landscapes across all assemblies. Importantly, we investigate how TE diversity intersects with genes involved in symbiotic nitrogen fixation. In *M. truncatula*, nodulation involves activation of gene clusters located within “symbiotic islands”, regions known to undergo dynamic epigenetic remodeling. We aim to establish whether natural variation in TE copy number and genomic positioning may influence the epigenetic landscape and transcriptional regulation of symbiosis-related genes. Our ongoing comparative analyses focus on TE diversity both genome-wide and within symbiotic islands, aiming to link structural variation with potential regulatory consequences. These newly generated assemblies and curated TE annotations establish a foundation for studying TE-driven genome evolution, epigenetic regulation, and environmental adaptation in legumes. This work is supported by National Science Centre (Poland) grant 2021/43/O/NZ2/01626, Preludium Bis NAWA3 and NAWA - PROM program.

Keywords: genome assembly, genomics, *Medicago*

GENETIC DIVERSITY OF COMMON BEAN LANDRACES VS. CULTIVARS: INSIGHTS FROM BORLOTTI BEANS IN NORTHERN ITALY TO GUIDE CONSERVATION STRATEGIES

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The adaptability of common bean (*Phaseolus vulgaris* L.) to diverse climatic and geographical conditions led to the development of numerous landraces, further shaped by local agricultural practices and dietary preferences. Over time, many of these traditional varieties have been replaced by high-yielding commercial cultivars resulting in a significant loss of genetic diversity. To safeguard this valuable heritage, it is crucial to characterize landraces at risk of extinction, both to support effective in situ and ex situ conservation strategies and as a first step toward their valorization and potential reintroduction into modern agriculture. One of the questions we addressed was whether native varieties have maintained their identity during prolonged on farm cultivation. In this study, a Borlotti bean panel including both landraces and modern cultivars —traditionally cultivated in Northern Italy was analysed. We developed an approach to distinguish native varieties from commercial cultivar, essential for establishing conservation priorities. Our results revealed distinct morphological and genetic profiles among the varieties, although only a subset of native varieties had maintained their original identity. Many others, despite being referred to by farmers as “old familiar varieties,” are more likely obsolete cultivars. Taken together, our data demonstrate that combining morphological and genetic analyses enables the development of a conservation priority framework. Furthermore, our results highlight the effectiveness of on-farm conservation, particularly in geographically isolated areas, in preserving the identity of traditional landraces. Therefore, on farm conservation can be considered an effective strategy to protect and potentially revitalize bean genetic resources.

Keywords: borlotti bean, landraces, genetic diversity, genotyping by sequencing

PHENOTYPIC VARIATION IN A CORE COLLECTION OF *LUPINUS MUTABILIS*

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The growing demand for sustainable, plant-based food systems highlights the importance of underutilized crops such as *Lupinus mutabilis* (tarwi, Andean lupin). Native to the Andean region, this species represents the only domesticated lupin crop in the New World and holds significant potential for improving food security, nutrition, and sustainable agricultural production. However, the majority of its genetic resources remain poorly characterized. In this study, we developed and characterized a core collection of approximately 750 accessions of *L. mutabilis* to capture the maximal genetic diversity. A comprehensive, multi-year phenotyping efforts were conducted using 39 agronomic traits, followed by statistical analyses to evaluate phenotypic variation. Our results demonstrate substantial phenotypic diversity within *L. mutabilis*, confirming its potential as a valuable genetic resource for crop improvement. To characterize genomic diversity, we generated low-coverage whole-genome sequencing data of the representative subset of accessions alongside high-depth sequencing of two accessions, and constructed a reference/high quality pangenome to explore patterns of variation, domestication, and evolutionary history. By combining evolutionary genomics with phenotypic characterization, this study provides new insights into the adaptation and diversification of lupins. The findings highlight *L. mutabilis* as a promising model for legume evolution, provide resources for association mapping and the genetic dissection of key agronomic traits, and a rich source of novel alleles for the development of climate-resilient, high-protein crops. This study was supported by the INCREASE project that received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 862862.

Keywords: lupin, biodiversity, variation, pan genome

ENHANCING NITROGEN FIXATION IN NORTHERN EUROPE BY USING SYNERGISTIC AND HOLOBIONT INTERACTIONS BETWEEN BACTERIA, NUTRIENTS AND PLANTS

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The cultivation of soybean (*Glycine max* L. Merr) is expanding with a latitudinal shift from Central to Northern Europe. Nevertheless, nitrogen (N) management remains a substantial challenge in cool regions where climate instability and biotic stressors often compromises both symbiotic activity and grain yields. To address the need for effective, sustainable agroecological N input practices, we evaluated measures (i) the selection of superior *Bradyrhizobium japonicum* strains; (ii) the application of additives, such as micronutrients, stickers, and sorbents; (iii) the development of holobiont synergies between *B. japonicum* and indigenous N-fixing endophytes; and (iv) the integration holobiont consortia of commercial associative bacteria. The results confirmed that inoculation is mandatory in regions where *B. japonicum* is absent in the soil, as the uninoculated control produced only 50% of the yield (1232 kg ha⁻¹) compared to treated plots. While the *B. japonicum* strain AGF78 emerged as a top individual performer, co-inoculation with *Azospirillum* spp. provided the highest overall grain yields (2311 kg ha⁻¹). Despite the physiological stability provided by chemical additives and diverse microbial consortia, their synergistic holobiont configurations resulted in seed yields comparable to individual elite *B. japonicum* strains. However, the study highlights a significant environmental advantage: inoculation did not increase N leaching during the growing season but instead, treatments involving *Bacillus atrophaeus*, stickers and *Arthrobacter pascens* provided a 50% increase in soil nitrate levels, during winter wheat germination. The integration of indigenous isolates like *Arthrobacter pascens* and *Serratia plymuthica* optimized both harvest quality and long-term soil fertility in Northern European crop rotations.

Keywords: BNF, N isotopes, endophytes, isolation, *Glycine max*

PULSEHEAT: ENHANCING LENTIL RESISTANCE TO HEAT STRESS

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The goal of this project is to identify and characterize heat-tolerant lentil genotypes with good seed quality, ensuring sustainable production under heat stress, from the plant genetic resources available in the USDA ARS National Plant Germplasm System and AGILE collection from Canada. To accelerate the identification of superior genotypes, we will integrate high-throughput field phenotyping tools and seed yield to assess heat tolerance across diverse lentil germplasm. A diverse lentil panel of 502 lines will be grown over three years under both typical and heat stressed conditions. Genotyping has been completed via exon capture (319 lines) and whole genome sequencing (183 lines). We will characterize genetic architecture underlying seed protein concentration and mineral (Fe, K, and Mg) accumulation in lentil under heat stress conditions, compared to non-stress conditions, by integrating genome-wide association studies (GWAS) with metabolic pathway analysis. Here we report the field and GWAS results from our first field season, 2025.

Keywords: lentil, heat tolerance, genetic diversity panel, GWAS

OPTIMIZING INTERACTIONS BETWEEN LEGUMES AND SOIL MICROORGANISMS FOR SUSTAINABLE AND HEALTHY LEGUME PRODUCTION

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The OPTILEG project, which stands for „Optimization of microbial interactions for sustainable production of legume proteins, beneficial for the environment and health“ is a research project funded by the French National Research Agency (ANR) as part of the France 2030 investment plan, over a 6-year period. This project aims to optimize the legumes interactions with beneficial soil microorganisms to promote protein production at low input levels (fertilizers and pesticides) for an environmental-health co-benefit. The project is based on a public-private partnership involving 14 academic research teams specialized in legumes, their associated microorganisms, agronomy and processing of legume products, nutrition and human health, and 5 private partners: the reference technical institute for protein crops, an union of agricultural cooperatives and leading companies in biostimulation, plant breeding and food processing. Pea and lentil, two major sources of protein for human consumption in France, are the main targets. This multi-disciplinary project is divided into 5 workpackages, which allows us to address different levels of organization (from the cell to the cropping system) to achieve its finalized objective. Knowledge accumulated on symbioses and beneficial microbial interactions over the last 20 years, progress in genetics and genomics, agronomy, and human nutrition feed this innovative holistic approach with multiple scale strategies bearing the ambition to accompany agroecological and food transitions via the increase of plant proteins in food.

Keywords: legumes, microorganisms, health, interactions, agroecology

IMPROVING ^{15}N -BASED ESTIMATES OF BIOLOGICAL N USE EFFICIENCY IN FIELD PEA

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Field pea (*Pisum sativum* L.) is an important legume in cereal-based cropping systems and a major source of biologically fixed nitrogen (N). The proportion of N derived from the atmosphere (%Ndfa) is commonly estimated using the ^{15}N natural abundance method. However, its accuracy depends on the selected B value ($\delta^{15}\text{N}$ of a fully N_2 -fixing legume) and the choice of a suitable non-fixing reference plant. This study assessed how B value determination and reference plant selection influence ^{15}N -based estimates of biological N use efficiency in field pea. A three-year unfertilized field experiment (2023–2025) was conducted under N_0 conditions. Field pea and three non-fixing species (*Chenopodium album*, *Echinochloa crus-galli*, *Sonchus arvensis*) were sampled within pea plots. In 2025, reference plants were also collected from preceding winter wheat plots to evaluate background soil N effects. A parallel controlled experiment was established using N-immobilised field soil and N-free vermiculite to determine B values under contrasting substrate N availability. Plant $\delta^{15}\text{N}$ was analysed by isotope-ratio mass spectrometry, and %Ndfa calculated using standard equations. The vermiculite-derived B value ($\delta^{15}\text{N} \approx -1.6\text{‰}$) was lower than values from soil-grown plants, confirming soil N uptake even under unfertilized conditions. %Ndfa estimates were highly sensitive to reference plant selection. Reference species sampled outside pea plots frequently produced biased estimates, whereas plants collected within pea plots provided more consistent values. Accurate ^{15}N -based evaluation of biological N use efficiency in field pea therefore requires carefully determined B values and appropriate in-plot reference plants.

Keywords: BNF, %Ndfa, ^{15}N isotope technique, B value, reference plant selection

ELEVATING THE ROLE OF PEA IN PLANT-BASED FOODS USING BREEDING, GENOMICS, AND FOOD SCIENCE APPROACHES

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Pea plays a major role in the expanding plant-based protein industries, second only to soybean. Improving the seed protein concentration (SPC), nutritional quality, and functionality of pea protein will facilitate the expanded use of pea in healthy foods and ingredients. To increase SPC we used multiple mapping populations for identification of associated QTLs and multi-parent crossing designs to pyramid the QTLs. We identified >10 moderate effect QTLs for SPC and 2 – 4 moderate effect QTLs each associated with the concentration of lysine, tryptophan, methionine+cysteine, and in vitro protein digestibility. Expression analyses identified 464 candidate genes associated with protein or sulfur amino acid concentration and some co-localized with the SPC QTLs. Using an 8-founder line Multiparent Advanced Generation Inter-Cross population of 850 inbred lines evaluated in four station-years we detected SPC concentrations ranging from 24 to 34% with a mean of 28%. We are utilizing Nested Association Mapping to fine map protein concentration, and Targeting Induced Local Lesions IN Genomes to identify mutants with increased protein concentration. We recently joined a consortium of public and private breeders to develop training models for selection of high seed protein concentration and yield in pea breeding lines. High protein and low protein sister lines did not differ substantially in their protein functionality when evaluated as flour or protein isolates. Consumers surveyed in Canada were willing to pay a premium for a simple ingredient list for plant-based burgers, and the current policy of allowing meat-related descriptions with qualifiers is generally effective.

Keywords: pea, protein, breeding, genomics, food science

EXPLOITING LIPOXYGENASE KNOCKOUT PEA LINES TO REDUCE PROTEIN OFF-FLAVORS

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Pea seeds are a valuable source of plant-based proteins and are increasingly incorporated into food products. However, the use of pea-derived ingredients, such as flour, isolates, or concentrates, is often limited by undesirable off-flavours that reduce consumer acceptance. Various mitigation strategies are commonly used, including flavour masking, solvent extraction, or technological processing. Exploiting both induced and natural genetic diversity in pea offers an alternative and complementary approach to address this challenge. One of the main off-flavours described for pea-derived ingredients is the characteristic “beany” off-note, which can be attributed to short-chain volatile compounds such as aldehydes (e.g., hexanal), alcohols, and ketones, largely produced through lipoxygenase (LOX)-mediated oxidation of polyunsaturated fatty acids. In pea, PsLOX2 and PsLOX3 are the two major seed lipoxygenases (Domoney et al., 1990), and recombinant proteins have shown distinct enzymatic properties, including differences in substrate preference and product specificity (Hughes et al., 1998). Pea TILLING mutant populations (Dalmais et al., 2009) were screened to identify loss-of-function alleles in PsLOX2 and PsLOX3, and we recently obtained the *lox2lox3* double mutant. The *lox2lox3* mutant produces mature seeds in which LOX activity is almost completely abolished. Analysis of volatile emissions from wild-type and mutant seeds by HS-SPME-GC-MS revealed a strong reduction in LOX-derived compounds in mutant seeds, including a 97% decrease in hexanal content. Sensory evaluation by a trained panel is currently underway to assess the impact of these volatile changes on the sensory properties of the mutant flour.

Keywords: pea, seed, off-flavour, lipoxygenases, TILLING, hexanal

MULTI-OMICS PERSPECTIVE ON ALKALOID PATHWAY ARCHITECTURE IN NARROW-LEAFED LUPIN

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The nutritional potential of lupin seeds is strongly constrained by quinolizidine alkaloids, which remain a priority target for crop improvement and molecular studies. In narrow-leaved lupin, breeding for low alkaloid cultivars has largely relied on the recessive *iucundus* locus, with RAP2-7 proposed as a candidate regulator, yet alternative sources of reduced alkaloid accumulation remain poorly understood. We integrated multi-omic analyses to compare the alkaloid pathway and its regulation across two low alkaloid sources in this species, the *iucundus* locus and the Bryansk background. As part of this approach, DAP-seq was used to characterize RAP2-7 DNA binding and identified an AP2/ERF like binding motif. This motif was strongly enriched in the *iucundus* background and Bryansk lines, but was not recovered in the *iucundus* background, consistent with reduced binding signals. To extend this analysis, promoter surveys highlighted AP2 family motif occurrences in a subset of alkaloid related candidate genes. Structural modelling of the RAP2-7:DNA complex further supported a mechanistic contribution of the *iucundus* associated R196S polymorphism to reduced RAP2-7 DNA binding. Together, these results support distinct regulatory routes to reduced alkaloid accumulation. In addition, they refine the working model of RAP2-7 DNA binding in a genotype dependent context and provide candidates and datasets to guide future functional studies and improvement of low alkaloid germplasm.

Keywords: narrow-leaved lupin, alkaloids, RAP2-7

DEVELOPMENTAL STAGE-DEPENDENT GENE EXPRESSION MODULATES MATERNAL CONTROL OF SEED VICINE BIOSYNTHESIS IN FABA BEAN

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Faba bean is a multipurpose legume valued for its high seed protein content and contribution to sustainable agriculture. However, its broader utilization is constrained by the presence of vicine and convicine (v-c), anti-nutritional compounds distributed throughout the plant. Although key genes involved in v-c biosynthesis have been identified, enabling genomic tools for breeding low v-c cultivars, the biosynthesis and accumulation of v-c within the plant remain incompletely understood. While previous studies reported maternal determination of seed v-c content, the underlying molecular mechanism remains unclear. In this study, we used multiple reciprocal crosses between five high and two low v-c parental lines to show that seed v-c content is determined by the maternal genotype, with F1 seeds consistently reflecting the maternal v-c phenotype. Expression analysis of VICINE-CONVICINE 1 and RIBOFLAVIN BIOSYNTHESIS PROTEIN 2 revealed maternal influence, with transcripts predominantly expressed in the seed coat, while the embryo remained transcriptionally inactive. Tissue-specific expression profiling confirmed that v-c gene activity is restricted to maternal tissues during seed development, suggesting that maternal determination is regulated by developmental stage-specific transcription. This absence of gene activity in the embryo explains the observed phenotypic delay, in which the seed v-c content reflects the genotype of the previous generation. Despite maternal control of v-c expression, we found that segregation of the v-c trait follows Mendelian ratios regardless of cross direction, indicating that maternal phenotype does not affect breeding outcomes. These findings provide a molecular basis for maternal inheritance of v-c content and offer practical insights for breeding strategies.

Keywords: anti-nutritional factors, maternal inheritance, tissue-specific expression, *Vicia faba*, vicine-convicine

PHENOTYPIC AND NUTRITIONAL EVALUATION OF GRASS PEA ACCESSIONS FOR LOW METHANE EMISSIONS AND HIGH FORAGE QUALITY

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The selection of low-methanogenic lathyrus is critical for sustainable livestock systems. Fifty grass pea (*Lathyrus sativus*) accessions with high biomass and low ODAP were evaluated for low-methanogenic potential and forage quality in trials conducted at ICARDA, Terbol-Lebanon (2024 and 2025) and Marchouch-Morocco (2025). Days to flowering (DF), Days to maturity (DM), plant height (PH), biological yield (BY), grain yield (GY), crude protein content (PC), acid detergent fiber (ADF), neutral detergent fiber (NDF) and methane emission after 24 h incubation (CH₄-24) were assessed. Two forage cuttings were collected at pre-flowering (C1) and late-podding (C2) stages and analyzed by NIRS and in-vitro digestibility method (HFT). Significant variation among accessions ($p < 0.05$ - 0.001) was observed for all traits. Longer growth cycles at Terbol (164-168 days) accompanied with higher averages of BY (7868 kg/ha) and GY (2223 kg/ha) compared to Marchouch; BY (1975 kg/ha), GY (932 kg/ha). ADFC1 (25.56 %) was slightly higher than ADFC2 (21.91 %) and NDFC1 (37.89 %) was slightly lower than NDFC2 (40.75 %). However, PCC1 (24.28 %) was higher than PCC2 (15.52 %). Spearman's correlation analysis showed that at early stages ADFC1 and CH₄-24 were positively correlated ($r = 0.40$, $p < 0.01$) and negatively correlated with PCC1 ($r = -0.5$, $p < 0.01$). Four elite lines combining high biomass, moderate fiber, and high protein with reduced methane emissions highlighting their importance for sustainable forage production.

Keywords: *Lathyrus*, forage, feeding, crude protein content, neutral detergent fiber, acid detergent fiber, methane

SEED SIZE VARIATION AND CHARACTERIZATION IN *LUPINUS ALBUS*

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Lupinus albus, as a representative of legumes, is recognized for its remarkably high seed protein content, offering significant potential as an alternative or complementary protein source for food, feed, and other industries. To characterize natural phenotypic diversity in special attention to seed size variation, we analyzed the *L. albus* Intelligent Collection, a curated panel designed to maximize genetic and geographic representation of the species. This collection allowed us to quantify variation in multiple seed traits and associate these phenotypes with underlying genomic variation. Here, we combined genome-wide association studies (GWAS) and transcriptomic profiling to investigate the genetic architecture underlying seed size variation in *L. albus*. GWAS revealed several genomic regions significantly associated with seed size, whereas differential gene expression analysis across developmental stages (globular, heart, and torpedo stages) and from particular seed compartment such as embryo, endosperm and seed coat allowed us to identify potential candidate genes. We provide a population-level genomic resource, including multi-omic genetic datasets, and characterize the phenotypic variation in seed-related traits. The integration of these data sets provides the basis to uncover regulatory pathways potentially shaped by convergent evolution, associating natural variation in wild accessions with domestication-related traits. This study was supported by the National Science Centre, Poland (grant no. OPUS 18 2019/35/B/NZ8/04283 to KS), and the INCREASE project that received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 862862 (to RP).

Keywords: lupins, seed size, GWAS, transcriptomics; natural variation, seed development

EARLY SEEDLING VIGOR AND END-USE QUALITY ASSOCIATIONS IN DRY BEAN

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Seed germination and seedling vigor are important characteristics for crop establishment in adverse environmental conditions. The aim of this study was to understand how dry beans (*Phaseolus vulgaris* L.) with genetic differences in cooking time respond to hot, humid postharvest storage conditions. The effect of high temperature and high humidity post-harvest seed storage on germination rate was tested on four parental bean lines identified as fast or slow cooking and selected top 20 and bottom 20 for cooking time in two recombinant inbred line (RIL) populations developed from the parental lines. Parental lines were tested for germination rate from seeds harvested from two growing environments, one with mild drought stress and one with sufficient water. Following exposure to post-harvest high temperature and high humidity, the germination rates of the fast-cooking genotypes grown in the dry environment were lower than the germination rates of the slow-cooking genotypes. By contrast, there were no significant differences between the germination rates of the slow- and fast-cooking genotypes grown with sufficient water following exposure to high temperature and high humidity. The fast-cooking genotypes had superior cooking times both before and after exposure to tropical storage, emphasizing their usefulness to dry bean consumers around the world, whereas the slow-cooking genotypes exhibited more tolerance to stress, as evidenced by their faster germination rates following exposure to pre-harvest drought stress and post-harvest high temperature and high humidity.

Keywords: bean, cooking time, germination, seed quality

ENHANCING LABLAB BEAN FLAVOR USING SUPERCRITICAL CO₂ EXTRACTION FOR SUSTAINABLE FOOD SYSTEMS

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As a climate-resilient pulse, Lablab bean (*Lablab purpureus*), is a promising yet underutilized protein source for sustainable food systems. However, off-flavor compounds, mainly volatile (ketones, aldehydes) and non-volatile (antinutrients such as phenolics or saponins) present in Lablab limit their acceptance and utilization. In contrast to conventional deodorization and antinutrient reduction methods, supercritical CO₂ extraction (SC-CO₂) offers a mild, selective, and environmentally friendly approach. This study aimed to optimize a green methodology using SC-CO₂ to produce a pulse ingredient with an improved flavour and reduced antinutrient content. Response surface methodology was used to optimize the SC-CO₂ process in order to maximize removal of volatile organic compounds (VOCs) and antinutrients. The impact of temperature (40–60 °C), pressure (10–40 MPa), and ethanol concentration (0–15% v/v) was evaluated. SPME-GC-MS was used to characterize the VOCs profile, while HPLC-DAD, LC-MS, and spectrophotometry were employed to evaluate the antinutrients (phenolics including tannins, saponins, trypsin inhibitors and phytic acid). Color changes were analyzed using a colorimeter. Ethanol concentration was identified as the variable with the most impact on VOCs removal, with a direct correlation between co-solvent level and extraction efficiency. The model provided a reasonable fit to the experimental data, effectively targeting key off-flavors such as 1-hexanol and nonanal. SC-CO₂ extraction successfully reduced phytic acid content. These results demonstrated that SC-CO₂ extraction is an effective green technology to valorize underutilized legumes by improving both their flavour and nutritional profile.

Keywords: lablab bean, supercritical CO₂ extraction, deodorization, antinutrients

FUNCTIONAL PROPERTIES ASSESSMENT OF A MINI-PIZZA MADE FROM MODIFIED CASSAVA STARCH IN PARTIAL SUBSTITUTION WITH LUPIN FLOUR

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The rising prevalence of diabetes and celiac disease, has led to a greater demand for foods that are both low in glycemic index and gluten-free. Nevertheless, baked goods formulated to meet these requirements often exhibit poor characteristics. The aim of this contribution was to evaluate the functional properties of a gluten-free, low-glycemic index mini-pizza made from modified cassava starch in partial substitution with lupin flour. Cassava (*Manihot esculenta*) roots (cultivar INIAP 651), and flour of lupine (*Lupinus mutabilis* Sweet) were obtained from a local supplier (Ecuador). The starch of cassava was extracted, sun-dried, and modified with alpha-amylase (6 U/g) and Ca lactate (6 mg/g). Blends with partial substitutions between modified cassava starch and lupine flour (0, 5, 10, 15 and 20%) were performed. To formulate mini-pizza doughs, water, oil, egg, and salt were added to the blends. Specific volume of the bread (SV), pasting properties and dough characterization by Mixolab were evaluated. Results showed that a partial substitution of up to 10% can be made with lupine flour. The blend with 10% of lupine had high SV (1.39 mL/g) and C2 (1.755 Nm) as well as a low C5 (4.898 Nm). The treatment with lupine 5% proved to be good too (SV (1.39 mL/g), peak viscosity (PV) (2259 Cp) and breakdown (BD) (1375 Cp)). Negative correlations were found between SV vs. C1 and PV as well as C2 and C3 vs PV, BD and final viscosity (FV). Also, positive correlations were found between PV and Setback vs FV and BD.

Keywords: gluten-free, low-glycemic index, lupine, modified cassava starch, pizza

PULSE-DERIVED BEVERAGES AS HIGH-PROTEIN DAIRY ALTERNATIVES: NUTRITIONAL AND FUNCTIONAL ASSESSMENT

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The growing demand for non-soy milk substitutes is driven by health and sustainability concerns. However, most plant-based beverages available in the European market contain low protein levels (<1.5%) compared with bovine milk (3.5%). Pulses, due to their high protein content, represent a promising alternative. Although a beany flavor may occur, current processing technologies can mitigate sensory limitations while improving digestibility and nutritional quality. This study compared the nutritional and bioactive properties of beverages produced from *Lupinus albus* L. and *Cicer arietinum* L. Seeds were processed into 10% (w/v) aqueous beverages, which were subjected to static in vitro digestion and subsequent analyses. Antigelatinolytic bioactivity was assessed to evaluate potential anti-carcinogenic effects. Chickpea and lupin beverages exhibited protein contents of approximately 3.6% and 4.7% (w/v), respectively. In vitro digestion resulted in a reduction in measurable protein content, consistent with proteolysis. Phytic acid levels decreased after digestion and did not impair protein digestibility or digestive enzyme activity. The low phytic acid concentrations in digesta did not negatively affect mineral bioaccessibility as well, as divalent cations remained available. Lupin digesta contained significantly higher levels of Ca, Mg, P, Mn, and S compared with chickpea. Overall, pulse-based beverages demonstrated high protein content, effective protein hydrolysis during digestion, and mineral bioaccessibility. Phytic acid and lectins did not inhibit digestive enzymes. Additionally, both beverages exhibited anti-inflammatory and potential anti-carcinogenic activity, as indicated by matrix metalloproteinase-9 inhibition.

Keywords: pulse proteins, upcycling food sidestreams

EFFECT OF ENSILING ON AMINO ACIDS, RIBOFLAVIN, AND SECONDARY PLANT METABOLITES IN WHOLE VETCH PLANTS HARVESTED AT DIFFERENT GROWTH STAGES

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Due to secondary plant metabolites, the use of whole vetch plants as home-grown roughage for pigs can be restricted. The aim was to examine whether pyrimidine glycosides and cyanoalanine toxins are degraded during ensiling, while amino acids and riboflavin are preserved. In 2022, two cultivars each of three vetch species were cultivated in a field trial: *Vicia villosa* Roth., *V. pannonica* Crantz., and *V. sativa* L. They were harvested in four growth stages (bud, flowering, pod formation, pod filling) and ensiled using buffered formic acid Amasil®NA (10 mL/kg FM; BASF) and homofermentative lactic acid bacteria BIO SIL® (1,2 mg/kg FM; Dr. Pieper Technologie- und Produktentwicklung GmbH). Concentrations of amino acids and riboflavin (HPLC-FLD), pyrimidine glycosides (according to Pulkkinen et al. 2015), and cyanoalanine toxins (HPLC-MS, according to Thavarajah et al. 2012) were analysed in fresh material and after ensiling for 90 d. Statistical analysis was conducted in R with linear models using generalized least squares with a correlation structure for the repeated measurements per plot with growth stage, cultivar, their interaction and block as fixed effects. Growth stadium affected the concentration of secondary plant metabolites, amino acids and riboflavin. With the start of pod filling, vicine and γ -glutamyl- β -cyano-L-alanine were detected in *V. sativa* plants. *V. pannonica* contained less vicine and no cyanoalanine toxins and *V. villosa* did not contain any of the tested secondary plant metabolites. Ensiling whole vetch plants with buffered formic acid decreased the concentration of cyanoalanine toxins and pyrimidine glycosides while preserving amino acids and riboflavin.

Keywords: vetch, ensiling, cyanoalanine, vicine, amino acids

PATTERNS AND DETERMINANTS OF GRASS PEA (*LATHYRUS SATIVUS*) CONSUMPTION AMONG RURAL HOUSEHOLDS IN MAJOR CULTIVATING DISTRICTS OF ETHIOPIA

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Grass pea (*Lathyrus sativus*), locally known as Guaya, is a protein-rich legume vital for food security in Ethiopia's drought-prone regions. However, its neurotoxin content poses a risk of neurolathyrism when consumed excessively. Evidence regarding current consumption patterns and associated risks remains limited. To assess grass pea consumption patterns and identify determinants of intake among rural households in major cultivating districts. A cross-sectional study was conducted (August 2022–September 2023) among 3,292 households selected through multistage stratified sampling in five zones of the Amhara region. Data were collected via interviewer-administered questionnaires and analyzed using descriptive statistics and multivariable logistic regression. Overall, 73.95% of households consumed grass pea, with 34.73% consuming it daily. Primary drivers included food scarcity (47.4%) and high costs of alternative pulses (46.5%). High intake was significantly associated with lower education (AOR = 4.97), larger family size (AOR = 1.41), and marital status (AOR = 1.47). The prevalence of neurolathyrism was 10.51%, peaking among males and food-insecure households. Risk factors included low wealth (AOR = 2.38) and farming (AOR = 2.9), whereas knowledge, crop mixing, and spice use like garlic were protective. Grass pea consumption remains high, especially among food-insecure households, with a substantial burden of neurolathyrism. Interventions should prioritize promoting low-toxin varieties and safe processing techniques to balance food security with public health safety.

Keywords: *Lathyrus sativus*, grass pea, consumption patterns, Amhara region, Ethiopia

INVESTIGATING GRASSPEA'S POTENTIAL AS A UK-GROWN POULTRY FEED CROP

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The UK poultry industry relies heavily on imported soya (*Glycine max*) as its primary protein source for feed. This can incur high economic costs for poultry farms, as well as significant environmental costs due to carbon emissions, deforestation and biodiversity loss associated with soybean production and transport. While soybean is unsuited to the UK climate, identifying alternative protein crops that can be produced locally is critical for improving the resilience of UK poultry systems and reducing reliance on soya imports. Grasspea (*Lathyrus sativus*) is a protein-rich legume crop renowned for its tolerance to stress such as drought and flooding. While it has not historically been cultivated in the UK, its performance in our recent field trials have shown great potential as a novel and sustainable domestic feed crop. In collaboration with Traditional Norfolk Poultry, we have trialled grasspea as a partial substitute for soya in chicken feed. The preference of the chickens for this feed, their behaviour and health were all monitored. Based on the success of this first experiment, we are now expanding the project by growing grasspea at scale for the first time in the UK, in a major field trial, to characterise yield and other agronomic traits. Additionally, further feeding trials, including turkeys as well, will examine the suitability of grasspea as a complete replacement for soya.

Keywords: animal feed, sustainability, grasspea, feed crops

VARIABILITY IN THE PROXIMATE COMPOSITION OF CAROB PULP FROM SOUTHERN CROATIA

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Carob pods, consisting of pulp and seeds, have diverse applications in the food industry and other commercial sectors, while the carob tree is also valued as an ornamental species. Carob pulp is processed into carob powder, an inexpensive ingredient widely used in bakery products and as a cocoa substitute in chocolate production. Therefore, the present study aimed to determine the proximate composition of carob pulp from 34 carob pod samples collected at different locations in southern Croatia. Seeds were manually separated from the pulp, which was then dried at 105 °C to constant weight, ground, and homogenized. The proximate composition of the pulp (moisture, ash, crude fat, crude protein and neutral detergent fiber) was determined using standard analytical methods. Based on these results, the total carbohydrates content and the proportion of insoluble fiber in total carbohydrates were calculated. The results showed that carob pulp was characterized by a high carbohydrate content (85.9-92.6% DM), followed by neutral detergent fiber (insoluble fiber; 12.2-30.2% DM), crude protein (3.7-9.3% DM), crude fat (0.34-1.92% DM), and ash (1.67-4.03% DM). The proportion of insoluble fiber in total carbohydrates ranged from 13.1 to 34.0%. Among the analysed components, neutral detergent fiber exhibited the greatest variability among locations, whereas carbohydrate content remained consistently high across samples. These findings confirm that carob pulp is a carbohydrate-rich raw material with variable fiber content, highlighting its potential value as an ingredient for food applications.

Keywords: *Ceratonia siliqua* L., pods, carbohydrates, fiber, crude protein

THE QUALITY OF DANISH-GROWN SOYBEAN AND LUPIN FOR HUMAN CONSUMPTION

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Rising interest in regionally sourced plant-based protein has intensified the focus on broadening the spectrum of grain legumes cultivated in Northern Europe. While Danish agriculture has traditionally relied on peas and, more recently, faba beans, crops such as soybean (*Glycine max* L.) and lupin (*Lupinus* spp.) have yet to gain significant agronomic or commercial traction. Previous attempts to introduce soybean to Danish growing conditions have been constrained by climatic limitations and inconsistent yields. The research project “Lupin&SoyFoods” addresses the entire value chain, from field management and quantitative analysis of (anti)nutritional compounds to functional characterization and pilot-scale food production using Danish-grown soybean and lupin, in close collaboration with industry partners. In 2025, nine cultivars of soybean and lupin (including both white and narrow-leaved lupin types) were cultivated under Danish conditions, yielding promising results. However, the late maturation and harvest time of certain soybean cultivars remain a challenge. For quality assessment, benchmark samples of soybean and lupin intended for human consumption were included to enable comparison with the experimental material. Protein content ranged from 38% to 48% in soybean and from 28% to 38% in lupin (dry weight), which is comparable to the benchmark materials. LC–MS/MS was employed to quantify six individual alkaloids and to calculate total alkaloid content. Both total and individual alkaloid levels varied markedly among lupin cultivars and also between the three biological replicates. Overall, the preliminary results are promising. The next phase will involve a more detailed characterization of protein composition and functional properties.

Keywords: soybean, lupin, plant-based food, nutrients, anti-nutrients

SCREENING OF PEA ACCESSIONS SUITABLE FOR THE DEVELOPMENT OF FUTURE FOOD PRODUCTS

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Grain legumes are staples in many cuisines world-wide, but in Europe, human consumption of grain legumes is relatively low. Grain legume production in Europe is used mostly for animal feed. In Sweden, for example, approximately 95% of harvested peas are used for animal feed. There is a great potential to shift much of the production into human consumption in the form of whole seed, flour, and protein extract. However, sensory evaluations and tests of consumer acceptance are crucial to fulfil this potential. By combining seed compositional content (protein, resistant and non-resistant starch, fatty acids, choline, phytate, saponins, and sucrose) and sensory evaluations (taste, aroma, mouthfeel, and aftertaste), we analysed a diversity panel of 15 pea accessions by means of canonical correlations to gain insights into preferential patterns. The study revealed that testa colouration strongly correlated to sensory evaluation: accessions having light-coloured testa were rated sweeter and juicier while accessions having dark-coloured testa were rated more odour intense and textured. Seeds with a wrinkled phenotype were markedly different compositionally to seeds with a smooth or dimpled phenotype and especially stood out on their sweet taste. Potential implications of the correlations on whole seed, flour, and protein extract were identified and the study serves as a stepping stone for future sensory explorations.

Keywords: sensory screening; seed quality; pea flour; diversity panel

GENETIC MAPPING OF SOLUBLE SUGARS AND MYO-INOSITOL CONTENT IN FRESH PODS OF COMMON BEAN

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Soluble sugars are key primary metabolites involved in growth, development, and cellular metabolism, with critical roles in abiotic stress responses and organoleptic quality. In this study, we investigated the genetic architecture controlling three major soluble sugars—sucrose, glucose, and fructose—and the related metabolite myo-inositol in fresh bean pods from a recombinant inbred line population derived from a cross between the dry bean cultivar ‘TU’ and the snap bean variety ‘Musica.’ Phenotypic data across the three environments revealed moderate to high heritability ($H^2 = 0.60-0.69$) for glucose, fructose, and myo-inositol, whereas sucrose content showed low heritability ($H^2 = 0.2$). QTL mapping identified nine QTLs clustered in three main regions on chromosomes Pv01 and Pv06. On Pv01, a single region contained QTLs for glucose, fructose, and myo-inositol content. Chromosome Pv06 harbored two regions: one associated with glucose, fructose, and myo-inositol, and another associated only with glucose. These regions co-localized with QTL for pod phenotypes and edibility. Candidate gene analysis underlying these QTLs identified 97 genes, including 43 enzymes involved in starch and sucrose metabolism, fructose-related pathways, glycolysis/gluconeogenesis, and inositol phosphate metabolism; 12 sugar and inositol transporters; and 42 transcription factors from the MYB, WRKY, and bHLH families. Seven of these candidate genes were prioritized based on the presence of amino acid substitutions or structural variants identified in the Allele Catalog (KBCommons-*P. vulgaris*) and predicted by in silico tools to have a functional impact. These results provide a basis for functional studies and the development of future breeding strategies.

Keywords: *Phaseolus vulgaris*, carbohydrate metabolism, QTL

VARIETAL AND SEASONAL EFFECTS ON FUNCTIONAL, SENSORY, AND APPLICATIVE PROPERTIES OF PEA PROTEIN ISOLATES (PROJECT PEA4EVER)

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This poster presents the experimental design and preliminary results from the 2025-2026 campaign of Workpackage 5 (WP5) in the Pea4Ever project. In France, the production of pea-derived protein ingredients predominantly relies on spring peas (PP), while winter peas (PH) have been historically perceived as heterogeneous in pod/meal globulins and protein content, limiting their suitability for standardized industrial wet-fractionation processes. Recent breeding advances in PH yield, protein concentration, and agronomic stability necessitate an objective reassessment of their technological potential for functional protein isolate production. WP5 of the Pea4Ever project addresses this gap by (i) comparing PH and PP transformation aptitude into functional protein isolates, and (ii) identifying biochemical and physicochemical determinants of functional disparities to support breeding programs. A multi-year experimental design deploys four contrasting varieties (2 PH, 2 PP) across four representative production zones, capturing pedoclimatic variability. Seeds undergo detailed characterization, biochemical (protein, crude cellulose, ...) and physical (thousand-seed weight, hydration capacity,). Isolates are rigorously evaluated for extraction yield, amino acid composition, solubility (pH/temperature isotherms), gelling properties, emulsifying capacity/stability, sensory performance (texture, off-flavor) in model food matrices (e.g., plant-based milk). Preliminary results from the 2025 campaign reveal no significant differences between PH and PP in terms of extraction yield, with the production area having a predominant effect. PP varieties have lower levels of antinutritional factors (ANF) than PH. Functional properties show differentiated responses between pea types, highlighting the importance of environmental factors. These findings provide industry benchmarks, facilitate PH supply chain integration, and guide breeding toward optimized food-grade profiles.

Keywords: pea, isolates, genotype-by-environment, wet fractionation, functionality

PASTA FORMULATED WITH HYPOALLERGENIC LUPINE FLOUR PROCESSED BY INSTANTANEOUS CONTROLLED PRESSURE DROP

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Lupine seeds (*Lupinus albus*) are widely used by food industry in bakery products, pasta, snacks, gluten-free and vegan products. Accordingly, allergenic reactions to this legume are increased, mainly in patients with allergy to peanut. The elimination or high reduction of lupine allergenic proteins may lead to reduce allergic risk. Our previous works suggest that processes combining heat and steam pressure on lupine seeds could nearly remove in vitro immunoreactivity of lupine. Therefore, instantaneous controlled pressure drop (DIC), combining high pressure for a very short time, could be an advantageous treatment. In this study, the effect of DIC (7 bar/170°C; 120 seconds) on lupine allergic reactions was evaluated. Then, considering that pasta as one of the most widely consumed food in the world, nutritional and sensorial properties of pasta elaborated by cold extrusion with 0, 5, 10 and 15% raw or DIC-processed lupine (*L. albus* var. Orden Dorado) and rice semolina (*Oryza Sativa*) were assessed. A clear reduction in immunoreactivity of lupine treated by DIC compared to raw material was observed by Western blot using pool sera from allergic patients reactive to storage proteins (Sprt) or nonspecific lipid transfer proteins (LPTs), as well as with IgG antibodies against 11S and 2S proteins. All elaborated pasta showed good cooking quality (cooking loss <10%). The inclusion of raw and DIC-processed lupine in pasta increased the protein, ash, and lipid content compared to pasta elaborated with 100 % rice. Considering sensorial analysis, pasta elaborated with lupine, particularly with DIC-processed lupine, were positively evaluated by panelists.

Keywords: DIC, instantaneous controlled pressure drop, *Lupinus albus*, pasta, food allergy

VALIDATION OF QTNs ASSOCIATED WITH SEED MINERAL COMPOSITION IN COMMON BEAN LANDRACES

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Due to its high protein content and significant levels of essential minerals, especially zinc, common bean is a key crop for improving dietary quality and addressing micronutrient deficiencies worldwide. The aim of this study was to determine the seed nutrient composition of a panel of 272 common bean landraces collected from diverse agroecological regions. The accessions were grown under uniform field conditions, genotyped using 5,833 polymorphic DArTseq-derived SNP markers and phenotyped for the seed content of 23 nutrients and trace elements. Collected data were subjected to genome-wide association study (GWAS), based on the Q + K model in TASSEL 5. The population structure matrix (Q) was derived using STRUCTURE, and the kinship matrix was generated from SNP data using the centered IBS method. Resulting significant QTNs were compared with the result of the similar previous study, in order to check for the repeatability of QTNs associated with seed mineral composition in a different year. Most of the newly detected QTNs are located elsewhere in the genome, therefore there is almost no repeatability between years. QTNs from both studies were then used to search for candidate genes using the reference common bean genome.

Keywords: *Phaseolus vulgaris* L., quantitative trait nucleotides, landraces, SNP

WHICH LEGUME SPECIES, GENOTYPES, AND FERTILISATION STRATEGIES ARE BEST SUITED FOR PLANT-BASED FOODS?

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The transition toward plant based diets is increasing the demand for sustainable, high quality crops. Legumes offer strong potential as raw materials and ingredients for human consumption, yet in Northern Europe they remain underutilised. Furthermore, existing fertilisation strategies for legumes often overlook the optimisation of nutritional value for human food use. The aim of this study was therefore to identify legume species, genotypes and fertilisation strategies best suited for plant-based foods. It was hypothesised that faba beans (*Vicia faba* L.) and peas (*Lathyrus oleraceus* Lam., formerly *Pisum sativum* L.) are particularly promising species, and that fertilisation can be optimised to improve growth, harvest yield, seed quality and nutritional value. Field and greenhouse trials were conducted to assess 10 commercial faba bean and pea genotypes, along with more than 350 pea gene bank accessions. Plants were grown under organic regulations and for commercial genotypes, protein concentrates and isolates were also produced and analysed. Selected genotypes and accessions were included in fertilisation experiments to optimise biological nitrogen fixation, harvest yield and seed quality. All commercial faba bean and pea genotypes showed strong agronomic performance and only minor differences in seed quality. However, faba beans differed significantly in antinutrient concentrations. Among pea accessions, greater variation was observed in nutrient concentrations with significant impacts of fertilisation strategy. In conclusion, faba beans and peas are highly suitable for plant based foods, though further work on genotypic variation and fertilisation strategies is needed to enhance their use in Northern Europe.

Keywords: legumes, faba beans, peas, fertilisation strategies, plant-based foods

REMEDIATION OF SLASH-PILE-BURNED AGRICULTURAL SOIL USING LEGUMES AND ORGANIC AMENDMENTS: EFFECTS ON FRESH AND ENSILED FIELD BEAN (*VICIA FABA*) FORAGE

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Slash-pile burning is commonly used to clear abandoned agricultural land in the Mediterranean, but it also causes soil degradation, which should be remediated with organic amendments and soil-improving crops. This study evaluated the effects of farmyard manure (40 t/ha), biochar (40 t/ha) + NPK 15:15:15 (450 kg/ha), and NPK 15:15:15 alone (450 kg/ha; control) on the chemical composition of fresh and ensiled field bean (*Vicia faba*) forage grown on slash-pile-burned soil (Terra Rossa). The experiment was conducted in Southern Dalmatia in 2024. Field bean was harvested at growth stage 207 (pod filling), and part of the biomass was ensiled in vacuum bags for 60 days. Chemical composition, tannin fractions, and fermentation characteristics were determined in both fresh forage and silage. Among the amendments, manure resulted in the highest crude protein (249.3 vs 223.8 g/kg DM) and crude fat (48.1 vs 36.0 g/kg DM). Ensiling markedly altered forage composition. Compared with fresh forage, silage had lower pH (4.78 vs 5.34) and lower contents of water-soluble crude protein (84.36 vs 145.48 g/kg DM) and sugars (4.86 vs 70.98 g/kg DM), with altered contents of protein- and fibre-bound tannins. Lactic acid, acetic acid, and ethanol were detected only in silage. Overall, manure showed the greatest potential to improve the feeding value of field bean forage and silage produced on slash-pile-burned abandoned land. Acknowledgments: This work was supported by the PRIMA Foundation through the SHARInG-MeD project (GA number 2211).

Keywords: field bean, organic amendments, fresh forage, ensiling, chemical properties

OVERVIEW OF GENE CO-EXPRESSION NETWORKS ASSOCIATED WITH ALKALOID ACCUMULATION IN NARROW-LEAFED LUPIN

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Quinolizidine alkaloids (QAs) are anti-nutritional compounds that limit the use of lupin seeds for food and feed. Despite their agronomic relevance, key aspects of the molecular basis of QA biosynthesis and accumulation remain only partially understood. To address this knowledge gap, we integrated transcriptomics, weighted gene co-expression network analysis (WGCNA), and quantitative QA profiling in genotypes whose alkaloid content is determined by the *lucundus* / *iucundus* locus. Preliminary analyses identified list of significantly differentially expressed genes in the tested samples. WGCNA revealed a module that was strongly and positively correlated with total QA content (Pearson $r = 0.8$, $P < 0.0001$). This module comprised 148 genes, including 35 highly connected hub genes. The module topology revealed two densely connected network centers, with the larger enriched in known and putative QA pathway components. The remaining genes in this center showed diverse functional annotations, not clearly linked to core QA biosynthesis, but including processes that could indirectly influence QA accumulation, transport, or regulation. Notably, putative upstream regulators of QA biosynthesis were not detected in the main QA-correlated module, suggesting that regulatory control may be partly decoupled from the core enzymatic QA module. Together, these results outline expression modules associated with QA accumulation and highlight key hub genes for further study.

Keywords: quinolizidine alkaloids, narrow-leafed lupin, WGCNA, transcriptomics

DEVELOPING CHICKPEA AS AN INTERMEDIATE CROP: FROM PULSE CROP TO PROTEIN RESOURCE

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Chickpea (*Cicer arietinum*) is the fourth most grown legume after soybean, peanut and common bean. It is an important human protein source, specifically in developing countries. It is rich in minerals, vitamins, and dietary fibre. Aquafaba is a valuable egg replacement in a vegan diet. Chickpea thrives in the semi-arid subtropical climate of Asia, with high heat and drought tolerance. Chickpea leaves have a high mineral and protein content and have potential for plant protein products. If used as an intermediate crop, farmers would benefit from opportunities to harvest and use it before sowing the next main crop. Therefore, this project focuses on harvesting chickpea at vegetative stage and evaluating biomass as a potential protein source in food and feed. To understand the fractionation efficiency in various genotypes, a small-scale extraction method has been developed, with only 10g of green biomass and was validated with an established method across seven crop species. This small-scale method will be utilized and genome-wide association studies (GWAS) will be conducted to identify genetic loci influencing leaf protein extractability. Furthermore, intermediates in the fractionation process will be evaluated for biogas potential. Additionally, imaging followed by machine learning was used to study seed morphology traits, which showed high accuracy in predicting seed weight, and is currently compared with phenolic compounds and antioxidant capacity. Preliminary results indicated higher phenolic content in Desi genotypes. Thus, in due course, the present project has the potential to influence farmers' income-opportunities from growing chickpeas in South Asia.

Keywords: chickpea, leaf protein extraction, Genome-wide association study, protein extractability

KEY ANTINUTRITIONAL COMPOUNDS IN AUSTRALIAN PULSES: VICINE-CONVICINE IN FABA BEANS AND QUINOLIZIDINE ALKALOIDS IN LUPINS

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Anti-nutritional compounds are natural plant metabolites that can limit nutrient utilisation or pose health risks, making their measurement important for safe food and feed use. Faba beans contain the glycosides vicine and convicine (vc^-), which can trigger favism—an acute haemolytic anaemia—in people with glucose-6-phosphate dehydrogenase deficiency. This study quantified vc^- concentrations in 22 Australian historical faba bean varieties grown across five sites. Both genotype and environment significantly affected vc^- levels ($p < 0.05$), revealing strong varietal diversity and notable environmental influence. Air-fractionated protein concentrates contained more than twice the vc^- concentration of their source flour, while wet-extracted protein isolates contained less than half, highlighting the need to breed low- vc^- cultivars and to monitor vc^- in processed fractions intended for sensitive consumers. Lupins produce quinolizidine alkaloids that defend against pests but can be toxic at high concentrations. Although Australian sweet lupins have been bred for low alkaloid content, analysis of seven lines across three sites showed strong genotype $\times$ environment effects. Western Australian sites (Merredin and Mingenew) produced very low alkaloid levels (< 35 mg/kg), whereas the Northern Mallee site yielded much higher concentrations (130–445 mg/kg). Six of seven Northern Mallee samples exceeded the Australian food threshold of 200 mg/kg, emphasising the need for routine testing when lupins are destined for food use. Overall, genetic and environmental factors strongly influence anti-nutritional compounds in Australian pulses. Effective management requires coordinated breeding, agronomic strategies, and processing oversight to ensure safe, consistent pulse-based food and feed products.

Keywords: antinutrients, vicine, convicine, alkaloids

COMBINING LEGUME FLOUR AND INNOVATIVE TECHNOLOGIES TO IMPROVE THE NUTRITIONAL QUALITY OF BAKED GOODS

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Legumes offer a nutritious, protein-rich alternative to animal proteins, and this study explored enhancing baked goods by incorporating legume flours. Various pretreatments—dry-fractionation, extrusion-cooking, and sourdough fermentation—were tested to improve functionality and nutritional profiles. Extruded-cooked lentil flour was found to be an effective, "clean label" substitute for hydroxypropylmethylcellulose in gluten-free pizza. Compared to native flour, extrusion increased water absorption, initial viscosity (69.3 vs 25.5 BU), and Mixolab consistency (1.77 vs 0.01 Nm), while reducing starch retrogradation. Incorporating 10% w/w extruded lentil flour raised the protein content of rice/maize-based pizza from 4.4% to 7.4%, enhanced antioxidant activity, and maintained consumer acceptance. Pea flour improved the nutritional profile of wheat-based focaccia, increasing protein, fiber, carotenoids, and phenolics, enabling "source of protein" and "rich in fiber" claims under EC Regulation 1924/2006. Fermenting pea flour with sourdough significantly reduced anti-nutritional factors, specifically raffinose (from 7.8 to 0.46 mg/g), stachyose (from 26.9 to 8.9 mg/g) and phytates (from 0.29 to 0.20 g/100g), while producing a more complex, desirable volatile profile. Furthermore, dry-fractionation of pea flour allowed raising protein content to 55%. Pea protein concentrate used at 5% w/w to fortify gluten-free rice/corn-based focaccia allowed to label the final product as a "source of protein" without compromising sensory quality. In conclusion, utilizing dry-fractionation, pregelatinization, and sourdough fermentation to process legume flours significantly improves the technological, nutritional, and sensory properties of baked goods, supporting the production of cleaner-label, healthier products.

Keywords: dry fractionation, extrusion cooking, sourdough, pea, lentil

STATUS OF MACROELEMENTS IN WILD CAROB FRUIT FROM DIFFERENT LOCATIONS IN SOUTHERN CROATIA

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Carob (*Ceratonia siliqua* L.) is a Mediterranean species from the legume family (Fabaceae) and its fruit (pod) is traditionally used in human and animal nutrition. The carob fruit is characterized by high nutritional value and contains significant amounts of dietary fiber, natural sugars and especially valuable minerals such as calcium, potassium, phosphorus, magnesium and iron. As far as is known, there is only one carob plantation in Croatia, and the fruits of wild carob are usually used for food. Such wild carob is not grown under standardized production conditions, so it is assumed that it may have different amounts of macroelements depending on the location of growth. The aim of this study is to determine the amount of macroelements in carob fruits (pods) from different locations in southern Croatia and to compare them. Samples of carob fruits (pods) at technological maturity were collected from wild trees in the southern part of Croatia. After drying at 105 °C, the samples were ground and homogenized. The amount of macroelements was determined in each fruit flesh sample. Nitrogen was determined by the modified Kjeldahl method. After decomposition with HNO₃ and HClO₄ in a microwave oven, phosphorus was determined by spectrophotometry, potassium by flame photometry, and calcium and magnesium by atomic absorption spectrometry. Results show significant variation in macroelements content in dry matter: 0.43-1.02 % N, 0.04-0.11 % P, 1.09-1.58 % K, 0.12-0.69 % Ca and 0.04-0.08 % Mg.

Keywords: *Ceratonia siliqua* L., mineral, nutrient, pod fruit, potassium

PEA PROTEIN ENRICHMENT IN 3D-PRINTED HYBRID PORK SNACKS: PHYSICOCHEMICAL AND SENSORY EVALUATION

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Excessive meat consumption has important and diverse impacts on the environment (e.g., greenhouse gas emissions, biodiversity, and land use) and on health (e.g., risk of developing stroke, diabetes mellitus, and cancer). The development of hybrid products combining meat and plant-based proteins offers several advantages, including cost-effectiveness, reduced calories and saturated fat, maintenance of high protein levels, increased fiber content, and the potential to improve environmental footprint and human health. However, sensory acceptability is extremely important for consumers and becomes a challenge. The 3D food printer could help in this process by creating more attractive textures, flavours and shapes. This work aimed to create a hybrid snack with pork and pea protein using 3D printing technology. Two hybrid formulations were produced and compared with a pork snack control: (i) 18% pea protein with 20% pork and (ii) 21% pea protein with 17% pork. Snacks were printed using a 0.8 mm nozzle and 0.35 mm layer height with 100% infill at 30 °C, then cooked at 125 °C for 10 min. Physicochemical properties (aw, pH, cooking water loss, and colour) were assessed, followed by sensory evaluation. Increasing pea protein content reduced aw (0.96 to 0.93), slightly lowered pH at 21% pea protein, and produced minor changes in cooking water loss. Colour shifted towards lower brightness and reduced redness relative to the control, consistent with legume incorporation. The perceived differences in sensory evaluation between the snacks were not significant ($p > 0.05$), suggesting that legume enrichment can be achieved without compromising perceived quality.

Keywords: 3D hybrid pork snacks, pea protein, nutritional and sensorial characterization

TURNING GRASS PEA INTO OPPORTUNITY: INNOVATIVE FERMENTED GRASS PEA PRODUCTS FOR PRODUCERS AND CONSUMERS

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Consumer and market-driven food production grounded in agrobiodiversity and agroecology contributes to the conservation of cultivated and native biodiversity while greatly strengthening the overall resilience of food systems. This collaborative study, developed under the H2020 DIVINFOOD project, aims to increase grass pea-based foods attractiveness by creating a set of innovative products and recipes that address local producers' and consumers' concerns for local, minimally processed, and tasty food. Three fermented products, salty and sweet grass pea-miso and grass pea-tempeh, were produced and compared with traditional soybean miso. These products were characterized and applied as ingredients in different food recipes. The overall liking of the grass pea-based products and recipes was assessed. Grass pea misos (sweet and salty) and tempeh exhibited improved nutritional and phytochemical profiles, showing higher methionine content, higher phenolic compound content, higher in vitro antioxidant activity, and lower trypsin inhibitor activity than the non-fermented cooked grains. Also, the texture parameters and viscoelastic moduli of grass pea-fermented products were similar to those of the control products. Different volatile profiles were obtained in miso and tempeh, suggesting distinct impacts of fermentation on the aromatic compounds produced. Sensory analysis of the fermented grass pea-based products/recipes made with misos (sweet and salty) and tempeh revealed that all received high overall liking, with positive responses exceeding 85%. These results indicate that grass pea fermentation can create sustainable and flavourful products with potential health benefits, aiding the development of innovative business opportunities and boosting demand for grass pea products, ultimately helping to reverse biodiversity loss.

Keywords: grass pea, innovative fermented products, biodiversity, consumer acceptance

GRASS PEA (*LATHYRUS SATIVUS*) FLOUR AS A PLANT-BASED EMULSIFIER FOR CLEAN-LABEL MAYONNAISE

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Growing demand for healthier and more sustainable foods has promoted the development of clean-label products and the replacement of animal-derived ingredients with plant-based alternatives. In mayonnaise-type emulsions, egg yolk plays a crucial stabilising role, creating the need for alternative proteins that ensure physicochemical stability and sensory acceptance. This study evaluated grass pea (*Lathyrus sativus*) flour as a natural emulsifier in clean-label mayonnaise formulations. Emulsions containing 0.5%, 1.0%, and 1.5% (w/w) flour were produced with 65% and 25% fat matrices and compared with egg-yolk controls. Physicochemical, rheological, textural, nutritional, antioxidant, and sensory properties were assessed. Data were analysed by ANOVA with Tukey's test ($p < 0.05$). The 0.5% concentration failed to form stable emulsions, while 1.5% showed phase separation after three months. The 1.0% formulation presented the best performance, with shear-thinning behaviour similar to the control. Initially, grass pea emulsions showed higher firmness and adhesiveness ($p < 0.05$), but consistency decreased during storage, unlike the control. Nutritionally, 25% fat samples had higher moisture and lower energy ($p < 0.05$), whereas 65% fat emulsions were more caloric. Protein content was highest in the 65% fat control ($p < 0.05$) and decreased with flour addition. Potassium was the predominant mineral, though not at claimable levels. The 1.0% formulations showed higher total phenolic content and antioxidant activity (DPPH, FRAP). Grass pea flour demonstrates potential as a clean-label ingredient, improving antioxidant capacity and short-term stability, despite sensory and long-term limitations.

Keywords: emulsions; grass pea flour (*Lathyrus sativus*); mayonnaise; clean label; plant-proteins

SEED COAT COLOUR AS A DRIVER OF ANTIOXIDANT AND MORPHOLOGICAL VARIATION IN PORTUGUESE COMMON BEAN GERMPLASM

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Legumes play a critical role in sustainable food systems, yet many traditional varieties remain underutilised. In Portugal, despite the wide diversity of *Phaseolus vulgaris* L. landraces preserved in germplasm collections, consumer demand is largely restricted to a few commercial varieties, contributing to the loss of local genetic resources. To address this gap, a Portuguese common bean collection comprising 259 accessions, was evaluated with a focus on seed coat colour (light, dark, and mix). Morphological descriptors (seed weight and width), seed mineral (calcium, potassium, magnesium, iron, zinc and phosphate), protein content, starch, and bioactive compounds (phenolics, condensed tannins, DPPH, saponins, phytic acid) composition were quantified. Seed coat colour significantly affected antioxidant related traits. Dark seeds exhibited the highest phenolic content (1.05 ± 0.35 mg GAE/g DW), condensed tannins (0.95 ± 0.38 mg CE/g DW), and DPPH activity (5.36 ± 1.72 μ mol TE/g DW), all significantly greater than light seeds (phenolics 0.69 ± 0.28 mg GAE/g DW; tannins 0.39 ± 0.43 mg CE/g DW; DPPH 2.85 ± 4.15 μ mol TE/g DW). Mix coloured seeds showed significantly larger seed weight (53.18 ± 13.96 g/100 seeds) and width (6.51 ± 0.77 mm), reflecting distinct morphological clustering pattern. In contrast, minerals, protein and starch did not differ across colours. Correlation analysis confirmed that Dark seeds aligned with antioxidant traits (DPPH $r=0.59$ and phenolics $r=0.47$, P -value <0.05), while Mix seeds aligned with seed size. Overall, seed coat colour is a reliable visual indicator of antioxidant potential, offering an immediate and low cost criterion to identify nutritionally superior Portuguese landraces and broaden consumer and breeding choices beyond the narrow range of currently marketed varieties.

Keywords: antiradical capacity, germplasm characterisation, nutritional profiling, phytochemical diversity

ENHANCING PEA PROTEIN CONCENTRATE FUNCTIONALITY: A COMPARATIVE STUDY OF PHYSICAL AND CHEMICAL MODIFICATIONS

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Pea protein concentrate (PPC) is a sustainable alternative to animal proteins, yet its limited functionality often hinders industrial application. This study evaluated four modification techniques, which are physical (dry heating and ultrasonication) and chemical (pH-shifting and Maillard conjugation), to improve the structural and functional properties of PPC. Results demonstrated that each method uniquely altered the protein's physicochemical profile. Ultrasonication and pH-shifting were most effective for solubility, achieving 96.4% and 92.6%, respectively. Conversely, Maillard conjugation primarily increased surface hydrophobicity through structural unfolding. Beyond fundamental characterization, the study assessed functional performance (emulsification, foaming, and gelling) under varying pH, ionic strength, and sucrose concentrations. Findings revealed that functional outcomes are highly dependent on both the modification technique and the specific solution environment, with pH-shifting consistently providing the most robust functional enhancements. Overall, this research shows that PPC can be effectively "tailored" for specific food systems through targeted modification. By linking structural changes to functional performance, these findings provide a practical framework for utilizing cost-effective pea protein concentrates in high-quality plant-based formulations.

Keywords: thermal processing, ultrasound-assisted treatment, alkaline treatment, pea protein–polysaccharide conjugation, protein structural modification

FARMERS' VOICES ON GRASSPEA: RESILIENCE ROOTED IN TRADITION

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Grasspea (*Lathyrus sativus* L.) plays a critical yet often undervalued role in the livelihoods of smallholder farmers across parts of Africa, Asia and Europe. Over time, it has emerged as a vital climate-adaptation crop thanks to its ability to thrive on marginal lands where other crops fail, and its remarkable tolerance to drought, poor soils, and low input conditions. Despite persistent challenges - including limited market access, production constraints, and insufficient policy recognition - farmers continue to cultivate grasspea. Its persistence reflects the importance of resilient, locally adapted crops in strengthening food security amid increasingly variable climates. Efforts were made to highlight the voices and perspectives of grasspea growing farmers from major producing countries, including Bangladesh, China, Ethiopia, India, Nepal, Portugal, and Spain. In these regions, grasspea is a historic crop woven into local culture and traditions, yet its potential remains hindered by inadequate research, extension services, and policy support. By featuring farmers' own experiences, we document how the crop fits into agricultural traditions, how cultivation practices and uses have evolved across generations, and what challenges and opportunities farmers identify today. This video offers an authentic, ground-level view of grasspea cultivation across diverse environments and captures firsthand insight to help inform policy and programmatic action aimed at promoting climate-resilient and nutritious crops.

Keywords: grasspea (*Lathyrus sativus* L.), farmers voices, neglected and underutilized species (NUS), low-input farming, sustainable agriculture

HARNESSING THE POWER OF PLANT-BASED MATERIALS: NATURAL ANTIMICROBIAL COMPOUNDS FOR A SUSTAINABLE FUTURE

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The shelf life of food is influenced by microorganisms, and various preservation methods ensure microbial safety while maintaining nutritional and sensory qualities. With growing consumer concerns about health, there is increasing demand for natural antimicrobial agents. Plant-based substances offer promising alternatives to antibiotics and preservatives. A better understanding of microbiota-food interactions can help producers in product development and quality assurance. In the project “Groenholdbar” we assessed significant differences in the abundance of food-associated pathogenic bacteria using both conventional plate count techniques and the rapid measurement system BactoBox. Microbial growth and viability rates were monitored over time and under different storage temperatures following supplementation with different plant-based materials, including plant-derived ingredients, raw materials, processed products and by-products. The tested materials exhibited variable effects on the growth and viability of different foodborne pathogens, demonstrating species-specific responses. Furthermore, varying concentrations of plant-based material revealed clear dose-response relationships, indicating concentration-dependent modulation of bacterial performance. Both analytical methods consistently detected significant shifts in microbial populations, confirming that the plant-based materials influenced bacterial dynamics. The combined application of classical cultivation methods and rapid real-time monitoring provides a robust framework for evaluating microbial responses to plant-based materials in food systems. The observed species-specific and dose-dependent effects highlight the potential of plant-derived ingredients as targeted modulators of food microbiota rather than broad-spectrum antimicrobial agents. These findings contribute to a deeper understanding of microbiota-food interactions and support the development of naturally preserved foods, enabling food producers to optimize safety, shelf life, and quality in a natural and sustainable manner.

Keywords: plant-based, antimicrobial, sustainability, food microbiota

FROM BY-PRODUCTS TO FUNCTIONAL FOODS: LEGUME PROTEIN-STABILIZED EMULSIONS FOR CIRCULAR FOOD SYSTEMS

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Valorization of food industry by-products constitutes a strategic approach to enhancing circularity and improving the efficiency of resource utilization within food systems. Pulse proteins represent a key technological resource for the development of innovative food products through the upcycling of industrial side streams, enabling the formulation of prototypes suitable for industrial scale-up and the commercialization of high-quality products with demonstrated health benefits. Our research group has more than 30 years of experience in the study and application of pulse proteins (Sousa et al., 1996; [<http://europepmc.org/article/AGR/IND20623577>]), with a strong focus on the development of food products in collaboration with industry partners. Legume- and fruit-based products constitute effective dietary sources of nutrients and bioactive compounds associated with health promotion, including plant proteins, dietary fiber, and antioxidants. In the present study, we report the outcomes of a large-scale, industry-led project aimed at developing novel food emulsions formulated with whey and pulse proteins, specifically faba bean and lupin isolates. The resulting formulations exhibited desirable textural characteristics and improved nutritional profiles, underscoring their potential for industrial application and market implementation. Emulsions were produced using apple and carrot pomaces (by-products of fruit juice processing), whey, and sunflower oil, and were stabilized with a 1:1 mixture of faba bean and lupin protein isolates. Emulsion stability was characterized by droplet size distribution, textural analysis, and rheological measurements. Antioxidant capacity was assessed through total phenolic content (TPC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) assays. The developed prototypes demonstrated technological robustness and readiness for industrial scale-up.

Keywords: pulse proteins, upcycling food sidestreams

ISOFLAVONES PROFILES OF ALFALFA (*MEDICAGO SATIVA* L.) AT DIFFERENT GROWTH STAGES

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Alfalfa (*Medicago sativa* L.) is the most important perennial forage legume worldwide. Besides being important forage for animals, alfalfa is a valuable source of isoflavones that play an important role in human health. The main objective of this study is to determine isoflavones profiles in six alfalfa cultivars at different growth stages. Samples of alfalfa were collected at three distinct growth stages in 2023 to evaluate developmental changes in isoflavone accumulation. At each sampling point, aboveground biomass was harvested, air-dried at room temperature, and homogenized to a fine powder using a laboratory mill. For isoflavone extraction, 500 mg of powdered lucerne material was weighed and extracted with 8 mL of methanol–water (4:1, v/v) containing 0.1% (v/v) acetic acid. Ultrasonic-assisted extraction was performed in a water bath at ambient temperature for 30 min to enhance the release of phenolic compounds. Following extraction, samples were centrifuged at 4000 rpm for 30 min. The supernatant was carefully collected and filtered through a 0.22 µm PTFE syringe filter prior to chromatographic analysis. The resulting filtrates were used for the determination of isoflavone contents. Separation, identification, and quantification of isoflavones were performed using high performance liquid chromatography (HPLC) on a C18 column (150 × 4.6 mm, 3 µm particle size). The mobile phase consisted of solvent A (0.1% [v/v] acetic acid in water) and solvent B (0.1% [v/v] acetic acid in acetonitrile). This experimental design enabled the evaluation of genotype-dependent variability and developmental stage influence on isoflavone profiles in lucerne across different growth stages.

Keywords: isoflavones, alfalfa, *Medicago sativa* L., growth stage, cultivar

GENETIC VARIATION IN PROTEIN SUBUNIT COMPOSITION OF BELGIAN FABA BEAN FLOUR DETERMINED WITH A NOVEL SIZE-EXCLUSION CHROMATOGRAPHY METHOD

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Faba bean (*Vicia faba* L.) is a promising European protein crop, but variability in protein quality, besides quantity, due to genotype poses challenges for reliable plant-based supply chains. Protein functionality in food systems strongly depends on the ratio of storage proteins, particularly legumin (11S globulin) and vicilin (7S globulin), which govern foaming, emulsifying, and gelling properties. To address the scarcity of systematic studies linking genotypic variation to protein characteristics, this study quantifies the impact of genotype on protein subunit composition. Fourteen winter and sixteen spring faba bean varieties, cultivated in Flanders (2024-2025), were analyzed. Protein content was determined via automated Dumas combustion, and subunit composition was quantified using a novel size-exclusion HPLC method combined with deconvolution. Protein content varied significantly, ranging from 23.1–25.3% for winter types and 22.5–31.1% for spring types. The 11S:7S ratios ranged from 0.96–1.31 (winter) and 0.94–1.39 (spring). Significant inter-varietal differences were observed for major subunits—convicilin, vicilin, legumin- α , and albumin—though legumin- β showed no significant variation among spring varieties. The 11S:7S ratio was primarily driven by vicilin ($r = 0.91$, $p < 0.001$) and legumin- α ($r = -0.90$, $p < 0.001$). These substantial compositional differences are expected to translate into distinct functional profiles. Varieties with a high vicilin fraction are anticipated to exhibit superior emulsifying capacity, while legumin-rich varieties are expected to display stronger gelling behaviour. Ongoing research will evaluate these functional properties to establish quantitative composition-functionality relationships, enabling targeted variety selection for specific food applications within the value chain.

Keywords: faba bean, genotype, protein subunit composition, protein functionality

WHAT MAKES CONVICINE IN VICIA SPECIES?

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Faba bean (*Vicia faba*) is an excellent source of protein, energy and fibre for humans and animals, but contains the anti-nutritional factors vicine and convicine (v-c). V-c is the product of an overflow mechanism from the riboflavin synthesis pathway, controlled by the RibAB gene. Vicine was first found in 1875 from *Vicia sativa* seeds, and 6 years later convicine was identified, differing only by a hydroxyl group replacing an amino group. Vicine can exist without convicine, but convicine is never found alone, and no pathway determining its presence has yet been determined. It is considered more bitter more harmful to rats than vicine. Hence we explored variation in convicine content in *Vicia* species. Vicine and convicine contents (mg / g) were obtained from 60 accessions representing 20 *Vicia* species (own laboratory) and from 82 inbred lines of faba bean (Khamassi et al., 2013, Plant Genetic Resources 11). The ratio of (convicine / (vicine + convicine)) was calculated. Most samples had less convicine than vicine. All that had less than 1 mg/g of vicine had no convicine, and all accessions that had over 2 mg/g of vicine had convicine. These point to convicine production being secondary to that of vicine, and necessary after the 2 mg/g threshold. The biochemical difference being a deamination, it appears that one of the many deaminases recognizes vicine. The presence of a threshold vicine content suggests that the deamination either requires a critical concentration of the vicine substrate, or assists in detoxifying or sequestering these compounds.

Keywords: faba bean, convicine, vicine, antinutritional

A CLINICAL–IMMUNOLOGICAL PERSPECTIVE ON THE IMPACT OF CULTIVAR CHOICE AND CULTIVATION METHODS ON THE ALLERGENICITY OF LEGUMES

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Legume allergies are an increasingly significant clinical challenge in allergology, particularly as plant protein consumption grows and their use in the food industry expands. Clinically relevant legume allergens are most commonly storage proteins from the 7S and 11S globulin groups, which are associated with persistent sensitisation, cross-reactivity among species, and potentially more severe clinical reactions. Although these proteins are well characterised from an immunological standpoint, the influence of agronomic factors on their expression and allergenic potential remains poorly understood. Available data indicate substantial differences in the composition and proportions of allergenically relevant protein fractions among cultivars of the same legume species, as well as in the sensitivity of their expression to environmental conditions. Abiotic stresses, such as drought, high temperatures, and nutritional imbalance during seed development, can modulate the synthesis and relative abundance of storage proteins, potentially affecting the IgE-binding properties of the final product. From a clinical perspective, this raises the question of whether raw materials of the same botanical species, but of different origin and cultivation practices, may pose different allergic risks for sensitised patients. The lecture emphasises the need for an interdisciplinary approach linking clinical allergology with agronomy and plant breeding, with the aim of improved risk assessment, safer production, and clearer communication along the “from field to patient” chain.

Keywords: allergy, seed storage proteins, cross reactivity

COMPOSITIONAL DIVERSITY OF AUSTRALIA'S MAJOR PULSES: FOUNDATIONS FOR FUTURE PROTEIN AND FOOD INNOVATION

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Global demand for plant based proteins is projected to rise from USD 20.3 billion in 2025 to USD 40.1 billion by 2034, driven by a 7.9% CAGR. This growth presents major opportunities for Australian pulse industries to supply high value food, feed, and industrial ingredients. While pulse breeding has traditionally focused on agronomic traits such as yield and disease resistance, expanding markets now require comprehensive data on nutritional composition. This study provides the first coordinated benchmarking of six key Australian pulses—chickpea, faba bean, field pea, lentil, lupin, and mung bean—grown across multiple environments. The assessment covered protein, starch, fibre, lipids, moisture, amino acids, and minerals using representative varieties. Marked interspecific differences were found: lupins had the highest protein and lowest starch contents, whereas field pea and lentil were starch dominant. Protein ranged from 17.2–39.5%, with lupin comprising the upper end. Carbohydrates were the major component (54–68%), and dietary fibre was notably high (12.6–58.0%), reaching up to four times that of wheat. Fat content was generally low (<1%) except in lupin and chickpea. Pulses were rich in lysine, two to three times higher than wheat, reinforcing their complementary role alongside cereal grains, which contribute sulphur-containing amino acids. Mineral profiling showed that a 250 g serving can meet or exceed daily requirements for potassium, magnesium, iron, and zinc. Overall, this benchmarking highlights the nutrient-dense profiles and substantial species-level diversity of Australian pulses, providing a foundation for breeding, processing innovation, and supporting their role in sustainable diets and the expanding global protein market.

Keywords: australian pulses, compositional traits

EFFECT OF TEMPERING AND MILLING ON FUNCTIONALITY OF YELLOW PEA FLOUR

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This study investigated how tempering moisture and milling speed influence the composition, particle size distribution, and functional properties of yellow pea flour. Yellow split peas were tempered to moisture levels ranging from 9.5% to 14% and subsequently pin-milled at speeds between 10,000 and 18,000 rpm. Analyses included proximate composition, particle size (D(0.5)), foaming capacity (FC) and stability (FS), and water-holding capacity (WHC). Results showed that lower tempering moisture or higher milling speed reduced flour moisture and protein content, while ash content was primarily affected by milling speed. Increased milling speed produced finer particles and enhanced FC, with minimal impact on WHC. Conversely, higher tempering moisture yielded coarser particles and improved WHC but reduced FC. FS remained largely unaffected by either factor. These findings highlight the role of processing conditions in tailoring yellow pea flour functionality for diverse food applications.

Keywords: tempering, milling, functionality, pea flour

WHOLE PLANT VETCH SILAGE AS ROUGHAGE FOR FATTENING PIG

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In two trials (MAY, DEC), 140 growing pigs were fed a compound feed containing cereals and grain legumes and either triticale straw (control = CON) or fresh whole plant vetch silage derived from Pannonian vetch (VP), common vetch (VS) or hairy vetch (VV). The pigs were slaughtered when weighing >119 kg. Retention time of the silage in the racks and use of the outdoor run were observed for CON, VS and VP using photographs automatically taken every 10 minutes. Generalized linear mixed models were performed with R. Pigs in the silage groups had a 3-5 d shorter growing and 3-5 d shorter finishing phase as well as an improved compound feed conversion in the finishing phase. Dressing percentage was between 79 and 80 % and lean meat percentage was 56.9 (CON), 57.7 (VS) and 58.2 (VP, VV). During daytime (DEC 08:30–20:30; MAY 07:00–21:00) pigs of VP and VS were observed more often in the outdoor run and in front of the racks. While CON racks were never emptied, VP and VS groups failed to empty the racks on 6 and 7 days in MAY, respectively. On the other days, VP and VS groups emptied their racks during 3.2 ± 0.65 h in MAY and 6.3 ± 3.34 h in DEC and 2.5 ± 0.65 h in MAY and 6.6 ± 3.34 h in DEC, respectively. In total, vetch whole plant silage is a suitable roughage that can contribute to the nutrient supply of fattening pigs and enrich their husbandry system.

Keywords: vetch, whole plant silage, nutrient supply

FROM GERMPLASM TO FEED: FLOWERING TIME AND QUALITY ATTRIBUTES OF 100 VETCH GENOTYPES

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Vicia sativa is an underutilized grain legume characterized by high grain protein content. It is widely adapted and thrives under adverse environmental conditions, including drought. Modern varieties achieve high and stable yields, making vetch grain a promising protein-rich feed component for climate-resilient cropping systems. However, high levels of antinutritional factors (ANFs) limit its use in animal nutrition. Gene bank accessions provide valuable genetic resources to overcome this constraint. A total of 100 genotypes are evaluated for agronomic performance and quality traits at two locations in Germany: Trenthorst (northern Germany) and Gatersleben (central Germany). From 2025 to 2027, 97 accessions from the IPK Gene Bank Gatersleben, together with three commercial varieties used as controls, are assessed in an augmented design. Traits under investigation include phenological characteristics (e.g., flowering time), morphological traits (flower and seed color), as well as grain protein content and ANF levels. Preliminary results indicate substantial variation in flowering time among accessions, with differences of up to 30 days. Initial quality analyses also reveal considerable genotypic variation, with crude protein content ranging from 23% to 39%.

Keywords: vetch, grain legumes, feed, quality, genetic resources

POTENTIAL BENEFITS OF USING LUPIN AND FABA BEAN SEEDS IN THE FEEDING OF NATIVE PIG BREEDS ON MEAT QUALITY AND PRODUCTION, AND ENVIRONMENTAL CONDITIONS

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Sustainable livestock production increasingly requires the development of feeding strategies that reduce environmental impacts while enhancing farm-level resilience to economic and climatic variability. In this context, the use of locally produced plant protein sources is gaining importance as an alternative to soybean meal, particularly within circular and low-emission agricultural systems. Among these alternatives, seeds of legumes, including narrow-leaved lupin (*Lupinus* spp.) and faba bean (*Vicia faba* L.), offer considerable potential due to their high protein content and role in biological nitrogen fixation, which contributes to reduced reliance on mineral fertilizers and lower greenhouse gas emissions. Legumes seeds have been widely studied as valuable protein and energy sources for monogastric animals and may also influence meat quality attributes in pigs. Specifically, native pig breeds, which are often raised under less intensive production conditions, may be especially well suited to legume-based feeding strategies. These breeds typically efficient utilization of locally available feed resources, and distinctive meat quality characteristics. Therefore, integrating legume seeds into their diets aligns with sustainability goals and enhancing product differentiation and market value. This study aimed to evaluate the effectiveness of diets containing narrow-leaved lupin or faba bean seeds in feeding a polish native pig breed. By assessing these parameters, the study seeks to contribute to a better understanding of the role of locally grown legume seeds in sustainable pig production systems and their potential to improve both production efficiency and meat quality. Research financed by the EU Horizon Europe research and innovation programme under grant agreement No.101059609.

Keywords: native pig breeds, meat production, meat quality, faba bean, lupine seeds

REPEATS AND REARRANGEMENTS IN *LENS* SPP. GENOMES HAVE IMPLICATIONS FOR BREEDING

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Numerous studies have demonstrated a reduction in genetic variability related to lentil breeding efforts carried out in different eco-geographic regions. To better understand the genetic variability available within the genus *Lens*, we developed both a pan-genome of seven diverse cultivated accessions as well as a reference genome for each of the six wild species. All species have seven chromosome pairs but have a size difference of over 1Gb – largely due to repeats. Synteny analysis suggests there are multiple inversions that are different among the domesticated accessions but once we look at the other species there are also multiple translocations and other rearrangements. These have been confirmed with oligo-FISH analyses. The implications of these findings for accessing this variability for plant breeding will be discussed.

Keywords: lentil, genomes, structural variation

THE FABA BEAN PANGENOME

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Faba bean (*Vicia faba* L.), one of the earliest domesticated crops dating back approximately 10,000 years, holds significant potential for sustainable agriculture due to its high yield and superior grain protein content. Despite its agronomic importance, progress in genetic research and crop improvement has long been constrained by the exceptionally large genome (~13 Gb), which has complicated genome assembly, variant discovery, and breeding applications. Recent advances in sequencing technologies and international collaborative efforts have resulted in a high-quality chromosome-scale reference genome, providing a foundation for deeper exploration of genomic diversity in this species. To capture global diversity, more than 2,000 accessions from worldwide germplasm collections were characterized using optimized genotyping-by-sequencing (GBS) approaches. Population structure analyses and high-density SNP resources enabled the systematic exploration of the species' diversity space. A representative set of 50 genotypes spanning diverse geographic origins, genetic backgrounds, and phenotypic variation was selected for chromosome-scale assembly. These assemblies form the backbone of the faba bean pangenome, enabling the identification of variants beyond the scope of a single reference genome. By integrating the pangenome with population-scale resequencing data, we enable genome-wide association studies of key agronomic and disease-related traits, improving variant discovery across diverse germplasm and facilitating the identification of candidate genes underlying trait variation. Together, these resources provide a foundation for understanding faba bean genomic diversity and accelerating genomics-assisted breeding for resilient and high-performing cultivars.

Keywords: Pangenome, associated mapping, diversity

FROM MENDEL'S PEAS TO GLOBAL CROP DIVERSIFICATION: THE ROLE OF GENE BANK-OMICS

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We recently published a groundbreaking work titled “Genomic and genetic insights into Mendel’s pea genes” [<https://doi.org/10.1038/s41586-025-08891-6>] In which we exemplified the utilisation of high resolution genebank genomics in peas. I will share findings with forward look on the new resource utilisation in global pea science and breeding. Mendel studied in detail seven pairs of contrasting traits in pea (*Pisum sativum*), establishing the foundational principles of genetic inheritance. Here we investigate the genetic architecture that underlies these traits and uncover previously undescribed alleles for the four characterized Mendelian genes, including a rare revertant of Mendel’s white-flowered allele. Primarily, we focus on the three remaining uncharacterized traits and find that (1) an approximately 100-kb genomic deletion upstream of the Chlorophyll synthase (ChlG) gene disrupts chlorophyll biosynthesis through the generation of intergenic transcriptional fusion products, conferring the yellow pod phenotype of gp mutants; (2) a MYB gene with an upstream O₂ element insertion and a CLE peptide-encoding gene with an in-frame premature stop codon explain the v and p alleles, which disrupt secondary cell wall thickening and lignification, resulting in the parchmentless, edible-pod phenotype; and (3) a 5-bp exonic deletion in a ClK-like co-receptor kinase gene, in combination with a genetic modifier locus, is associated with the fasciated stem (fa) phenotype. Furthermore, we characterize genes and alleles associated with diverse agronomic traits, such as seed size and the ‘semi-leafless’ form. This study establishes a foundation for fundamental research, education in biology and genetics, and pea breeding practices.

Keywords: genebank, genomics, pea, mendel, gwas

NUCLEAR AND TOTAL PROTEOMIC ANALYSES OF TENDRILS IN TWO PEA (*PISUM SATIVUM* L.) CULTIVARS REVEAL DISTINCT REGULATORY PROGRAMS UNDERLYING CONTRASTING PHENOTYPES

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Field pea (*Pisum sativum* L.) is an important legume crop valued for its high protein content and role in sustainable agriculture, particularly in crop rotations across the Canadian Prairies. Beyond photosynthesis, pea leaves provide mechanical support through tendrils, yet the molecular basis of tendril development remains poorly understood. In this study, we performed integrated nuclear and total proteomic analyses of leaf tissues in two contrasting cultivars—a semi-leafless type with highly developed tendrils (Cooper) and a leafed type with residual tendrils (Trapper)—to define distinct developmental trajectories. Across tissues, we identified approximately 7,800 total and 8,600 nuclear proteins. Nuclear proteomes showed greater conservation (~37% differentially abundant proteins) compared with total proteomes (~78%), with young tissues contributing disproportionately to proteomic variation. Genetic variation at the *afila* locus strongly influenced tendril proteome composition. Cooper exhibited extensive proteome reprogramming consistent with its branched tendril phenotype, whereas Trapper showed more constrained tendril development shaped by nuclear regulatory signatures. Differentially abundant proteins included transcription factors (WDR, C2H2, bZIP, PHD, TF-B3), redox regulators, phytohormone-associated proteins, chromatin modulators, and structural proteins. Together, these findings reveal divergent regulatory programs underlying tendril development and provide a systems-level resource for future functional and breeding studies.

Keywords: pea, compound leaf, tendrils, total and nuclear proteomics, transcription regulators

NEXT GENERATION DECISION SUPPORT FOR LEGUME BREEDING THROUGH DOMAIN-SPECIFIC ARTIFICIAL INTELLIGENCE

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Common bean, *Phaseolus vulgaris*, is a cornerstone grain legume for global food security, yet the accelerating growth of genomic, agronomic, and climate research has made it increasingly difficult for breeders and scientists to synthesize evidence efficiently and translate knowledge into actionable decisions. We present BeanGPT, a domain-specific retrieval augmented artificial intelligence system designed exclusively for common bean research that transforms natural language questions into citation-backed and verifiable scientific responses. BeanGPT integrates more than three hundred thousand curated legume-focused peer-reviewed publications with eleven years of multi-location performance trials, structured climate projections extending to 2090 under multiple emission scenarios, and a genomic knowledge graph built from authoritative gene and protein databases. The system combines semantic vector retrieval, deterministic intent classification, structured parameter extraction, and constant-time genomic validation to ensure that every molecular entity referenced is either validated against primary databases or explicitly grounded in literature evidence. An asynchronous architecture supports concurrent users with low latency, while dynamic visualization modules generate interactive analyses directly from structured datasets. Evaluation across genetics, agronomy, and climate queries demonstrated ninety-five percent retrieval success within the top eight documents, one hundred percent gene verification accuracy, and ninety-seven percent citation precision, with average response times under five seconds. BeanGPT establishes a transparent and scientifically rigorous framework that accelerates evidence-based breeding decisions while maintaining traceability, scalability, and domain fidelity for next-generation legume research.

Keywords: climate adaptation, common bean, genomic validation, multi environment trials, retrieval augmented generation

DIVERSITY AND GENETIC CONTROL OF SPECIALIZED METABOLITES SHAPING STRESS DEFENCE AND QUALITY IN PEA SEEDS

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Pea (*Pisum sativum* L.) is a major European legume crop producing protein-rich seeds increasingly used in food applications. Its specialized metabolites (SMs) play key roles in stress defence while influencing organoleptic and nutritional quality, highlighting the need to consider this dual function in breeding programs. Despite their importance, the genetic and biochemical bases of SM biosynthesis in pea seeds remain poorly understood. To address this, we performed untargeted metabolomic analyses (LC-MS/MS) on mature dry seeds, examining seed coats and embryos from a panel of 204 pea genotypes, including wild and domesticated species and subspecies. Metabolomic profiling revealed large metabolic diversity dominated by nitrogen-containing compounds, with seed coats enriched in phenolics (especially flavonoids) and embryos characterized mainly by lipids and related structures. Comparative analyses across species indicate evolutionary history shapes these metabolic profiles. To uncover genetic determinants, we conducted a metabolome-based genome-wide association study (mGWAS) combining metabolomic and genotyping data to identify loci and candidate genes involved in SM biosynthesis, modification, and regulation. Significant associations were found for defence-related metabolites in seed coats and for metabolites linked to seed quality traits in embryos, leading to several candidate genes. The antimicrobial activity of selected SMs is being characterized, and functional validation of these genes is underway via heterologous expression in *Escherichia coli*. These results provide new insights into the metabolic landscapes of pea seed coats and embryos and their genetic control, paving the way for integration of SM traits into breeding strategies aimed at improving seed quality without compromising stress resistance.

Keywords: seed metabolome, metabolite plasticity, untargeted metabolomics, GWAS, seed defence

GENETIC AND MOLECULAR APPROACHES TO MODULATE B-ODAP BIOSYNTHESIS IN *LATHYRUS SATIVUS*

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Grass pea (*Lathyrus sativus* L.) is an annual cool-season grain legume with high nutritional value and exceptional resilience to abiotic stresses, making it a promising crop for climate change adaptation. However, its wider adoption is constrained by the presence of the neurotoxin β -N-oxalyl-L- α,β -diaminopropionic acid (β -ODAP), long-term consumption of which is associated with neurolathyrism. Despite efforts to develop low-ODAP varieties, environmental instability and limited breeding tools have hindered their widespread use. In our study, we generated novel low-ODAP mutants of the cultivar Pusa 24 using EMS mutagenesis, with the mutagen dose optimized to balance mutation frequency and plant viability. Screening across M₂ seedlings and M₃ seed generations identified stable low-ODAP lines that retained normal protein content, demonstrating that nutritional quality can be preserved while reducing antinutritional load. Comparative metabolite profiling revealed pronounced genotype and developmental stage specific shifts in amino acid, organic acid, and sugar metabolism, particularly during early pod development, highlighting key regulatory windows in ODAP biosynthesis. To accelerate functional genomics and gene-based improvement, efficient regeneration and transformation protocols were established. A multiple shoot induction system using cotyledonary nodes enabled rapid plant regeneration, while an *Agrobacterium*-mediated CRISPR/Cas9 hairy root transformation platform facilitated targeted genome editing and rapid gene function assessment in *Lathyrus*. Together, these integrated approaches provide valuable genetic resources, metabolic insights, and biotechnological tools for developing safer, nutritionally robust grass pea varieties, supporting food security in climate-vulnerable regions.

Keywords: β -ODAP, mutants, EMS, metabolomics, hairy root

CRISPR/CAS9 EDITING OF GMSULTR3;3 REVEALS A NOVEL STRATEGY TO MODULATE PHYTIC ACID ACCUMULATION IN SOYBEAN

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Legumes are central to sustainable agri-food systems, yet their nutritional value is limited by phytic acid (PA), the main seed phosphorus storage compound. PA chelates essential minerals such as Fe, Zn, Ca, and Mg, reducing their bioavailability in monogastric diets and increasing phosphorus excretion into the environment. Developing low-phytate (lpa) genotypes without compromising plant performance remains a key breeding challenge. Here, we targeted the phosphate/sulfate transporter gene family SULTR3;3 in soybean using CRISPR/Cas9 genome editing to modulate phosphorus allocation and reduce PA accumulation in seeds. Independent edited lines carrying mutations in both GmSULTR3;3 paralogues were generated and molecularly characterized. Selected lines exhibited up to 20% lower seed phytic acid content without significant alterations in plant growth, seed development, or yield-related traits, identifying GmSULTR3;3 as a promising target for lpa breeding. To investigate the broader biological consequences of GmSULTR3;3 editing, integrated omics approaches were applied. Metabolomic profiling of seed flours confirmed significant metabolic changes associated with reduced PA levels. Consistently, RNA-seq analyses of developing seeds revealed extensive transcriptional reprogramming beyond phosphorus homeostasis, suggesting that these transporters may function as regulatory nodes in seed development and metabolism. Ongoing studies aim to determine the subcellular localization of GmSULTR3;3 proteins and assess how editing-induced mutations affect their targeting and activity. Overall, these findings provide mechanistic insights into phosphorus partitioning in soybean seeds and support GmSULTR3;3 editing as a viable strategy to enhance mineral bioavailability while preserving agronomic performance and improving nutritional quality.

Keywords: SULTR, biofortification, phytic acid, sustainable agriculture, gene editing

FROM GENOMICS TOOLS TO VARIETIES: A CUSTOM GENOMIC SELECTION FRAMEWORK TO DEVELOP SUPERIOR DRY BEAN VARIETIES WITH SPECIFIC ADAPTATION

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The integration of genomic selection (GS) into public breeding programs represents a critical step toward accelerating genetic gain through data-driven decision making. However, transforming high-density genomic information into affordable, program-ready tools for routine selection decisions remains a major constraint. This study presents a genomic selection framework applied to the North Dakota State University (NDSU) dry bean (*Phaseolus vulgaris* L.) breeding program through the development of a targeted SNP chip development informed by genotyping-by-sequencing (GBS) and genome-wide association analysis (GWAS). A diverse training population of 427 advanced breeding lines representing eight market classes across the Middle American and Andean gene pools was evaluated from 2020–2023 for key agronomic, quality, and disease-resistance traits. After rigorous marker optimization and quality control, a custom genotyping chip containing 3,867 SNPs was developed, distributed across all 11 chromosomes, with the greatest marker densities on chromosomes 11 (18%) and 8 (16%). Notably, 5.6% of the selected SNPs were shared across multiple traits, suggesting potential multi-trait utility. Genomic prediction models produced accuracies ranging from 0.33 to 0.92, with the highest predictive ability observed for 100-seed weight and the lowest for days to maturity. Validation in independent populations demonstrated strong predictive performance, including accuracies of 0.80 for soybean cyst nematode (SCN) resistance and 0.53 for protein content. This customized SNP chip provides a cost-effective, high-throughput platform for selection. Its implementation in early-generation negative selection enables breeders to potentially discard inferior lines at preliminary stages, reduce field/testing population size, and concentrate resources on advancing superior genotypes.

Keywords: *Phaseolus*, common bean, genomic selection

UNCOVERING ADAPTIVE TRAITS IN LEGUMES THROUGH NOVEL GENOTYPING APPROACHES

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In the context of climate changes, legumes play a crucial role in promoting sustainable agriculture and food security, due to their high protein content and nitrogen-fixing ability. Despite these advantages, the highly repetitive nature of many legume genomes complicates large-scale sequencing of germplasm accessions, thereby limiting the exploitation of their genomic potential. The GENUINE (Genomic mEthods to ideNtify fUture cllmate resilient geNotypes in lEgumes) project aims to overcome these constraints by applying an innovative and cost-efficient genotyping platform. The approach is based on a sequencing pipeline incorporating a Cas9-mediated repeat depletion system, designed to selectively remove highly repetitive and less informative genomic regions while enriching functional sequences. We applied this strategy to genetically diverse panels representative of different agro-environmental regions of lentil (*Lens culinaris*), pea (*Pisum sativum*), and faba bean (*Vicia faba*), three nutritionally and economically important crops characterized by large and highly repetitive genomes. Preliminary results in lentil and pea showed that Cas9-driven repeat depletion concentrates sequencing efforts toward single-copy regions, thereby improving genome-wide genotyping efficiency. The resulting genomic data were integrated into a landscape genomics framework together with environmental variables, shedding light on the genomic basis of adaptation to different ecosystems. Future genome-wide association studies (GWAS) will further investigate the links between genomic variation and key agronomic traits. These outcomes will provide valuable tools to guide breeding programs, supporting the development of climate-resilient legume varieties and contributing to more sustainable agricultural systems under future global warming scenarios.

Keywords: legumes, CRISPR-Cas9, landscape genomics

SNP GENOTYPING FOR LEGUMES

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Single nucleotide polymorphism (SNP) genotyping is now an indispensable tool for breeding and genetic analysis of legumes. Based on large SNP data and in part through collaboration with academic partners, we have established several genotyping panels for important European legumes. Most of these panels contain optimized marker sets in terms of markers from legacy arrays, high data quality, high levels of polymorphism over a wide range of germplasm, haplotype structure and genome distribution. These new panels include a 10K pea array, an 8K array for faba bean and a 6K array for soybean. All three arrays are on the Illumina Infinium XT platform which permits a flexible and reproducible generation of SNP data in a 96-sample format at reasonable costs. Since the development of these arrays, we have analyzed ten-thousands of legume samples for purposes such as marker/trait association studies, characterization of germplasm and varieties, genetic mapping and genomic selection in plant breeding. Beside the three mentioned optimized arrays, we have developed optimized tools to analyze all other public legume genotyping arrays on the Illumina platform including lentil, chickpea and blue lupin or on the Axiom platform (like soybean, green bean and pea). Emerging breeding methodologies require SNP analyses with more focused marker sets. Traditionally, this has been achieved through single marker analysis using the KASP platform. To bridge the gap between single marker and array genotyping, we now use targeted genotyping-by-sequencing (tGBS) to enable customized SNP profiling in the medium marker range of approximately 100 to 500 markers.

Keywords: SNP, genotyping, marker analysis

GENETIC ARCHITECTURE AND NETWORKS UNDERLYING AGRONOMICAL TRAITS IN SOYBEAN

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Soybean (*Glycine max* (L.) Merr.) is a major global source of plant protein and oil, and increasing consumption requires the development of high-performing, well-adapted cultivars. Breeding is challenged by complex correlations among agronomic traits and genetic interactions influencing their expression. Most key traits are quantitative, controlled by multiple genes and strongly influenced by the environment. Although many main-effect quantitative trait nucleotides (QTNs) have been reported, limited information exists on QTN-by-environment interactions (QEIs) and epistatic QTN–QTN interactions (QQIs), resulting in incomplete explanations of phenotypic variation. In this study, 182 genetically diverse soybean lines were genotyped with over 30,000 SNP markers and phenotyped for 12 agronomic traits related to yield, grain quality, plant architecture, and local adaptation across two environments (Serbia and Austria) over two years. Genome-wide association analyses were performed using the 3VmrMLM method. Single-environment analyses identified 133 QTNs, multi-environment analyses revealed 178 QTNs and 95 QEIs, and epistatic analyses detected 57 QTNs with 142 QQI pairs, highlighting the substantial contribution of non-additive effects. Integration of GWAS results across traits enabled construction of a genetic association network, uncovering nine highly connected loci influencing at least three traits. Several loci colocalized with previously reported maturity and plant height regions. These findings provide insights into the genetic architecture of soybean agronomic performance and identify valuable loci for functional validation and breeding applications. This research was supported by the Science Fund of the Republic of Serbia (SoyPredict, 6788) and the EU Horizon Europe project BELIS (101081878).

Keywords: GWAS, soybean, 3VmrMLM

INSIGHT INTO MICRORNA REGULATORY NETWORKS INVOLVED IN FLOWERING INDUCTION OF WHITE LUPIN (*LUPINUS ALBUS* L.)

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White lupin (*Lupinus albus* L.) is an important grain legume crop, appreciated for its high seed protein and low alkaloid levels. Synchronization of flowering time in seasonal environments is critical for plant adaptation and its reproductive success. To ensure correct timing of flowering, its induction is under complex genetic regulation that addresses environmental cues, including photoperiod, vernalization, and temperature, as well as gibberellin, autonomous, and age-related pathways. It also involves post-transcriptional regulation of gene expression, maintained by small non-coding RNAs, including microRNAs. Recently, we selected a set of white lupin lines differing in flowering time and their responsiveness to vernalization and photoperiod, showing significant phenological associations of marker loci localized in gene and microRNA coding regions. Consequently, we investigated the expression profiles of miRNAs and mRNAs under contrasting photoperiods (8 h and 16 h) and vernalization treatments across two developmental stages (juvenile and floral onset) in nine phenotypically diverse lines. A total of 136 miRNA clades revealed significantly different expression patterns between photoperiods, vernalization treatments, developmental stages, and genotypes. Among these, 32 clades were explicitly associated with the vegetative-to-generative phase transition. Key candidates, including miR156, miR2111, miR395a, miR398, miR408, and miR4415, were identified as potential regulators of flowering pathways, providing new insights into miRNA-mediated regulation of flowering in white lupin. This study was financially supported by the National Science Centre (Poland) project “Shall I interfere? A short story of small RNAs involvement in flowering regulation of white lupin (*Lupinus albus* L.)” no. 2021/43/D/NZ9/00293.

Keywords: white lupin, miRNA, flowering time

TOWARDS PAN-NLRome IN COMMON BEAN

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Common bean (*Phaseolus vulgaris* L), is an ideal evolutionary model to study adaptive diversity in wild populations. Its remarkable evolutionary history has led to the emergence of three eco-geographical gene pools: Mesoamerican, Andean and northern Peru/Ecuador. The first two populations include both wild and domesticated forms, while northern Peru/Ecuador one has only wild forms. Plants and pathogens engage in continuous co-evolution through reciprocal adaptation. Therefore, many members of the plant immune system, as the Nucleotide-binding leucine-rich repeat (NLR) genes are highly diverse. NLRs constitute a rapidly evolving multigene family, exhibiting substantial variation in copy number across species, with approximately 400 members identified in common bean. NLRs are typically organized into large gene clusters, such as the I cluster or the Co-2 cluster in *P. vulgaris*. Here we present high-quality chromosome-level genome assemblies of 17 wild *P. vulgaris* accessions (7 Mesoamerican; 6 Andean; 4 northern Peru/Ecuador). 15 genotypes were sequenced with Ultra Long reads based on ONT technologies and 2 with PacBio-HiFi. All of them were de novo assembled and organized into chromosomes. Gene prediction was performed and the NLRs were annotated based on 2 different specialized annotation softwares and manual inspection. A gradient in NLR abundance was observed along the distribution transect of wild bean populations, with higher numbers in the Mesoamerican and reduced numbers in the Andean center. Several NLR clusters, such as the I cluster, present a dramatic structural variation. These results provide valuable genomic resources to established common bean Pan-NLRome.

Keywords: pan-NLRome, common bean, wild

KEY REGULATORS OF FLORAL INDUCTION IN FABA BEAN (*VICIA FABA* L.) ARE REVEALED FROM SPATIO-TEMPORAL GENE EXPRESSION ANALYSIS OF CONTRASTING GENOTYPES

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Flowering time is a crucial trait for crop adaptation and yield stability, yet its genetic regulation in *Vicia faba* remains poorly understood. In this study, we performed RNA-sequencing of shoot apex (SA) and leaf tissues to investigate the genetic mechanisms underlying variation in flowering time between two contrasting cultivars, Gubbestad (early flowering) and Honey (late flowering). Based on differential gene expression analysis, K-means clustering and weighted gene co-expression network analysis (WGCNA), we identified key transcriptional modules enriched in genes associated with gibberellin (GA) signalling, photoperiod response, and floral induction. Notably, FT homologs exhibited distinct expression patterns: two FT genes were expressed in Gubbestad, whereas only one was detected in Honey, in the SA during the reproductive stage. The late-flowering cultivar displayed high expression of the transcriptional cofactor TFL1 and transcription factor AP2 in the SA at the vegetative stage, which likely contributed to late floral initiation by suppressing downstream activators of flowering such as SOC1, AP1, and SPL/miR156. In contrast, the early-flowering cultivar showed FT gene expression in both leaf and SA, promoting LFY and SOC1, which accelerate floral transition. Through co-expression analysis, WGCNA identified SOC1 and AG as key hub genes within flowering-related modules, co-expressed with multiple genes encoding regulators of floral development. Our findings highlight the interplay between GA-mediated flowering pathways and photoperiod-responsive genes, revealing a complex regulatory network that controls floral induction. Unravelling these molecular mechanisms provides insights into breeding faba bean cultivars that are better adapted to different geographical regions.

Keywords: *Vicia faba*, floral induction, protein crop, legume, co-expression, gibberellin, photoperiod

SINGLE-NUCLEUS AND SPATIAL TRANSCRIPTOMICS REVEAL SPATIAL EXPRESSION AND FUNCTION OF PVYSL3 IN POD-SEED IRON TRANSPORT IN COMMON BEAN

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Common bean (*Phaseolus vulgaris* L.) is a globally important grain and vegetable crop, ranking top among legume vegetables in China. This crop also represents a key target for iron biofortification. Iron transport has been extensively studied in rhizosphere, root-shoot, and leaf-fruit systems, but poorly understood in internal structure of fruit. Spatial omics (spatial transcriptomics and single-nucleus RNA-seq), enables in situ analysis of cellular heterogeneity and gene expression patterns, providing precise molecular atlases for dissecting tissue micro-regions. Here, we elucidate that in reproductive organs of common bean, iron progressively relocates from pod wall to grain during development. This dynamic distribution driven us to establish spatial and single-nucleus transcriptomic atlases of pods at 15 and 20 days after flowering, identifying 9 and 13 cell types, respectively. Integrative analysis identified PvYSL3a as a strong candidate regulating pod-seed iron partitioning. Molecular characterization demonstrated that PvYSL3a is a membrane-localized transporter with 14 transmembrane domains. Its heterologous expression in yeast and Arabidopsis altered iron distribution, confirming its Fe transport activity, while its expression is transcriptionally regulated by PvWRKY3. Our study provides valuable high-resolution omics resources and advances understanding of iron distribution and its regulation in reproductive organs of common bean. The mechanism of PvYSL3a-mediated iron distribution provides theoretical and practical guidance for iron biofortification tailored to dual pod and seed consumption.

Keywords: spatial transcriptomics, single-cell RNA-seq, iron distribution, PvYSL3a

UNMETHYLATED REGION PROFILING IN FABA BEAN

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Faba bean (*Vicia faba*, $2n=12$) is a cool season legume crop with high (approx. 30%) protein content. Despite being one of the oldest cultivated crops, its genetic improvement is limited because of its large (13-Gb) and highly repetitive genome, with only 2% gene density. Given its size, whole genome sequencing at high resolution or on a large scale (eg. populations) remains challenging. DNA methylation is a heritable epigenetic variation that involves the addition of methyl groups to the 5' cytosine of DNA, without changing its sequences. It regulates gene expression by changing DNA accessibility to transcription factors and polymerases. Notably, low to no DNA methylation levels have been shown to correlate with the presence of functional DNA, including both protein coding genes and regulatory regions. Therefore, this project focusses on identifying Unmethylated Regions (UMRs) to investigate both epigenomic and functional space diversity across a panel of 80 faba bean accessions. We will generate a pan-UMR-ome from a diverse panel of elite faba bean breeding lines and identify core-UMRs (conserved across all accessions) and accessory-UMRs (unique to a subset of accessions), and their link to genes and regulatory elements. This UMR-focused study is expected to enhance our understanding of faba bean epigenomic diversity and to provide a foundation for future faba bean investigations and breeding strategies to produce resilient and high yielding varieties.

Keywords: epigenetics, DNA methylation, faba bean, unmethylated regions

GENOME-WIDE MUTATION DISCOVERY THROUGH OPTIMIZED WHOLE-GENOME SEQUENCING IN A NOVEL EMS-BASED COMMON BEAN TILLING PLATFORM

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Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption worldwide and a major target for nutritional and agronomic improvement. However, genomic resources and functional genomics platforms for this species remain comparatively limited. To expand the genetic diversity available for trait analysis, a large EMS-mutagenized population (PhasTILL) was developed in the Italian cultivar Mecerarly (dwarf Borlotto type, Verisem Italia), generating 2,345 M1 lines. M3 seeds from 2,360 M2 plants, derived from 640 M1 lines, are currently available. Early phenotypic screening identified visible alterations in germination and plant development, establishing a valuable platform for forward and reverse genetics. For genome-wide mutation discovery, DNA from four M2 plants per each of 370 reproduced M1 lines was collected and subjected to whole-genome sequencing (WGS). A pilot study compared variant-calling pipelines using short-read sequencing of pooled M2 samples (4× pools at 40× coverage), individual mutants (10× coverage), and wild-type controls. Five variant-calling approaches were evaluated, including alternative Freebayes strategies and a HaplotypeCaller gVCF workflow. After multi-step filtering, an average of approximately 26,700 variants per sample was retained. HaplotypeCaller detected more canonical EMS-type transitions, whereas Freebayes showed higher validation rates in low-coverage datasets, highlighting the trade-off between sensitivity and precision. These results demonstrate that EMS-induced mutations can be reliably identified through optimized short-read WGS. The PhasTILL platform expands genetic variability in common bean and supports functional genomics and breeding programs targeting yield, resilience, and nutritional quality.

Keywords: common bean (*Phaseolus vulgaris* L.), functional genomics, TILLING by sequencing, whole-genome sequencing (WGS), variant calling optimization

DEVELOPMENT OF A PRACTICAL HAPLOTYPE GRAPH (PHG) DATABASE FOR FABA BEAN USING MULTIPLE GENOME ASSEMBLIES

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Faba bean (*Vicia faba*) is an important grain legume with high nutritional value. Despite its agronomic importance, genomic-assisted breeding in faba bean is challenging because of its very large (~13 Gb) and highly repetitive genome. The Practical Haplotype Graph (PHG) is a graph-based representation of multiple genomes which covers the whole diversity of a breeding program. It consists of a graph of haplotypes anchored to a reference genome and enables genotype imputation from low-coverage sequencing data. This approach has been successfully applied in other crops such as wheat and maize for efficient genotype storage and accurate imputation. In this study, we developed a PHG database for faba bean using 19 genome assemblies, with Hedin v2 as the reference genome. Genome assemblies were aligned to the reference, and haplotypes were defined across genic and intergenic regions. These haplotypes were stored in a trellis graph structure, where each node represents a haplotype for a genomic interval and edges connect adjacent intervals. This structure captures sequence diversity across the included assemblies within a unified graph framework. To evaluate the imputation efficiency of the PHG database, 0.1x coverage sequencing data were used for genotype imputation. The results demonstrated reliable imputation performance, supporting the ability of the PHG to recover accurate genotypes from low-coverage data. Considering the large and repetitive faba bean genome, this highlights the robustness and potential of the PHG approach for complex legume genomes. This PHG database provides a resource for accurate and affordable genotype imputation to support faba bean breeding.

Keywords: Practical Haplotype Graph (PHG), genotype imputation, pangenome, low-coverage sequencing, faba bean

CRISPR/CAS-BASED MANIPULATION OF TRANSCRIPTIONAL REPROGRAMMING AND STOMATAL IMMUNITY AGAINST PATHOGEN INVASION OF AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA*) AND GRASSPEA (*LATHYRUS SATIVUS*)

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African yam bean (*Sphenostylis stenocarpa*) and Grasspea (*Lathyrus sativus*), important leguminous crops, are known to be resilient and tolerant to abiotic stress but susceptible to bacterial infections. Upon pathogen invasion, plants have evolved sophisticated immune systems such as transcriptional reprogramming and stomatal closure to respond to microbial threats. QUAN SHOU KINASE 1 (QSK1), a conserved serine/threonine kinase is known to regulate plant immune responses upon pathogen invasion of Arabidopsis by the phosphorylation of some immunity-related transcription factors. This study was designed to investigate the role of the transcription factor, QSK1 in modulation of stomatal immunity and transcriptional defense against bacterial pathogens in *Sphenostylis stenocarpa* and *Lathyrus sativus*. Using CRISPR/Cas9 genome editing technology, we are currently working on generating loss-of-function mutants and overexpression lines of QSK1 to elucidate its regulatory role in pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), with a focus on stomatal closure and apoplastic defense responses. The study will explore cross-kingdom immune signaling by testing whether QSK1-mediated defenses are effective against not only the devastating pathogen of African yam bean, *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas syringae* but also the fungal pathogens, *Uromyces pisi* and *Erysiphe species* in *Lathyrus sativus*. This work will provide novel insights into conserved immune mechanisms that can be leveraged as biotechnological strategies for enhancing crop resistance against infectious plant pathogens for sustainable agriculture and food security.

Keywords: african yam bean, CRISPR/Cas9, pathogens, transcriptional defense, stomatal immunity

DEVELOPING FUNCTIONAL GENOMIC RESOURCES TO ACCELERATE *VICIA SATIVA* (COMMON VETCH) IMPROVEMENT

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Global population growth is increasing demand for affordable and sustainable protein sources for both animal feed and human consumption. Common vetch (*Vicia sativa*) is a hardy legume suited to poor and arid environments and contributes to soil health through nitrogen fixation, weed suppression, and moisture retention. Its seeds contain 24–32% protein as well as essential fatty acids and vitamins, making it a promising plant-based protein source. However, global vetch production remains below 5% of legume crop production due to the presence of two toxic anti-nutritional compounds, β -cyano-alanine (BCA) and γ -glutamyl- β -cyano-alanine (GBCA), which cause excitotoxicity in monogastric animals and limit its suitability for feed and food markets. To accelerate common vetch improvement, we developed a suite of genomic and genetic resources. We generated a high-quality 1.65 Gbp chromosome-level reference genome ($n = 6$) using Oxford Nanopore, Illumina, and Hi-C sequencing, achieving 98% completeness and annotating 53,218 protein-coding genes (Xi, Nguyen et al. 2022). We also whole-genome resequenced 285 wild accessions and identified a seed coat colour-related gene with potential use as a supply chain marker for vetch varieties (Xi et al., unpublished). Additionally, we established a rapid *Rhizobium rhizogenes* hairy root transformation system (Nguyen and Searle 2022), optimized a stable *Agrobacterium tumefaciens* transformation method achieving 7% efficiency within 16 weeks, and developed Cas12a and Cas9 CRISPR-based genome editing toolkits (Wang et al., unpublished). Together, these resources provide a strong foundation for accelerating vetch improvement and enhancing its value as a safe, sustainable plant-based protein crop.

Keywords: common vetch (*Vicia sativa*), plant-based protein, supply-chain marker gene, genome assembly and transformation, CRISPR genome editing toolkits

Genebank Genomics: Resurrecting Utility for Nordic Soybean

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To unlock the potential of plant genetic resources, genomic characterization must supplement germplasm conservation. To this end, NordGen (the regional genebank of the Nordic countries) and the Center for Quantitative Genetics and Genomics at Aarhus University are whole genome resequencing 4,500 high-protein accessions including soybeans, peas, clover, and faba beans. The deep characterization of regionally relevant germplasm will allow NordGen to better facilitate conservation and germplasm utilization. In soybean, the 155 accessions conserved by NordGen – originating from a Swedish soybean breeding program running from the 1930-1980's has allowed for the identification of a core of just four accessions capturing approximately 76% of the observed genomic variation. Notably, the globally important cultivar 'Fiskeby III' represents only half of this adaptive variation, suggesting untapped sources of heritable variation linked to defense responses, stress resistance, and early maturation may lie dormant in the genebank. Characterization of E1–E4 maturity genes revealed haplotypes driving extremely early maturation in the Nordic soybean germplasm. Further, deep genomic characterizations allowed for the identification of a novel frameshift mutation (e4-fs) in the third exon of the E4 gene resulting in a suspected loss-of-function mutation of the E4 gene. This variant is absent from all Fiskeby lines, but remains conserved across cold adapted accessions across Sweden (84 accessions), Canada (13 accessions), mainland Europe (2 accessions), and Japan (1 accession).

Keywords: Glycine max, genebank, whole genome resequencing, maturity

TRANSFERABLE GENOTYPING AND GENETIC DIVERSITY ASSESSMENT APPROACHES FOR LEGUME GENETIC RESOURCES

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Legume crops are central to sustainable agricultural systems owing to their nutritional value, symbiotic nitrogen fixation, and contribution to soil fertility and climate smart farming. The effective use of legume genetic resources depends on robust, scalable approaches to genotyping and diversity assessment that support the integration of molecular, phenotypic, and environmental data. This contribution presents methodological and conceptual experience in the molecular characterisation and data driven management of plant genetic resources, with particular emphasis on the transferability of these approaches to legumes. The experience is based on the application of the DArTseq™ genotyping platform (Diversity Arrays Technology sequencing), a crop independent and scalable solution suitable for legume species with diverse genome sizes and varying levels of reference genome availability. DArTseq combines genome complexity reduction, next generation sequencing, and standardised bioinformatic pipelines to generate high density SNP and SilicoDArT markers, enabling the analysis of genetic diversity, population structure, and relatedness within germplasm collections. Methodological workflows were validated using a wheat genetic resource collection as a model system, solely to demonstrate transferable analytical principles for diversity assessment, redundancy detection, and evaluation of collection representativeness. These principles are directly applicable to legume genetic resources and support the characterisation of variation relevant to key legume traits, including adaptation to abiotic stress, yield stability, resource use efficiency, and traits associated with symbiotic performance. The integration of molecular data with passport information, phenotypic records, and information systems is highlighted as a prerequisite for systematic legume genetic resource management and for strengthening pre breeding and breeding programmes under changing environmental conditions. This work was supported by the EVATECH project – Intelligent technologies for the evaluation and utilization of plant genetic resources for the present and future (No. 09I01-03-V04-00078), funded by the EU NextGeneration EU through the Recovery and Resilience Plan of the Slovak Republic.

Keywords: legumes, plant genetic resources, genetic diversity, genotyping, DArTseq, data integration, crop adaptation

DISSECTING GENETIC AND ENVIRONMENTAL REGULATION OF FLOWERING TO ENABLE CLIMATE-RESILIENT MUNGBEAN BREEDING

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Mungbean (*Vigna radiata*) is an important sub-tropical grain legume, yet its prolonged and asynchronous flowering window increases vulnerability to abiotic stress and reduces harvest efficiency. Optimising flowering behaviour represents a major breeding target for improving adaptation and yield stability under climate variability. We integrated multi-environment field trials, controlled photoperiod experiments, and transcriptomic analyses to dissect flowering regulation across genetic, physiological, and molecular scales. In a nested association mapping population evaluated across four environments in Queensland, Australia, substantial variation was observed for days to flowering (DTF; 35–70 d after sowing) and flowering duration (FD; 20–60 d). Genome-wide association analyses identified eight QTL for DTF and one for FD, with no overlap, demonstrating that flowering initiation and duration are genetically independent traits with distinct environmental sensitivities. Controlled photoperiod experiments (8 h, 12 h, 16 h) across 12 diverse genotypes revealed strong genotype × photoperiod interactions. Photoperiod-sensitive genotypes (n = 7) exhibited substantial flowering delays under long-day conditions (up to 1887 °Cd) accompanied by coordinated shifts in bud appearance timing and node of first flower. In contrast, photoperiod-insensitive (n=5) genotypes maintained stable flowering time across treatments despite early developmental delays, indicating compensatory acceleration during post-initiation phases. RNAseq of excised apical meristems was performed to gain insight into transcriptional changes associated with floral transition and test the involvement of conserved flowering pathway homologs in regulating photoperiod responsiveness. Together, these findings and approaches establish an integrated framework to inform breeding strategies targeting synchronised flowering and enhanced climate resilience in mungbean.

Keywords: mungbean, flowering time, photoperiod, flowering duration

HARNESSING HAPLOTYPE DIVERSITY AND AI-DRIVEN GENOMICS TO ACCELERATE SUSTAINABLE GENETIC GAIN IN CHICKPEA

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As a key grain legume for global food security, genetic progress in chickpea (*Cicer arietinum* L.) remains constrained by narrow breeding gene pools and underutilization of advanced genomic technologies. To address these constraints, we deployed haplotype-based and AI-driven approaches to a newly assembled panel of 304 chickpea accessions, combining globally diverse accessions and Australian breeding material, uncovering untapped haplotypes with potential to accelerate genetic gain. Leveraging 1.4 million genome-wide SNPs, we implemented a graph-connectivity framework to delineate 631,488 LD-informed haplotype blocks capturing recombination patterns. Block-level haplotype effects were then estimated using multi-environment yield data collected across five locations. We identified haplotypes with large effects on yield in our panel, ranging from -120 to 180 kg/ha (estimated breeding values). A highly informative subset of 158 blocks was selected, accounting for 86.5% of the total variance of haplotype effects across blocks. By tracing the geographic origins of haplotypes and evaluating their representation in breeding material, we found that 37% of high-impact haplotypes originated from overseas landraces and were absent from contemporary elite Australian germplasm. Using AI-guided parent selection and in-silico simulations, we optimized crossing schemes to accumulate favourable haplotypes within breeding pools or design new high-potential base populations. Simulated outcomes indicate significant yield gains, up to 40% higher than traditional truncation selection, while maintaining genetic diversity, a critical objective for resilient legume breeding. Together, this framework delivers a practical decision-support tool for chickpea improvement and offers a transferable strategy for accelerating genetic gain in other grain legumes.

Keywords: haplotype-based breeding, AI-assisted breeding, genetic gain optimization, diversity

RAPID AND IN-DEPTH GENOTYPING OF BOTH SNPS AND SVS ALLOWS CALLING OF ALLELES AT FOUR E GENES, KEY DETERMINANTS OF MATURITY IN SOYBEAN (*GLYCINE MAX*), VIA AMPLICON SEQUENCING

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Mutations in E (Earliness) genes have opened the door to a tremendous expansion in the cropping area of soybean, *Glycine max*, allowing it to extend into regions at high latitudes relative to its center of origin. Therefore, the capacity to precisely determine the alleles present at the most important of these genes is extremely valuable to soybean breeders. While some of the causal polymorphisms in these genes are SNPs and can be easily genotyped using KASP assays, targeted GBS or SNP arrays, some major alleles are due to structural variation (insertions or deletions). In such cases, the information derived only from genotyping SNPs can prove insufficient and potentially misleading. For example, when a plant carries the e1-nl allele (a very common “early” allele due to a 130-kb deletion encompassing the E1 gene), KASP assays can produce erroneous results due to the presence of E1-like paralogs which act as templates in the absence of the intended target. In this work, we describe an amplicon sequencing approach that enables the capture of both types of alleles (SNPs and SVs), thus enabling a more comprehensive analysis and a precise allele call. In addition, we describe an efficient bioinformatics pipeline tailored to translate haplotypes at each E gene into an actual allele call. While the genetic determinants of earliness are not perfectly conserved across legumes, the ability to jointly and efficiently call SNPs and SVs at loci of interest is a broadly shared concern.

Keywords: soybean, genotyping, structural variants, SNPs, amplicon sequencing

PEA4EVER, A MASSIVE INVESTMENT PROGRAM TO RECLAIM COMPETITIVENESS OF THE PEA CROP THROUGH ACCELERATED BREEDING FOR YIELD POTENTIAL AND STABILITY

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Lack of investment in breeding has been identified as the major bottleneck and inversing the fall of field pea cropping in French arable agriculture. Microeconomic analysis indicates farm yields need to be increased by circa 1 t/ha. To achieve this, three private breeding companies have mutualized breeding efforts and partnered with farmer representative institutions to create the Pea4Ever program, aimed at delivering accelerated breeding progress, leveraging an objective of 50 M€ through public-private partnership over 5 years. We provide an overview of this program, its strategic objectives and their scientific foundations, its first achievements and next steps. Launched in September 2024, the program includes implementation of speed breeding, genomic selection and phenomics into routine breeding ; mobilizing worldwide genetic diversity and stacking multiple resistances to stresses. Milestones already attained include the creation of an accelerated breeding pipeline with thousands of fixed lines at now at the field nursery stage, a worldwide collection of over 3000 accessions multiplied and genotyped, from which 15 representative individuals are being used to generate advanced back cross populations, and the creation of multiple donors stacking resistance to at least 2 stresses through marker assisted selection. The program is still in the process of leveraging funding and extending its collaborations to pursue and scale these pipelines, as well as tackle important knowledge gaps on major biotic and abiotic stresses affecting pea production in France and Europe in the current context of climate change.

Keywords: pea, breeding, yield, resistance, collaboration

EXPLORING WORLDWIDE GENETIC DIVERSITY IN WILD AND CULTIVATED *MEDICAGO SATIVA*: A RESOURCE FOR BREEDING

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Medicago sativa is a complex of four subspecies (*sativa*, *falcata*, *caerulea*, *xvaria*) whose taxonomic delimitation remains uncertain, limiting the rational exploitation of diversity. We genotyped 1584 accessions with 9,761 SNPs to assess genetic structure and clarify the relationships between subspecies, wild forms and cultivars. A Discriminant Analysis of Principal Components (DAPC) confirmed the difference among the four subspecies. Within each subspecies, a clear geographical structure was evidenced, even if, for cultivars that all belonged to *ssp. sativa*, the regional groups markedly overlapped. Within each regional group of each *ssp.*, the largest number of private alleles was found in the regional groups of *ssp. falcata* (>125), and *ssp. xvaria* (>59). Groups of *ssp. caerulea* also contains between 17 and 30 private alleles each. In contrast, regional groups of *ssp. sativa*, either cultivated or wild, contained between 0 and 10 private alleles, except the group of cultivated accessions from China (65) and Scandinavia (27). To identify the geographical origins of domestication, we projected cultivated groups onto the genetic space of wild pools. The Chinese and Afghan-Persian accessions projected almost exclusively onto the Central Asia group, while cultivars from the Indo-Middle Eastern group show proximity to Central and South Asian groups. Western cultivars derived mainly from the Mediterranean group. These trajectories suggest at least two distinct centers of domestication. These results open up identification of regionally specific gene pools that remain under-exploited and suggest concrete opportunities for more exploitation of genetic diversity in breeding schemes.

Keywords: lucerne, alfalfa, genetic resource, breeding, diversity, marker

A GENOMIC TOOLBOX FOR IMPROVING GRASSPEA

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Grasspea (*Lathyrus sativus*) has a long history of cultivation in challenging environments. Accelerating climate change impacts are driving a resurgence of interest in this highly resilient legume crop. We report a chromosome-scale reference assembly of the 6.2Gbp genome of grasspea, which we have used to analyse a diverse panel of 344 global accessions through whole-genome re-sequencing. This extensive dataset serves as a valuable resource for high-resolution GWAS and EcoTILLING. With this platform, we characterized a loss-of-function mutation in a bHLH transcription factor (orthologous to Mendel's A locus) governing a pleiotropic phenotype of white flowers, cream seed coats, and reduced condensed tannin concentrations. Our biochemical assays demonstrate that condensed tannins are the primary inhibitory determinant of iron bioavailability in grasspea, linking this nutritional trait to a specific genetic variant and easily trackable morphological markers. To address the neurotoxin β -ODAP, we used a Recombinant Inbred Line (RIL) population to map a major quantitative trait locus controlling this trait, facilitating marker-assisted breeding for of safe elite varieties. We are establishing CRISPR-mediated gene editing to expedite functional genomics. In collaboration with our partners, we are now using these breeding resources to develop improved varieties (including the first UK-adapted variety), and we are researching pathways to integrate them into local seed systems in Ethiopia.

Keywords: grasspea, genomics, breeding

DEVELOPMENT OF HIGH YIELDING IMIDAZOLINONE HERBICIDES TOLERANT FIELD PEAS FOR SOUTHERN AUSTRALIA

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Australian grain growers widely rely on Group-2 Imidazolinone (IMI) herbicides to control diverse weed species in major cropping systems. Their importance has increased with the expansion of pulse crops, which contribute to food sustainability, improve crop rotation, enhance weed management, and provide high protein alternatives. Field peas, in particular, benefit from IMI-tolerant varieties that enable sustainable weed control and effective management of herbicide residues where IMI herbicides are routinely used. Agriculture Victoria's National Field Pea Breeding Program (NFPB) incorporated IMI-tolerant mutant germplasm developed by the South Australian Research and Development Institute (SARDI) to develop better-adapted IMI-tolerant field pea varieties. In 2025, two advanced IMI-tolerant breeding lines (APB2502 and APB2503) were evaluated in a multilocation trial of 385 genotypes across eight sites in Victoria and South Australia. This included two commercial IMI-tolerant cultivars (GIA Ourstar and GIA Kastar) and 11 non-IMI tolerant cultivars. A linear mixed model was used to quantify genotype × environment interactions, and empirical BLUPs were generated to compare yields among IMI tolerant breeding lines, commercial IMI-tolerant checks, and leading conventional cultivars. Significant genotype, environment and genotype x environment effects were observed, with site mean yields ranging from 1.16 to 3.33 t/ha. The IMI tolerant breeding lines APB2502 and APB2503 yielded 90–107% of the best conventional cultivar (APB Bondi), and 101–141% of the commercial IMI-tolerant cultivars. These results demonstrate strong yield competitiveness and highlight the potential of these advanced breeding lines to expand sustainable IMI based weed management options for field pea growers in Australia.

Keywords: imidazolinone herbicides, weed management, residues, herbicide tolerance

HARNESSING THE GENETIC BASIS OF PHOSPHOROUS UTILIZATION EFFICIENCY AND ROOT TRAITS IN AN INTERSPECIFIC CHICKPEA POPULATION

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Phosphorus (P) availability is a major constraint to chickpea (*Cicer arietinum* L.) production, as it is essential for plant metabolism, nodule development, and nitrogen fixation. Improving phosphorus utilization efficiency (PUE) offers a sustainable strategy to enhance nutrient acquisition and improve biomass production under P-limited conditions. We evaluated an interspecific population consisting of 200 inbred lines derived from a cross between cultivated *C. arietinum* and different accessions of wild *C. reticulatum* to dissect the genetic variations for root traits, plant biomass, and PUE. Plants were grown under controlled conditions using hydroponic systems to enable precise phenotyping and minimize environmental variation. Root traits were quantified using WinRhizo imaging software alongside manual measurements. Both photosynthetic and physiological PUE were quantified. The photosynthetic PUE was measured using a CIRAS-3 gas exchange system as well as manual measurement. Shoot biomass and phosphorus content were determined and used to calculate physiological PUE (g g⁻¹), which ranged from 169 to 399 g g⁻¹. There was a significant positive correlation between cumulative root length and PUE ($p \leq 0.001$). Several interspecific lines that maintained high biomass and high PUE under low-P conditions were identified. GWAS identified multiple SNPs across several chromosomes associated with root traits, shoot biomass, and PUE.

Keywords: phosphorus utilization efficiency, SNPs, chickpea

LESSONS LEARNT FROM SAFLUFENACIL (HRAC: GROUP 14) HERBICIDE TOLERANCE SCREENINGS IN LENTILS: A CASE STUDY FROM THE UOFS LENTIL BREEDING PROGRAM

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Our program has been exploring finding increased group 14 herbicide tolerance in lentils (*Lens culinaris* L.). We aim to provide producers with a suitable option for in-crop weed management. The industry has relied on the Imidazoline herbicides for 20 years and current weed resistance to this group of herbicides poses a significant threat to yield. Over the past two years, valuable lessons learned along this journey have led to the development of foundational work for pre-breeding required to comprehensively advance this work due to the complex nature of this trait. We share the major lessons learned with this work in hope for meaningful collaborations in this area. Firstly, the use of pre-emergent herbicides brings challenges associated with varying herbicide activity and varying environmental conditions affecting accuracy of in-field screening. Thus, the generation of an appropriate indoor screening protocol is vital for the accurate screening of genotypes and validation of field results. Secondly, herbicide tolerance is quantitative in nature. Thus, quantitative traits like NDVI collected using UAV imagery have proved to be useful for capturing phenotypic variability more accurately. Multi-replicated and multi-location trials are also important in ensuring genotypic variability is accurately captured. Finally, the integration of high-throughput phenotyping technology is proving to be the next frontier in the accurate identification of 'true' genetic tolerance. Thus, future research in the area, much like most agricultural research today, requires collaboration with experts in other relevant fields like computer science and big data.

Keywords: group 14, saflufenacil, lentils, lessons

FROM WILD TO CULTIVATED: DOMESTICATION RESHAPED FUNCTIONAL DIVERSITY IN *VIGNA* GENUS

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As climate change intensifies environmental stresses and shifts crop distribution areas, underutilized tropical legumes are gaining renewed attention for adaptation of cropping systems. *Vigna*, a pantropical genus offers strong diversification potential as food and service crops. Although over 100 species have been identified, only about ten are domesticated out of which only *Vigna unguiculata* and *Vigna radiata* have perceived breeding attention while the other species have undergone limited selection pressure. Two field trials were conducted in Morocco in 2021 and 2022 to evaluate a collection of 368 accessions representing seven cultivated and 30 wild *Vigna* species. Following an initial qualitative characterization of the full collection (El Graoui et al., 2025), 40 accessions representing 11 species and subspecies corresponding to different degree of domestications were selected for. 15 traits related to seed morphology, plant architecture, phenology, productivity, and leaf stoichiometry were measured. Principal component analysis (PCA) and hierarchical clustering identified three functional groups: one predominantly wild, one predominantly cultivated, and one intermediate group with different trait profiles. Hedges' indices revealed significant domestication effects on flowering time and seven traits related to plant size and biomass allocation, with cultivated accessions showing greater allocation to reproductive organs. Allometric relationships involving leaf nitrogen concentration and $\delta^{13}\text{C}$ confirmed that domestication favored adaptation to resource-rich environments, enhancing nitrogen uptake capacity and promoting less conservative water-use strategies. This study provides a comprehensive phenotypic characterization of *Vigna* diversity and offers a retrospective assessment of domestication effects, highlighting potential pathways for reintroducing stress-adaptive traits under climate change.

Keywords: *Vigna*, domestication level, functional strategy, traits, diversity

BREEDING FOR SYNERGY: ESTIMATION OF A LEGACY VALUE IN A LENTIL-WHEAT ROTATION TRIAL

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Modern agriculture's heavy reliance on synthetic nitrogen fertilizers poses significant economic and environmental challenges. While integrating legumes into cereal rotations is a proven strategy to offset nitrogen demand, maximizing this benefit requires a paradigm shift: breeding crops specifically for rotational synergy, rather than isolated performance. To address this, we established a multi-environment crop rotation experiment to evaluate the inherited effects of 100 lentil genotypes (year-1) on the subsequent yield of 100 wheat genotypes (year-2). To overcome the challenge of evaluating 10,000 lentil-wheat combinations, we used a combining ability strategy on 1,000 random lentil-wheat combinations twice at each site in each rotation cycle. This design allows us to statistically disentangle the genetic potential of lentils based on their contribution to the following wheat. Agronomic and nitrogen use efficient traits were evaluated in both crops. We also integrated soil microbiome sampling to assess the role of microbial communities in mediating nitrogen transfer from lentil to wheat. Preliminary results from the first complete rotation cycle demonstrate that specific lentil genotypes possess a consistent rotational value that correlates with improved agronomic performance in the subsequent wheat. Microbiome analysis indicates that bacterial alpha-diversity depends on lentil genotype. Those that promote greater bacterial diversity often act as better rotation partners, conferring a significant legacy benefit to wheat, even when the lentil itself is a low-yielding variety. With the second rotation cycle currently underway and showing promising trends, this methodological framework provides a viable way to select for legacy effects in legume and cereal breeding programs.

Keywords: crop rotation, lentil breeding, nitrogen use efficiency, microbiome

THE ORTHO_KB KNOWLEDGE GRAPH, AN INNOVATIVE TOOL FOR BOOSTING TRANSLATIONAL APPROACHES AND THEIR APPLICATIONS FOR CROP IMPROVEMENT - DEMONSTRATION WITH THE INSTANCE DEDICATED TO LEGUMES, ORTHOLEGKB

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A key objective of the ongoing agroecological transition is to encourage the incorporation of legumes into agricultural and food systems. The symbiotic fixation of nitrogen by legumes provides an effective alternative to synthetic fertilisers, and legumes are a sustainable source of proteins for food and feed. However, legume production is challenged by various biotic and abiotic stresses, particularly in low-input cropping systems. Advances in sequencing technologies and reductions in cost have enabled the development of genetic and -omic resources for certain grain and forage legumes. As these species are both closely related and numerous, it seemed essential to develop a tool that facilitates knowledge transfer between them and from model species. Here, we report on the development of a knowledge graph database that allows users to query genetic and -omic data in an intelligent and efficient manner, within and between target species. A pipeline known as Ortho_KB was created to combine heterogeneous data available for different species, including gene structures, functional annotations, trait genetic bases, as well as genotyping and transcriptomic data. Genes from different species are connected via relationships of orthology and synteny. The OrthoLegKB graph database, which is derived from Ortho_KB and is dedicated to legumes, is now available and routinely used. Examples of outputs demonstrating the conservation of loci that control flowering time, seed quality and resistance to frost and *Aphanomyces* root rot will be provided. The discussion will focus on the value of data generated by comparative and translational approaches to varietal improvement, including for agroecology.

Keywords: translational biology, graph database, heterogeneous data, grain and forage legumes, plant breeding for agroecology

NONADDITIVE GENE EXPRESSION IMPACTS HETEROSIS IN SYNTHETIC FABA BEAN CULTIVARS

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Yield instability presents a considerable challenge in Faba bean cultivation, primarily due to the unstable nature of cytoplasmic male sterility (CMS), which complicates hybrid breeding. To address this issue, the development of partially heterotic synthetic cultivars has been proposed to exploit heterosis at least partially. Synthetic cultivars are produced by selecting a set of superior inbred lines, which are then grown under open pollination conditions, referred to as the Syn-0 generation. The resulting mixed seed lot, with both homozygous and F1-hybrid offsprings, is harvested and sown as the Syn-1 generation. In our study, we examined 40 Syn-1 families and phenotyped them for yield-related traits. Our findings revealed strong positive heterosis for seed weight, flowering time, pod setting, and plant height. To elucidate the molecular mechanisms underlying heterosis in the F1 generation of Syn-1 components, we conducted a transcriptomics study on the same plants. For this purpose, we analysed RNA expression in five Syn-1 families across two tissues—leaf and flower. We identified approximately 900 non-additively expressed genes, exhibiting expression patterns distinct from their mid-parent values across different tissues and time points. We also established a significant association between the number of non-additive genes and mid-parent heterosis. Moreover, we highlighted that non-additive genes enriched in gene co-expression modules are associated with seed weight and flowering time. In summary, this study provides a comprehensive understanding of the nature of non-additive genes and their role in enhancing the performance of F1 in Syn-1 families and may assist in improving synthetic cultivar breeding.

Keywords: heterosis, faba bean, synthetic cultivars, Non-Additive Gene expression

A REPRODUCIBLE TISSUE CULTURE AND TRANSFORMATION PROTOCOL FOR FABA BEAN (*VICIA FABA* L.) ACROSS DIVERSE GENOTYPES

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Faba bean (*Vicia faba* L.) is a high-value grain legume for sustainable cropping systems, yet molecular breeding in this species remains limited by recalcitrant and genotype-dependent *in vitro* responses. We developed and validated an optimised tissue culture and transformation pipeline for *V. faba* using a broad panel of 33 genotypes. Multiple hormone regimes were evaluated to enhance shoot and root regeneration, with particular emphasis on achieving uniform root formation suitable for efficient plant recovery. Across genotypes, indole-3-acetic acid (IAA) at 1.5 mg/L produced the most consistent root induction, reducing a major bottleneck associated with variable rooting performance. Genetic transformation was performed by particle bombardment, using excised embryos as target tissues. In cultivar Tiffany, we obtained a transformation efficiency of 11.7% and verified stable inheritance of the transgene. Beyond efficiency, the workflow reduced key culture-associated limitations, including phenolic compound production and erratic root induction, supporting improved reproducibility. This protocol establishes a reliable foundation for molecular breeding, and gene editing applications in faba bean, and can guide optimisation strategies for other legumes with similar regeneration constraints.

Keywords: faba bean, tissue culture, *in vitro* regeneration, particle bombardment, genetic transformation

GENOME WIDE ASSOCIATION STUDY IN A GLOBAL COLLECTION OF LENTIL (*LENS CULINARIS*)

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Lentil (*Lens culinaris* Medik.) is the third most cultivated cool-season grain legume worldwide and a key protein crop for sustainable agriculture. Leveraging genome-wide variation data available from the INCREASE project Single Seed Descent (SSD) lines' collections, we carried out comprehensive analysis of lentil genetic structure. In addition, we performed multi-year phenotypic evaluation to identify superior accessions and perform genome wide association (GWA) studies for several economically important traits. Discriminant analysis of principal components (DAPC) revealed patterns of genetic diversity reflecting geography and seed morphological features. Genomic regions underlying major economic traits were investigated, including plant and first-node height, response to the parasitic weed *Orobanche crenata*, and content of minerals and trigonelline, a potentially bioactive seed metabolite. Based on the analysis of linkage disequilibrium decay and information available in scientific literature, candidate genes were identified for some of the traits under study, including a palmitoyl transferase for first-node height, and a cysteine proteinase inhibitor for resistance to *O. crenata*. Overall, these findings provide a basis to enhance germplasm valorisation in lentil genetics and breeding.

Keywords: breeding, Lentil, GWAS, genetic structure, germplasm valorisation

BEYOND PREDICTION: UNCOVERING YIELD-FORMING STRATEGIES THROUGH INTEGRATED DATA IN SOYBEAN

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Improving yield prediction in soybean remains a major challenge due to the complex and plastic nature of yield formation, which arises from multiple physiological strategies and their interaction with the environment. High yield can be achieved through diverse mechanisms, including variation in canopy development, resource-use efficiency, biomass allocation, and metabolic composition. Capturing this diversity requires predictive frameworks that integrate complementary sources of biological information. We developed framework of combining genomic prediction(GP), drone based multispectral field phenotyping(HTPP) and plant near-infrared reflectance spectroscopy(NIRS) to improve yield prediction and to better understand yield-forming strategies in soybean, in cost effective way. GP capture molecular variation among genotypes, HTPP reflects dynamic growth and canopy-level physiological responses, while NIRS provides information on tissue chemical composition resulting from cumulative metabolic processes. Yield prediction models were developed using each data source independently and combined, allowing satisfactory predictive accuracy and complementarity. Specific NIRS spectral regions were linked to proteins, lipids, carbohydrates, and water, allowing perspective of nitrogen and carbon allocation strategies among different genotypes. These associations provided insight into how distinct metabolic profiles contribute to yield performance, combining with field growth parameters, revealed that high-yielding genotypes may rely on alternative physiological and biochemical strategies. Overall, integrated predictive approaches represent a promising direction for soybean breeding, as they capture genetic potential, physiological plasticity, and metabolic status underlying yield formation. Beyond improving prediction accuracy, this approach may provide biological insight that can support accelerated genetic gain toward resilient, high-yielding soybean cultivars adapted to diverse and changing environments. Funding: SoyPredict[6788], BELIS [101081878]

Keywords: soybean breeding, resource-use efficiency, multi-source phenomics, genomic prediction

DIVERSITY AS INFRASTRUCTURE: INTEGRATING GENETIC VARIATION INTO MODERN PULSE BREEDING

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Genetic diversity is widely acknowledged as essential for resilient and innovative plant breeding, yet it is often underutilized in practical improvement programs. Here, we present a functional diversity platform based on more than 300 pea accessions evaluated over three years in two contrasting field environments. The panel has been characterized using 6,600 genome-wide markers, targeted SNPs in starch- and protein-related genes, and multiple trait-associated markers identified through genome-wide association studies. Multi-year phenotyping includes agronomic performance, seed composition, and quality-related traits. We have developed predictors for traits that are difficult or costly to phenotype directly and established correlation structures linking agronomic, compositional, and functional properties. Importantly, this diversity panel extends beyond exploratory research. It is actively integrated with commercial breeding material, used as test material in sensory evaluation and product development studies, and forms the backbone of interconnected projects spanning genomics, proteomics, quality research, and breeding methodology. The same genetic resource has enabled locus discovery, quality differentiation, identification of favourable allelic combinations, and the development of breeding-relevant knowledge and decision-support frameworks. This work illustrates how structured, deeply characterized diversity panels can function as long-term research and breeding infrastructure. By integrating multi-environment phenotyping, genomics, predictive tools, and applied breeding contexts, genetic diversity becomes an operational asset for sustainable crop improvement under changing climatic and market demands.

Keywords: genetic diversity, pre-breeding, genome-wide association study (GWAS), multi-environment phenotyping, pea (*Pisum sativum*)

DEVELOPING VIRUS-INDUCED GENOME EDITING (VIGE) FOR GRASSPEA AND PEA

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Plant genome editing (GE) typically depends on *Agrobacterium* to transform CRISPR-Cas machinery into explants, which are regenerated via tissue culture. The inefficiency of this process in most legume crops seriously impedes modern research and breeding. However, delivering this machinery virally instead, using viral vectors as expression platforms in whole plants, offers an opportunity for accelerated and potentially transgene-free GE that bypasses tissue culture. The recent, successful application of this technique (VIGE, virus-induced genome editing) is already impactful in model species and several crops, but heritable VIGE has never been transferred to legumes, where the efficiency gains would be even greater. Our work aims to develop VIGE in grasspea and pea using PEBV (pea early-browning virus), a vector already well characterised in pea. Now we have established that PEBV expresses cargo systemically in grasspea, including – significantly – reproductive tissues (flowers and pollen). One VIGE approach is to deliver the complete CRISPR-Cas machinery into wild-type plants using a viral vector. To this end, we have constructed PEBV vectors encoding miniature endonuclease enAsCas12f (to reduce cargo size) and guide RNAs targeting PDS, as well as similar vector designs for *Nicotiana benthamiana* using closely-related TRV (tobacco rattle virus). Additionally, we are trialling a more widely achievable VIGE approach: virally delivering just sgRNAs into Cas9-expressing transgenic plants. We found through RT-PCR analysis that our PEBV-sgRNA vectors were stable at least 10 weeks post-inoculation in grasspea, giving a promising outlook on the possibility of heritable VIGE in Cas9-expressing grasspea and pea.

Keywords: Virus-induced genome editing (VIGE); PEBV; genome editing (GE); viral vectors

HARNESSING HETEROSIS: SIMULATION-BASED EXPLORATION OF FABA BEAN BREEDING STRATEGIES

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Faba bean (*Vicia faba*) is an important grain legume that exhibits substantial heterosis due to its partially outcrossing nature. However, because hybrid breeding is not currently feasible, alternative strategies are needed to capture this heterosis. Synthetic varieties provide one such approach to capture heterosis, but it remains unclear when synthetic breeding is more effective than line breeding, especially with the growing use of genomic selection. To address this, we used stochastic simulation modelling to compare the long-term performance of line and synthetic breeding strategies in faba bean. Using AlphaSimR, six breeding strategies were simulated over 20 years, including phenotypic selection, genomic selection applied in early generations, and a two-part genomic selection strategy designed to accelerate genetic improvement. These strategies were evaluated under genetic scenarios with varying levels of dominance, which influence the expression of heterosis. Genomic selection consistently increased genetic gain compared to conventional phenotypic selection. When dominance effects were absent or low, line breeding achieved the greatest long-term improvement. Synthetic breeding showed a short-term advantage at intermediate dominance but was eventually overtaken by line breeding. Only under strong dominance did synthetic breeding maintain the highest genetic gain throughout the simulation period. These results based on simulations suggest that the effectiveness of synthetic breeding depends strongly on the underlying genetic architecture and highlight the importance of considering dominance when designing breeding programmes. This work provides practical guidance for optimising faba bean breeding strategies and demonstrates how genomic selection can be used to accelerate genetic improvement in partially outcrossing legumes.

Keywords: breeding simulation, faba bean, *Vicia faba*, synthetic varieties, genomic selection

ASSESSING REALIZED GENETIC GAIN FOR GRAIN YIELD IN LENTIL ACROSS DRYLAND REGIONS USING INTERNATIONAL MULTI-ENVIRONMENT NURSERY TRIALS

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Lentil (*Lens culinaris*) is a key pulse crop in dryland agro-ecosystems, providing an important source of protein, dietary fiber, and essential micronutrients. Efforts across national and international breeding programs have led to the development of improved cultivars adapted to diverse environments worldwide. Quantifying realized genetic gain is essential for evaluating the effectiveness of breeding pipelines, guiding future investment and improvement strategies. The objective of this study was to quantify realized genetic gain for lentil grain yield (kg ha^{-1}), a fundamental metric for assessing long term breeding progress, using multi-year data from ICARDA's lentil breeding program analyzed through the Excellence in Breeding (EiB) BIOFLOW platform and conducting data quality control to remove outliers and anomalous yield observations, breeding values were estimated. The analysis used ICARDA's International Nurseries, which consist of elite breeding lines assembled in incomplete block designs and evaluated across 30 locations in 16 countries over 10 years (2016–2025). A total of 85 datasets from 47 trials were grouped into four Target Product Profiles: Small-Seeded Red Lentil for South Asia, Small-Seeded Red Lentil for East Africa, Large-Seeded Red Lentil for West Asia, and Large-Seeded Yellow Lentil for North Africa. Tested genotypes were developed between 2000 and 2014. Grain yield gains reached 1.14% for South Asia, 2.15% for East Africa, 3.25% for West Asia, and 3.54% for North Africa. These results demonstrate measurable breeding progress and highlight the impact of ICARDA's international germplasm testing network and collaborative partnerships in accelerating lentil genetic improvement and delivering improved varieties to farmers across dryland regions.

Keywords: lentil, genetic gain, dry land, multi-environment, international yield trials

AGROBACTERIUM MEDIATED TRANSFORMATION OF *LATHYRUS SATIVUS*

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Grass pea (*Lathyrus sativus*), often referred to as the “poor man’s meat” in Bangladesh, is a vital source of affordable protein for millions of low-income households. It is uniquely adapted to drought-prone and marginal environments and is widely cultivated across Bangladesh. However, its potential remains constrained by low productivity and the presence of the neurotoxin β -ODAP, which can cause lathyrism in humans and animals. Though, grass pea is a climate resilient legume with agronomic and nutritional importance, yet progress in genetic improvement has been hampered by the absence of reproducible transformation systems. As a woman scientist from a developing country and as a young mother, I am deeply motivated to transform this climate-resilient legume into a safe, nutritious, and high-yielding crop that can sustainably support vulnerable communities under a changing climate. A major technical bottleneck in grass pea research has been its recalcitrance to genetic transformation. Here we report a robust *Agrobacterium* mediated half seed transformation method coupled with RUBY cassette–based visible selection and in vitro grafting to regenerate fertile T₀ plants. This protocol reliably yields PCR and phenotype confirmed transformants and provides a tractable platform for gene functional studies and genome engineering in grass pea. By enabling genome editing in grass pea, this research seeks to bridge cutting-edge plant biotechnology with real-world food security challenges faced by developing countries.

Keywords: *Lathyrus sativus*, *agrobacterium*-mediated transformation, RUBY reporter, in vitro regeneration, betalain pigmentation

WHITE LUPIN BREEDING FOR INTERCROPPING WITH CEREALS: RESPONSE OF EVOLUTIONARY POPULATIONS AND MORPHOLOGICALLY CONTRASTING LINES TO DIFFERENT PURE OR MIXED STAND ENVIRONMENTS

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Grain legume-cereal intercropping has high interest for European agriculture. A key question is whether intercropping could profit of specific breeding instead of pure stand (PS) selection. Little is known also on the value of evolutionary populations (EPs), usable in Europe as Organic Heterogeneous Material. This study aimed to assess the grain yield in PS and mixed stand (MS) environments of Northern Italy of two EPs that evolved in PS or MS and two elite lines representing a tall and a semi-dwarf plant type. The study included three PS and nine MS environments, with MS including wheat or triticale mixed within lupin rows, autumn or late-winter sowing, and moderate or nil N fertilization. Lupin was at severe competitive disadvantage in just a few MS environments, while approaching 50% grain yield proportion in most environments without N fertilization. Lupin genotypes ranked for mean lupin yield as follows: EP evolved in MS > EP evolved in PS > tall inbred line > semi-dwarf inbred line. Lupin genotype × environment interaction for yield was fairly modest. Its modelling by joint regression analysis revealed little cross-over interaction and a response pattern suggesting intrinsically greater yielding ability and good complementarity with associated cereals of the EP evolved in MS. This population featured a tall stature along with high plasticity allowing for further stature increase under MS. Our results highlighted the high value of EPs and, for intercropping, the added value of evolutionary adaptation to this condition. A general advantage of tall over semi-dwarf lupin was evident.

Keywords: intercropping, evolutionary population, *Lupinus albus*, plant-plant interaction, selection environment

BOOSTING INNOVATION IN PEA BREEDING THROUGH PRE-COMPETITIVE RESEARCH AND BREEDING COLLABORATION

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Pea (*Pisum sativum* L.) is the most widely grown cool-season grain legume across Europe and the UK, with growing importance for sustainable protein production, novel food markets, and high-value feed sectors. Yet despite its historical role as a model genetics organism, the rate of genetic improvement in pea has lagged far behind cereals, reducing farm-level competitiveness and discouraging growers from including pea in rotations. Targeted public investment in pre-competitive pea breeding research is essential to correct this imbalance. The Pea Innovation Community (PIC), within the EU-funded Legume Generation project, addresses this challenge by connecting researchers and breeders across Europe and the UK in a sustainable framework for pre-competitive collaboration. Our strategic goal is to identify and deliver novel genetic diversity and validated breeding tools that improve pea yield, resilience, and disease resistance across a range of environments. To achieve this, we have established a curated diversity panel of ~250 accessions including wild *Pisum* relatives, landraces, and modern cultivars which are phenotyped in multi-location field trials across the UK, Germany, France, Poland, and Spain. Controlled environment assays and high-throughput phenotyping complement field evaluations, targeting traits including drought and heat tolerance, resistance to root rots, powdery and downy mildew, and adaptation to autumn sowing. High-depth whole-genome sequences across the panel enable pangenome-based GWAS, through which we have already identified novel loci for thousand grain weight, mildew resistances, and seedling drought tolerance. Markers derived from these findings are being developed and will be shared with breeding partners for deployment in marker-assisted selection.

Keywords: *Pisum sativum*, breeding, GWAS, resilience

INTEGRATING GLOBAL GERMPLASM INTO FRENCH LENTIL AND CHICKPEA BREEDING PROGRAMS

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Lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) are key crops for sustainable agriculture and plant-based protein production. In France, breeding programs for these legumes are recent and small-scale, requiring access to diverse genetic resources to support varietal development and adaptation. This three-year project, started in December 2022 and coordinated by Terres Inovia in partnership with ICARDA, aims to broaden the genetic base of French lentil and chickpea germplasm through introduction, field evaluation, and utilization of ICARDA genetic resources. Each year, 33 accessions were evaluated in nursery conditions, with the most promising advanced to microplot trials in years 2 and 3 to assess yield potential. While many lentil accessions were too early for French conditions and some chickpea accessions were susceptible to *Ascochyta* blight and *Fusarium* wilt, several resources showed useful variability and traits of interest. These materials provide opportunities to enrich and diversify French breeding programs. The project also promotes knowledge exchange, capacity building, and training in controlled crossing techniques. Overall, it illustrates how integrating global genetic resources into small-scale national breeding programs can support the development of new lentil and chickpea varieties.

Keywords: lentil, chickpea, genetic resources, breeding

POLLINATOR-MEDIATED YIELD GAINS AND GENOTYPE × ENVIRONMENT INTERACTIONS IN GRASSPEA UNDER DIFFERENT GROWING CONDITIONS IN CENTRAL INDIA

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Reciprocal interactions between flowering plants and pollinators are central to biodiversity and ecosystem stability. Insect pollination, especially by bees, is globally important as it supports plant reproduction, enhances crop yields, maintains genetic diversity, and reduces inbreeding depression. Grasspea (*Lathyrus sativus* L.) is a protein-rich, often cross-pollinated legume grown under low-input systems but suffers from low yield due to poor pod set under stress conditions. Despite its resilience, the role of pollinators in grasspea yield has not been quantified previously. In this study, 30 grasspea genotypes were evaluated across four conditions (open field, caged in field condition, heat stress, and polyhouse) to measure honeybee contributions to reproductive success. Bee-pollinated open field plots produced the highest yield (1002 kg/ha) with 16,230 bees/ha, while pollinator exclusion (caged) reduced yield by 33.8%. Heat stress reduced bee visits by 42% and caused a 69.9% decline in yield, along with reduced plant height (-30%), biomass (-26%), and seed size (-10.7%). Polyhouse conditions promoted the tallest plants, biomass and boldest seeds, but decline seed yield (-35%) in grasspea genotypes. Significant positive correlations were found between biomass and grain yield ($r = 0.48-0.56$) and between bee abundance and yield ($r=0.12-0.16$). Significant genotypic variation and genotype × environment (G×E) interactions were identified, with stable and high-performing genotypes such as IFLA2441, Mahateora, and BANG-310. Overall, this study shows that bee activity is essential for maximizing grasspea yield, reducing heat-related losses, and minimizing inbreeding depression. Declines in pollinator populations could severely impact grasspea productivity and food security in climate-vulnerable regions.

Keywords: grasspea, pollinator, bee, heat stress, grain yield, *Lathyrus sativus* L.

STREAMLINING PEA GENETIC RESOURCES INTO COMMERCIAL PLANT BREEDING : FIRST RESULTS FROM THE PEA4EVER PROGRAM

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Mobilizing genetic diversity is key to long term success of any breeding program, and often provides sources of resistance to specific stresses, but it remains a difficult and costly endeavour due to the gap in agronomic performance with elite lines. The Pea4Ever program has allowed an investment in mobilizing genetic resources to be made by commercial breeding programs. We initially obtained passport data from 27588 accessions in 10 genebanks, from which we ordered 5000 accessions maximizing taxonomic and geographic diversity. We successfully received 3517 that were then multiplied and genotyped with a 10K array. From this collection, we wished to derive a core set of genotypes maximizing diversity. Using Core Hunter, we determined an acceptable core set of 15 genotypes, that are being used to create advanced back-cross populations that will serve as resources for future breeding. We also identified 2 core panels, for spring and winter pea, of 294 and 154 genotypes respectively, that have been documented for atleast one resistance or agronomic trait of interest in passport data or bibliography. These panels are being multiplied for phenotyping and potential future use as resistance sources. Some of these sources are already included in additional breeding schemes targeting biotic stresses. Our approach could be improved with better genotypic characterization of the *P. fulvum* and *P. abyssinicum* accessions, due to the limitations of the array used for this initial work.

Keywords: pea, breeding, genetic ressources, breeding scheme

THE EFFECT OF DROUGHT STRESS ON DISEASE PATTERNS IN LENTIL: IMPLICATIONS FOR CROP RESILIENCE AND MANAGEMENT

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Plants experience multiple stresses throughout their lifecycle, either sequentially or simultaneously, which most likely results in a combined stress effect (CSE). Therefore, plants must adapt to various combinations of stresses while managing the crosstalk among known stress factors. Additionally, the CSE can be constructive or destructive, depending on the type, intensity, and sequence of the stress factors, as well as the genotypes involved. We explored the CSE of drought and Ascochyta Blight (AB) disease under two stress patterns, sequential and simultaneous, in lentil genotypes ILL6002 and ILL7537, in controlled growth-chamber conditions, as two separate experiments for each stress pattern. Physiological and disease severity traits were assessed across five drought intensities (D0-D5), including combined drought and AB treatment. Results showed that in sequential stress, disease severity decreased with increasing drought in ILL6002, suggesting drought-induced resistance. In contrast, simultaneous stress allowed early AB establishment, nullifying this effect. Genotype ILL7537 has maintained high resistance, except under extreme drought, when AB lesions formed on leaves without disrupting resistance under both stress patterns. The findings highlight that the stress sequence and genotype critically modulate the plant's response to CSE. This study provides a framework for breeding lentil cultivars with improved resilience to climate-induced multifactorial stress scenarios.

Keywords: lentil, drought, Ascochyta blight, combined stress effect, sequential stress, simultaneous stress

GENETIC VARIABILITY AND KEY ADAPTIVE TRAITS IN *TRIFOLIUM GLOMERATUM* AND *TRIFOLIUM CHERLERI* FOR PASTURE IMPROVEMENT

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Pioneer legume species are key to improving the adaptability and productivity of permanent pastures, as they rapidly colonize early successional ecological niches, enhance soil fertility through nitrogen fixation, and facilitate the establishment of later-successional forage species. This work outlines the objectives of a pre-breeding programme for the pioneer, annual self-reseeding clovers *Trifolium glomeratum* L. (Tglo) and *Trifolium cherleri* L. (Tche), focused on identifying germplasm with high adaptive and productive potential to strengthen the resilience and sustainability of Mediterranean permanent pastures. At INIAV Elvas experimental fields (Southeast Portugal), the vegetative and phenological performance of 25 Tglo and 24 Tche genotypes (six plants per genotype, over two years) was evaluated. The aim was to select individual plants showing key traits for pasture improvement: high winter vigour/rapid establishment, short growth cycle/early flowering, and high biomass and seed production. The Tglo working collection displayed broad variability both between and within genotypes. Winter vigour showed a wide range of expression, and flowering onset varied considerably, with a 39 day gap between the earliest and latest genotypes, indicating the coexistence of early, intermediate, and late types—an important feature for breeding. Tche results similarly revealed substantial variation in vegetative vigour and flowering phenology. Marked differences in earliness were observed, with flowering duration ranging from 13 to 44 days. Some individuals showed prolonged flowering, while others displayed short flowering periods, including among early types.

Keywords: annual clovers, pre-breeding, mediterranean pastures

HIGH-THROUGHPUT PHENOTYPING AT THE SCALE OF A PEA BREEDING PROGRAM USING UAV-BASED IMAGING

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Pea4Ever is a collaborative breeding initiative involving three major French breeding companies — Florimond Desprez, Limagrain, and RAGT — aimed at accelerating genetic gain in pea through the integration of advanced breeding technologies. Within this framework, UAV-based high-throughput phenotyping (HTP) is being deployed across multi-environment field trials to support large-scale, quantitative trait assessment. Key objectives include: (1) improved characterization of disease response, with a focus on *Aphanomyces euteiches* root rot, (2) enhanced understanding of yield formation and its component traits, and (3) development of predictive breeding approaches through phenomic prediction. The first full campaign was conducted in 2025 and comprised more than 80 UAV flights over 20+ experimental trials, including both advanced yield trials and early-generation nurseries. Multispectral and RGB imagery were collected at multiple developmental stages to capture canopy structure, vigor, senescence dynamics, and stress-related spectral signatures. This presentation will report initial findings on the heritability and repeatability of UAV-derived phenotypes, their correlation with conventional ground measurements, and their integration into predictive models. We will discuss which traits can be robustly estimated from aerial imagery and evaluate their potential to improve selection accuracy and accelerate breeding cycles in pea.

Keywords: pea, breeding, UAV, high-throughput phenotyping, phenomic prediction

GENETIC MAPPING OF VERNALIZATION-INSENSITIVE AND PHOTOPERIOD-INDEPENDENT FLOWERING IN YELLOW LUPIN (*LUPINUS LUTEUS* L.)

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Yellow lupin (*Lupinus luteus* L.) is a Mediterranean annual legume valued for its high protein content and sandy soil adaptation. However, yellow lupin populations usually require strong vernalization to induce flowering, whereas breeders prefer vernalization-independent genotypes already adapted to spring sowing in temperate climate. To facilitate studies on vernalization pathway in yellow lupin, a recombinant inbred line (RIL) population was developed from a cross between a thermoneutral, rapid flowering breeding line PRH444/14 and a vernalization-responsive, moderately late flowering cultivar Parys. This population was subjected to phenotyping of flowering phenology traits under long-day and short-day photoperiods in the controlled environment. Time-to-flowering differed significantly between early and late flowering RILs, reaching from 39 days under 16-h photoperiod to 68 days under 8-h. Following genotyping with PCR-based and DArT markers, a genetic linkage map was constructed, carrying 1448 non-redundant loci, spanning 2009.8 cM and distributed across 63 linkage groups assigned to 26 chromosomes. Composite interval mapping revealed a major QTL on chromosome YL-16, explaining up to 34% of phenotypic variance under both photoperiods. One of Flowering Locus T homologs, LlutFTc1, co-localized with this QTL, whereas markers anchored in this gene revealed high correlation with flowering time ($r = 0.73\text{--}0.94$). Moreover, two minor QTLs, explaining up to 9% of phenotypic variance and specific for long and short day conditions, were mapped on chromosomes YL-04 and YL-14, respectively. Candidate genes for these QTLs include known regulators of flowering time such as EAF1B, CIB2 and bHLH93. Funding: National Science Centre, Poland, project OPUS21 2021/41/B/NZ9/02226.

Keywords: yellow lupin (*Lupinus luteus* L.), vernalization, flowering time, quantitative trait loci (QTL), LlutFTc1

BREEDING OF PEAS IN THE CZECH REPUBLIC

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Lists of recommended pea varieties are compiled in the Czech Republic to help users navigate the wide range of available cultivars, to provide growers and processors with objective and independent information, and to enable growers to minimize risks associated with varietal characteristics. The aim of this study was to verify the level of resistance of breeding lines to a complex of fungal (*Fusarium* spp., powdery mildew) and viral pathogens (pea enation mosaic virus – PEMV and pea seed-borne mosaic virus – PSbMV). Molecular techniques for the rapid and accurate identification of resistance gene carriers to viral and fungal diseases were introduced into the pea breeding program. In a field trial, 170 pea genotypes were evaluated. Phenological stages, resistance to lodging, yield, thousand-seed weight (HTS), protein content, and trypsin inhibitor activity (TIA) were assessed. Protein content ranged from 18.5 % to 27.5 %. The content of the antinutritional factor TIA is one of the criteria for variety registration, with a registration limit of 9 mg/g. Four pea genotypes exceeded this threshold: AGT 219_19 (9.2 mg/g), Gotik (9.84 mg/g), AGT 219_16 (11.24 mg/g), and Empire (13.09 mg/g). A promising breeding line, AGT 220_15, exhibiting complex disease resistance was developed. Resistance was verified using an internal methodology for detecting carriers of resistance to PEMV and powdery mildew in pea. The line was described according to official descriptors and included in the Czech GRIN gene bank. This work was supported by the Technological Agency of the Czech Republic, Programme TREND, project No. FW10010461.

Keywords: pisum, breeding, resistance, qualitative parameters

BREEDING OF LUCERNE FOR A NORDIC ENVIRONMENT

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Lucerne is not widely used in the Nordic region but is of interest due to its drought tolerance and the need for crop diversification. We have explored methods of high-throughput phenotyping and genomic prediction, while at the same time characterizing two sets of genetic material in small plots of 25 plants: i) a diverse collection of half-sib families resulting from crossing of European elite material of non-Nordic origin and ii) a collection of populations representing Norwegian lucerne breeding from 1979 to 2021. Preliminary analyses of the Norwegian historic breeding material showed that persistence has been improved; this has also been the main selection criterium. Flowering has become earlier, while the changes in yield are unclear. In both sets persistence was associated with dry matter yield in the production years, while there was no or a negative association with dry matter yield and autumn growth in the establishment year. The European material displayed lower persistence than Norwegian cultivars and therefore had yields that declined over the years. It also had less autumn dormancy and later flowering. The European material was variable, with some families yielding similarly to the Norwegian cultivars. Using the European material, we developed genomic prediction models with prediction abilities ($r_{GEBV,y}$) of 0.48 for total dry matter yield over two production years. Using both sets of materials, we developed prediction models for dry matter yield based on RGB and multispectral data. The best predictions (r^2 up to 0.86) were obtained for the first harvest of the first production year.

Keywords: alfalfa, breeding, genomic prediction, lucerne, phenomics

GENETIC ARCHITECTURE AND GENOMIC PREDICTION OF SEED PROTEIN CHARACTERISTICS IN MUNGBEAN

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Mungbean is a climate-resilient legume with high seed protein content, making it promising for diversifying plant-based protein supply. However, understanding of genetic variation in seed protein characteristics, which determine its utility, remains limited. We characterized the genetic basis of variation in seed storage protein profiles and total protein content in mungbean and evaluated genomic selection for trait improvement. We conducted a field trial with 150 diverse mungbean accessions. Total protein content was determined from seed nitrogen, and relative abundance of 21 seed storage proteins was quantified by mass spectrometry. Using 3,310 SNP markers, we fitted genomic best linear unbiased prediction (GBLUP) models to assess heritability, genetic correlations, and prediction accuracy. Thirteen of 21 proteins showed significant variation between accessions ($\alpha = 0.0001$). Mean relative abundance ranged from 6.04% to 44.20%, with heritabilities of 0.26 to 0.96. Total protein content ranged from 23.3% to 29.2% with heritability of 0.70. Yield showed low to moderate genetic correlations with total protein content (0.08) and storage proteins (−0.09 to 0.31), while total protein content had variable correlations with individual storage proteins (−0.32 to 0.53). Cross-validation prediction accuracies ranged from 0.28 ± 0.07 to 0.66 ± 0.05 . Results demonstrate substantial genetic variation in seed protein traits, providing a foundation for genetic improvement. Low to moderate correlations with yield suggest simultaneous improvement of protein content and composition is achievable without yield penalty. Genomic selection shows promise for accelerating genetic gain in mungbean protein quality, supporting development of varieties with enhanced nutritional and techno-functional value for sustainable plant-based food systems.

Keywords: mungbean, genomic prediction, protein profile, total protein content

BOOSTING EUROPEAN PHASEOLUS BREEDING IN LEGUME GENERATION

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Phaseolus beans are the second most important cultivated legume species in the world and the most important legume species for human consumption. Common and runner beans [*P. vulgaris* (Pv); *P. coccineus* (Pc)] are also traditional food crops grown in Europe, with 18 geographical indications. Our main goal is to generate resources to boost *Phaseolus* breeding by i) collecting and disseminating information about the available bean genetic resources; (ii) screening diversity panels to provide sources of traits; (iii) developing and providing innovative resources as breeding populations (bi- and multi-parental, inter-specific lines), including novel bean genotypes using cutting-edge methods; and (iv) validated user-friendly genetic markers as breeding tools. To achieve this goal, we established two panels that bring together a wide diversity: panel1 with 200 lines from previous EU projects and panel2 with 200 bean lines. Both panels, with 30 overlapping lines, are being phenotyped in multi-location field trials for morpho-agronomic traits. Additional phenotyping including root architecture, is conducted under controlled conditions in response to biotic and abiotic stresses. We also completed the development of a population of interspecific recombinant lines derived from the cross Pv x(PvxPc) (145 F7Bc1 lines) as new sources of traits, and a MAGIC population from 8 parents is in progress. Resistance to anthracnose in one of these lines was characterized. A set of functional-friendly markers for breeders is being developed simultaneously. Finally, the markers are being tested for the grouping of resistance genes to four diseases in a genotype.

Keywords: phenotyping, stresses, multifield trials, new breeding tools

LARGE-SCALE STRUCTURAL VARIATION BETWEEN TWO COMMON BEAN GENOMES

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The common bean (*Phaseolus vulgaris* L.) is the most widely consumed legume worldwide and exhibits substantial genetic diversity structured into two major gene pools: Mesoamerican and Andean. The Andean genotype G19833 was the first assembled genome and is used as reference genome in many studies. However, the availability of additional genome assemblies, such as the Spanish landrace Fabada, genotype A25, provides new opportunities to explore intraspecific structural variation. A25 is characterized by very large, white, oblong seeds and shows predominantly Andean genetic background, with 30% of Mesoamerican introgression. In this study, we performed a comparative genomic analysis between G19833 and A25 using SyRI to identify structural variants (SVs). Overall, both genomes shared 82.09% synteny, although collinearity varied among chromosomes. The chromosome Pv04 exhibited the lowest level of collinearity, whereas Pv09 showed the highest degree of synteny. At the inter-chromosomal level, we detected 230 SVs larger than 10 kb, spanning 19.5 Mb, including 40 inversions, 178 translocations, and 10 duplications. Pv08 accumulated the highest number of SVs (47), predominantly translocations. Additionally, 86 intrachromosomal SVs larger than 10 kb were identified (21.2 Mb), primarily translocations, 45 of which were inverted. Beyond large-scale rearrangements, we identified 2,366,403 single nucleotide polymorphisms (SNPs) distributed across the 11 chromosomes. Our findings reveal extensive structural variation within the Andean gene pool and emphasize the importance of incorporating variety-specific genome assemblies to enhance gene discovery and support breeding programs.

Keywords: bean, genome, intraspecific variation, structural variants, comparative genomics

FROM LEGACY TO ACTION: HOW THE FERNAND LAMBEIN FUND ADVANCES GRASSPEA

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The Fernand Lambein Fund (FLF), established in 2023, continues the legacy of Prof. Dr. Fernand Lambein (Ghent University, Belgium), whose work placed *Lathyrus sativus* (grasspea) at the crossroad of plant science, nutrition, and human health. The Fund addresses three key needs within the grasspea community: strengthening global collaboration, empowering early-career researchers, and assessing the crop's potential as a climate-resilient species beyond its traditional production areas. The FLF advances its mission through several initiatives. Community engagement is supported by a semi-annual newsletter, now at six issues and 471 subscribers, featuring research updates, community highlights, and promotion of related initiatives. Early career researchers benefit from competitive funding schemes. To date, 24 travel grants have enabled participation in international conferences, two PhD researchers received direct financial support and one postdoctoral researcher was funded a two-year fellowship. To promote global dialogue, the FLF has established the biannual *Lathyrus* Day, the first meeting exclusively dedicated to grasspea, which fosters collaborations across disciplines and countries. Finally, through the GROW-Grasspea initiative, the Fund intends to strengthen the digital infrastructures within the grasspea community by supporting updates to two existing platforms: the Germinate database and grasspea.net. Germinate is an open access repository designed for the storage and visualisation of validated agronomic, phenotypic, and breeding datasets, while grasspea.net connects laboratories worldwide and provides a platform for science communication. Through capacity strengthening, community building and targeted scientific support, the FLF advances Prof. Lambein's vision and positions grasspea as a promising climate-smart crop for the future.

Keywords: grasspea, *Lathyrus sativus*, climate resilient crops, capacity building, international networking

DISSECTING THE GENETIC BASIS OF RED SEED COAT COLOR IN A RECOMBINANT INBRED LINE POPULATION OF COMMON BEAN

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Seed coat color in beans (*Phaseolus vulgaris* L.) results from the accumulation of pigments, mainly phenolic compounds. Classical inheritance studies have described a complex network of genes that control seed color and distribution. The goal of this study was to investigate the genetic control of red seed color and identify candidate genes controlling this trait by combining genetic mapping and expression analysis. A genetic map was developed for the RIL ABM [AB136 (Red) × MDRK (Red)] with 850 SNP distributed across the 11 chromosomes. The observed segregation revealed three phenotypic classes: red parental phenotype (N=49), dark purple (N=29), and pink (N=27). The observed segregation fitted a 2:1:1 ratio ($\chi^2=0.54$, $p=0.76$), suggesting the involvement of two independent epistatic genes. Contingency tests revealed three significant regions associated with seed coat color; two regions were located on Pv02 (35.16-41.73 Mb and 48.48-49.64 Mb), and one region was located on Pv08 (1.82-3.49 Mb). These positions overlapped with those reported for the major genes Rk, B, and complex C. Differential expression analysis was performed during seed development in both parents. A total of 3450 differentially expressed genes (DEGs) were identified considering $\text{LogFoldChange} \geq |2.5|$, and 74 were located in the regions delimited in chromosomes Pv02 and Pv08. Six DEGs associated with the polyphenol pathway were detected: one anthocyanidin reductase on chromosome Pv02 and a cluster with three phenolic glucoside malonyltransferases and two UDP-glycosyltransferases on chromosome Pv08. Functional markers were developed from these candidate genes for future analysis.

Keywords: *Phaseolus vulgaris*, phenolic compounds, RNA-Seq

PvE1 REGULATES PHOTOPERIOD SENSITIVITY AND FLOWERING TIME ADAPTATION IN COMMON BEAN

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Flowering time is a key adaptive trait in common bean (*Phaseolus vulgaris* L.) influencing yield stability and geographic adaptation through photoperiod sensitivity. Despite progress in identifying photoperiod regulators such as PvCOL2 and PvPHYA3, key determinants of long-day (LD) flowering remain unknown. To dissect this response, we fine-mapped the major flowering-time QTL DTF9.4/DTF9.5 in a recombinant inbred line (RIL) population derived from an early, photoperiod-insensitive Spanish breeding line and a late, photoperiod-sensitive Bolivian landrace. The region was narrowed to a 223-kb interval on chromosome 9, prioritizing PvE1 (Phvul.009G204600), a homolog of the soybean floral repressor E1. A naturally occurring 34-bp deletion in the PvE1 3' UTR (e1-del) was associated with early flowering and reduced photoperiod sensitivity. Near-isogenic lines (NILs) carrying the functional PvE1 allele flowered later specifically under LD and showed greater plant height and node number than NILs carrying e1-del. Expression analyses indicated that PvE1 represses FT genes (florigens; PvFTa3, PvFTb1 and PvFTc), and genetic comparisons support partially redundant, parallel repression by PvE1 and PvCOL2. In a diversity panel, two rare PvE1 loss-of-function alleles (e1-del and a frameshift allele, e1-fs) were linked to early, LD-neutral flowering, with additive effects when combined with col2 mutations. Together, these results establish PvE1 as a conserved legume-specific floral repressor in common bean and provide alleles to breed early-flowering, photoperiod-insensitive cultivars for temperate and high-latitude environments. This work was funded by PID2023-147984OB-I00 and PID2023-151867OB-C31, with technical and scientific support from GENE CROP (IN607A 2024/02; Xunta de Galicia).

Keywords: gene regulation, flowering time, photoperiod response, legume adaptation, breeding

POWERFUL PEANUTS: EXPLORATORY BREEDING OF WILD ARACHIS SPECIES FOR OIL CONTENT AND DROUGHT TOLERANCE

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Peanuts (*Arachis hypogaea* L.) are a major protein source for humans, especially in countries where food security is a concern. Recent research shows that the crop also has potential as a renewable energy feedstock. Peanut oil is being evaluated as a renewable fuel source, and current breeding strategies focus on developing cultivars with increased oil content and improved drought tolerance. Cultivars grown for oil would not be required to meet food-grade standards, allowing for reduced irrigation and chemical inputs. These lines would be adapted for dryland systems and targeted to marginal lands to prevent competition with cultivars grown for human consumption. This project seeks to generate a complex hybrid involving *A. vallsii* Krapov. & W.C. Greg. (accession VSW 9902-1) and an intersectional hybrid between *A. major* W.C. Greg. & Krapov. (accession GK 10573) and *A. pflugeae* C.E. Simpson et al. (accession SWmCbRb 3945). Successful hybridization will be assessed using taxonomic characteristics, pollen viability, and chemometric analysis of Raman spectral data, including PCA and PLS-DA classification models. Hybrid vegetative cuttings and germinated seedlings will be treated with a 0.2% (5 mM) and 0.02% (0.5 mM) colchicine solution, respectively, for 8 h to induce chromosome doubling. Resulting amphidiploids will be verified by pollen staining, then crossed with high-oil peanut cultivars to evaluate cross-compatibility.

Keywords: arachis, hybridization, drought, oil content, renewable fuel

ASSESSMENT OF GENOTYPE × ENVIRONMENT INTERACTIONS FOR GRAIN YIELD AND RELATED TRAITS IN CHICKPEA USING A MIXED MODEL APPROACH

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Chickpea (*Cicer arietinum* L.) productivity in Morocco is strongly constrained by environmental variability, leading to substantial genotype × environment (G×E) interactions that complicate the identification of stable, high-yielding cultivars. This study evaluated 40 genotypes across four contrasting environments using a randomized complete block design with three replications. Grain yield and key agronomic traits were recorded, and a mixed model approach based on Best Linear Unbiased Predictions (BLUPs) was applied to estimate genotypic performance and stability. Results revealed significant environmental and G×E interaction effects for grain yield and several yield-related traits. A subset of genotypes combined high yield potential with broad stability, representing promising candidates for wide adaptation across Morocco's diverse agro-climatic conditions. These findings provide valuable insights for chickpea breeding programs aimed at developing resilient, high-performing cultivars responsive to the country's heterogeneous production environments.

Keywords: chickpea, genotype × environment interaction, mixed model, yield stability, Morocco

PRELIMINARY INVESTIGATION OF FOLIAR APPLICATION OF BORON ON POLLEN VIABILITY AND DEVELOPMENT IN THE CULTIVATION OF RED CLOVER IN THE FIELD

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Red clover (*Trifolium pratense* L.) is a crop used as forage that possesses an exceptional nutritional profile and digestibility. Unfortunately, this crop has low seed yield. Within the framework of the “Legume Generation” EC-funded project, our team aimed to investigate the role of foliar boron application on pollen viability and pollen tube development, and to assess its overall effect on red clover cultivation. Plants of six commercial diploid red clover cultivars, Nika 11, Sofia 52, AberClaret, Milvus, Global, and S123, were field-grown and boron-treated by spraying with the commercial product “Lebasol”, 11% active water-soluble boron. To reach our purpose we counted pollen grains collected from anthers of flowers developed in months May and June; measured the expression level of 8 genes related to flower, pollen, and pollen tube development, boron transport and intracellular auxin transport activators: Auxin Response factor (TprARF17); TprAPETALA3; Walls are thin (TprWAT1 and TprWAT2); NIPs genes (Nodulin Intrinsic Protein) TprNIP4;2, TprNIP7;1, TprNIP5;1, TprNIP6;1 in florets taken from early and mature flower heads collected from field plants treated and untreated with boron. The total nitrogen content in leaves detached from field-grown boron-treated and untreated plants was assessed and compared with the expression levels of two TprNIP5;1 and TprNIP6;1 transporters. In addition, the fresh and dry biomass weight from the first and second cut was collected, as well as the seed yield of the red clover plants. Seed germination rate and vigor of seedlings were examined in vitro for both boron-treated and untreated groups of two specific cultivars.

Keywords: red clover, foliar boron application, transcript analyses, pollen viability, green biomass yield, total nitrogen content, seed yield

THE LEGUME GENERATION CLOVER INNOVATION COMMUNITY

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The Clover Innovation Community is a part of the “Legume Generation” consortium [<https://www.legumegeneration.eu>]. Our goal is to improve the breeding of red and white clover. Our strategy is to create a lasting framework for much-needed pre-competitive investment in pre-breeding. In white clover, we are identifying accessions and genotypes that may harbour valuable characters such as persistence and disease tolerance that are not available in modern cultivars. 204 accessions of wild populations and elite cultivars are being assessed in replicated trials in the UK (Germinal and IBERS) and in Germany (LfL) in contrasting environments. We are collecting yield and quality data. The Earlham Institute (UK) is carrying out whole-genome sequencing on populations of 100 genotypes of each accession. We are using a GWAS approach to understand the mechanisms underlying yield and persistency. Increasing seed yield, especially in red clover, will boost innovation from new germplasm and the use of red clover on farms. We are assessing two approaches to improve seed yield. Foliar boron application, carried out at the Agrobiointstitute in Bulgaria and Germinal/IBERS (UK), will test effects on pollen viability and pollen tube growth, and seed production. Our other approach is to utilize the already characterised S-allele markers and S-allele frequencies in the same poor seed-yielding varieties. We will use the facilities at IBERS to perform this work. Our IC is also committed to the elucidation of the S-alleles in white clover. This work is being carried out at AgResearch (NZ), and Aarhus University (DK).

Keywords: “Legume Generation” consortium, Clover Innovation Community, white clover, red clover

SELECTION OF HIGH-YIELDING CHICKPEA GENOTYPES USING MULTI-ENVIRONMENT TRIALS DATA

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Plant breeders are employing stability parameters for selecting cultivars within the context of genotype $\times$ environment interaction (GEI). Understanding GEI and assessing genotypic stability are essential for selecting genotypes that exhibit optimal performance across diverse environments. However, identifying genotypes that demonstrate high performance and stability across environments continues to pose a challenge. The objective of this research was to select chickpea genotypes that demonstrate high yield, adaptability, and genotypic stability. The mixed models restricted maximum likelihood (REML)/best linear unbiased prediction (BLUP) methods were employed in this study. A total of 240 genotypes were evaluated using an Alpha lattice design at three locations (Morocco, India, and Lebanon in both spring and winter) during the 2023-2024 season. The REML/BLUP analysis revealed significant differences in the effects of genotypes (G), environments (E), and the genotype $\times$ environment interaction (GEI). The GGE biplot method explains about 69.32% of the total variation. In this study, the genotypes S190429, S190422, S190377, S190511, S190282, S190609, S190315, and S190436 were identified for their higher yield performance and good early maturity trait. The S190429 genotype exhibited the greatest stability across diverse environments. The GGE biplots enabled the visual comparison and identification of the ideal genotype (S190511) for winter planting and the genotype (S190429) for spring planting. The selected genotypes would be used to release a new source in the breeding program.

Keywords: high-yielding, chickpea, GGE, multi-environments

IMPROVING LENTIL HERBICIDE TOLERANCE TO SUPPORT EXPANDED CHEMICAL WEED CONTROL OPTIONS FOR GROWERS IN WESTERN CANADA

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Canadian lentil producers have relied heavily on acetolactate synthase (ALS) inhibitor herbicide tolerant lentil varieties for weed management. The rise of herbicide-resistant weed populations across Western Canada - particularly those resistant ALS inhibitors - are a growing threat to lentil production. Quantitative phenotypic variation for lentil resistance to metribuzin, a photosystem II inhibiting herbicide, was observed in preliminary screening experiments. This project screened two diverse lentil populations at multiple site years for metribuzin induced phytotoxicity. The first population is a diverse collection of lentil varieties, genebank accessions, and landraces from around the world. The other is comprised of elite lentil germplasm developed at the University of Saskatchewan's Crop Development Centre (CDC). Genome wide association studies (GWAS) were used to identify multiple quantitative trait loci (QTL) responsible for metribuzin resistance in lentil tolerance in both populations across multiple site-years. Kompetitive allele specific PCR (KASP) markers are being developed based on these QTL for screening within the CDC lentil breeding program. Multiple small effect genes will be stacked through marker assisted to develop metribuzin resistant lentil varieties.

Keywords: lentil, herbicide tolerance, genome wide association studies, marker assisted selection

GENETIC PREDICTION OF LUCERNE GERMINATION UNDER EXTREME TEMPERATURES

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While lucerne is a vigorous forage plant, its slow establishment often leads to sowing failures. These challenges are exacerbated by extreme temperatures—such as late cold or early heat in spring—or when lucerne is sown in mixtures with forage grasses. A set of accessions, previously genotyped with GBS markers, was phenotyped for germination rate at normal temperature (15°C) and under extreme temperatures (5°C and 34°C). Accessions with germination below 70% at 15°C were discarded, leaving a set of 364 accessions. To correct the germination at extreme temperatures by the germination at optimal temperature, the trait “alteration of germination at extreme temperatures” was calculated as the relative difference compared to 15°C. A large diversity was found across all traits. Narrow-sense heritability was moderate for germination alterations at 5°C (0.30) and 34°C (0.35), but low for germination at 15°C (0.15). Notably, germination performance at extreme temperatures were positively correlated but showed weak correlation with germination at 15°C. Predictive ability was higher for the alteration of germination at 5°C (0.35) and 34°C (0.30) than for the germination at 15°C. The germination at the optimum temperature of 15°C is already high so its genetic improvement is probably not a priority. However, these findings suggest that breeding for improved germination at extreme temperatures is feasible. Phenotypic evaluation of germination, relatively easy at 5°C in a cold chamber, is more complex at 34°C. Genomic prediction could facilitate the identification of genotypes tolerant to both cold and heat temperatures during germination, ultimately enhancing lucerne establishment.

Keywords: *Medicago sativa*, germination, temperature, genomic prediction, cold, heat

NEW PERSPECTIVES OF POST-GWAS ANALYSES: FROM MARKERS TO CAUSAL GENES

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Genome-wide association studies (GWAS) have become a cornerstone in identifying genomic regions associated with important agronomic traits; however, the transition from statistical association to causal genes and variants still remains a major challenge. In this work, we review recent advances in post-GWAS approaches designed to bridge the gap between marker-trait association and causal mutation (CM) discovery. We discuss key limitations of traditional GWAS, including sample size, variability in phenotype and genotype quality, and insufficient power to detect rare and multiple alleles. We evaluate current bioinformatic pipelines and genomic strategies, including fine-mapping, integration of multi-omics data (e.g., transcriptomics and metabolomics), and machine learning. These approaches bridge the gap between variant discovery and practical breeding applications, enabling the development of optimized marker panels that enhance the efficiency and precision of legume and crop breeding programs. We also highlight the challenges in the biological validation of candidate genes, emphasizing that more reliable prediction methods are essential to justify and guide resource-intensive functional validation experiments. Finally, we summarize future directions for predicting CMs in complex quantitative traits, such as yield stability and biotic stress resistance. We advocate for the generation of high-quality reference genomes and pangenomes, adherence to FAIR principles, and improvements to data storage and computing infrastructure. The shift toward CM prediction is vital to precision breeding and to ensuring that legumes meet the demands of global food security and climate resilience.

Keywords: post-GWAS analysis, casual mutation prediction, precision breeding

LEGUME GENERATION: BOOSTING LEGUME BREEDING IN EUROPE

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This presentation is about how the Legume Generation project and its six innovation communities (ICs) will boost legume breeding in Europe. Innovation is risky and requires an entrepreneurial spirit to drive and manage change. Breeding is a long-term, risky endeavour, while research scientists typically operate on shorter project timescales with different incentives. With the decline of public-sector breeding, harnessing public research to boost breeding is not a trivial undertaking, especially as commercial rewards for improved cultivars are low. Legume Generation bridges this gap by combining breeders' entrepreneurial drive with research inventiveness. The six ICs focus on soybean, pea, lupins, lentil, common bean, and clovers, linking research to breeding and pre-breeding programmes. Organising ICs by breeding focus rather than science disciplines is intended to create lasting structures, partnerships, and drivers for legume genetic improvement. Legume crops remain underutilised in Europe because cereals perform better under temperate and high-latitude conditions. Improving competitive yields through efficient resource capture (light, water, nutrients) and enhancing quality traits for sustainable, healthy diets is therefore essential. Each IC is supported by knowledge of resource capture, ideotypes, beneficial traits, breeding methods, and a catalogue of species and cultivars. Novel resources are produced and validated, germplasm is tested and demonstrated, training is provided, and activities are coordinated via the European Legume Hub. Long-term sustainability is reinforced through governance studies and business plans, ensuring innovation continues up to the point where newly-bred germplasm and cultivars are proven on farm.

Keywords: boosting breeding, legume crops

THE LEGUME HUB: EUROPE'S KNOWLEDGE PLATFORM FOR LEGUMES

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The Legume Hub is an open access platform dedicated to sharing knowledge and best practices across the entire legume value chain - from plant breeding and agricultural production to processing and consumption. It serves as multilingual, permanent publishing platform for projects, articles, technical papers, videos and photos. Research teams can use the Hub as a long-term platform to publish, document and disseminate their work to a broad international audience. Managed by the European Legume Hub Community, the platform brings together stakeholders involved in the development, production and utilisation of legumes. Experts from research, practice, industry, advisory services and policy are invited to register as members and contribute to the Hub. Membership provides extended access to resources and enables users to create a personal profile highlighting their expertise and activities related to legumes. Members can publish articles, present project outcomes and share relevant information, thereby increasing visibility and impact of their work. Legume Hub can be used as: Project platform: EU and other publicly funded projects supporting legume development are encouraged using the Hub as a permanent documentation and dissemination platform instead of investing in project websites. It serves as a reliable outlet for publishing results and as a long-term archive. Publication and dissemination platform: New or previously published contributions can be uploaded to gain additional visibility and reach diverse target groups. All contributions are citable. Community platform: The Legume Hub Community provides a collective voice in public discussions on the development of legume-based cropping systems, uniting innovators, scientists and technologists.

Keywords: sharing knowledge, permanent publishing platform

DEVELOPMENT OF LINKAGE MAPS FOR NARROW-LEAFED LUPIN MAPPING POPULATIONS EMIR×LAE-1 AND GRAF×AU, AND QTL MAPPING OF FLOWERING TIME, MATURITY, PLANT HEIGHT, YIELD, AND THOUSAND GRAIN WEIGHT

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Narrow-leaved lupin, *Lupinus angustifolius* L., is a recently domesticated grain legume currently cultivated in Europe and Australia. As its genetic improvement was based on a few donors of key agronomic traits, it experienced significant domestication bottlenecks that limited genetic diversity and constrained further adaptation. To counteract progressive genetic erosion in narrow-leaved lupin breeding, novel germplasm resources were developed, including two recombinant inbred line (RIL) populations, descending from the crosses between Emir and LAE-1 (129 RILs), and between Graf and AU (128 RILs). These populations were phenotyped at two locations for 3 years, then genotyped by sequencing using SeqSNP and DArT-seq methods, yielding several thousand markers. Linkage map constructed for Emir×LAE-1 contained 2143 unique loci distributed into 21 linkage groups with a total length of 2947 cM, whereas that for Graf×AU - 2178 unique loci distributed into 22 linkage groups with a total length of 2555 cM. Using narrow-leaved lupin genome assembly version 1.0 as a reference, 2004 markers from the first linkage map and 2288 from the second one were assigned to pseudochromosomes and contigs, respectively. QTL mapping in Emir×LAE-1 revealed, apart from numerous minor loci, a major locus common for flowering time, maturity, plant height, yield, and thousand-grain weight, stable across years and localizations, located on chromosome NLL-06. For Graf×AU, major QTL loci for yield were identified at chromosomes NLL-01 and NLL-20, whereas for thousand grain weight at NLL-11 and NLL-09. Research was funded by Polish Ministry of Agriculture and Rural Development, Biological Progress in Plant Production, Task 18.

Keywords: linkage mapping, narrow-leaved lupin, genotyping, phenology, yield

THE APPROACH OF THE LEGUME GENERATION LENTIL INNOVATION COMMUNITY

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Lentil (*Lens culinaris* Medik.) is traditional in European farming systems and cuisine, with strong potential for sustainable, healthy diets. Although European consumption is rising, much of the lentil consumed in the EU is still imported, particularly from Canada. Lentil is a short- and cool-season legume that can occupy niches in cropping systems, with spring sowing in northern and central Europe and autumn sowing in the south regions. The EU lentil market (~300,000 t/a) is growing, creating a business opportunity. Our goal is to establish an entrepreneurial innovation chain linking biologists, agronomists, and breeders to boost lentil breeding. During 2023–2024, multi-site field trials were conducted to phenotype diverse lentil germplasm, autumn-sown trials in Greece and Italy, and spring-sown in France and Germany. Our strategy follows two approaches: 1) to improve traditional landraces linked to regional PDO/PGI products; 2) to expand lentil production using higheryielding cultivars with improved seed size, colour, quality, and adaptation to diverse agro-ecosystems. Germplasm evaluated includes 180 IPK accessions tested at 5 sites, 220 ICAR-DA accessions evaluated in Greece and 200 advanced lines from previous projects in Italy and Germany. Standard phenotyping protocols applied across all locations. Follow-up work will focus on the development of single-seed descent lines and on identification of genotypes with broad adaptability across environments. Results from 2023-24 and 2024-25, combined with prior project data, will guide breeding in the Lentil IC. Genomic prediction models will help selecting lines with desirable traits, aiming to produce high-performing, quality cultivars for further evaluation in farming practice.

Keywords: Lentil Innovation Community, legume generation

LIMA BEAN: DEVELOPING A NEW PULSE CROP FOR CANADA

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Dry white baby lima beans (*Phaseolus lunatus* L.) are a pulse crop used to produce Shiroan, a high-value bean paste preferred by Japanese consumers. The moist climate and relatively short growing season in Ontario, Canada, differ substantially from those of other North American regions that produce dry baby lima beans; therefore, novel production practices and adapted varieties will be required to establish this industry in Canada. To address this need, phenotypic variation within the species was evaluated using genetic resources sourced from genebanks. Biotic and abiotic stress tolerance, productivity, and end-use quality were assessed to inform parental selection decisions within the breeding program. The results of these experiments, along with the associated breeding objectives, are presented to outline a path forward for the development of new, adapted varieties.

Keywords: lima bean, *Phaseolus*, breeding, quality

UNDERSTANDING HISTORICAL BREEDING PROGRESS FOR MULTI-STRESS RESISTANCE IN PEA

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Pea cultivation is highly susceptible to numerous diseases and pests, the impact of which is exacerbated by climate change. Root diseases remain difficult to control, and foliar diseases and insects are becoming increasingly damaging to pea crops due to the withdrawal of certain pesticides and fungicides. Developing multi-resistant pea varieties is therefore a major objective for research and breeding programs today, in order to expand the crop. This study aims to identify historical genetic progress made in breeding and the potential for improvement of future pea varieties, for multi-resistance to stress. We have established a panel of 241 pea genotypes, comprising 203 French and/or European varieties registered over the last 60 years and 38 control genotypes selected for their resistance to various stresses. The entire panel was genotyped using the multi-species SNP array developed within the European BELIS project. This array enables genotyping for five grain legumes, i.e. pea, faba bean, white lupin, chickpea, and lentil. For pea, it includes 15,564 SNP markers well distributed across the pea V2 reference genome sequence. Six hundred of these markers have been associated with key traits, such as resistance to biotic and abiotic stresses, according to genetic studies. Work is in progress to analyze the genetic diversity in the panel and identify resistance alleles selected throughout the history of pea breeding, using the genotyping data obtained. Initial results regarding genetic progress achieved for resistance to *Aphanomyces* root rot in pea varieties will be presented.

Keywords: varieties, resistance, quantitative trait loci, genetic diversity, *Pisum sativum*

IDENTIFYING COMMON BEAN YIELD-RELATED GENOMIC REGIONS USING UNDEREXPLORED PORTUGUESE GERMPLASM DIVERSITY

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Climate change and rising food security concerns increase the need for sustainable protein sources. Common bean (*Phaseolus vulgaris* L.) is a summer legume well adapted to European farming systems, requiring low inputs while improving soil structure and fertility. Despite its role in traditional diets, farmer interest has declined due to unstable and limited yields, threatening local production. Breeding programmes aligned with farmers' needs are therefore essential. Portuguese common bean germplasm represents a valuable yet underused source of genetic diversity for pre-breeding. To characterize this diversity, we evaluated 182 Portuguese genotypes for yield and its components (seed-number, single-seed-weight, seeds/pod) in an alpha-lattice field trial, conducted in summer 2025 under drip-irrigation at ISA (Lisbon, Portugal). A panel of 12,631 high-quality SNP markers was used for association analysis using R package GAPIT. Substantial phenotypic variation was observed. Yield/plant ranged from 0.79 g to 352.8 g (mean 84.8 g; CV 41%), seed-number/plant from 1.7 to 1190.5 (mean 142.8; CV 79%), single-seed-weight from 0.079 g to 0.752 g (mean 0.449 g; CV 25%), and seeds/pod from 2.39 to 9.07 (mean 4.77; CV 21%). Genotype effects were significant for all traits. Broad-sense heritability was 0.50 (yield/plant), 0.59 (seed-number/plant), 0.27 (seed weight), and 0.85 (seeds per pod). Four SNPs were associated with single-seed-weight and two with seed-number/plant, explaining 2.0-6.0% and 12.9-23.1% of trait variance, respectively. Candidate genes are under investigation, and multi-season trials will assess GxE effects and improve detection power. These results support molecular selection and the development of improved pre-breeding materials from Portuguese bean diversity.

Keywords: common bean, Portuguese germplasm, GWAS, yield components, molecular breeding

ACCELERATING GENETIC GAIN THROUGH INCREASED RECOMBINATION

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Could modifying recombination accelerate legume breeding?

Meiotic recombination is the process by which maternal and paternal genetic material are intermixed. This is achieved through the formation of crossovers, however the rate of crossover formation is naturally limited in plants. This imposes a limiting factor on legume breeding efforts. While increasing recombination has been an area of research in some model plants, its potential has been little-explored in legumes. Here, we are assessing the potential of using genetic changes to increase rate of recombination in a legume. A transgenic approach will test the candidate gene HEI10 via overexpression cassette. Cowpea (*Vigna unguiculata*) will be utilised as a transformable model legume. Once transformed lines are generated they will be assessed via crossing, selfing and sequencing. The self-progeny of an F1 hybrid will demonstrate the rate of recombination in their genotype. They will also be assessed for effects to fertility and fitness. If increased recombination is observed with reasonable efficiency, this could potentially be used for accelerated breeding. Higher recombination resulted in increased genetic gain in breeding, faster introgression of novel alleles into elite lines, and more high definition trait mapping.

Keywords: recombination, reproduction, cowpea, meiosis, transformation

THE APPLICATION OF NEW MOLECULAR MARKERS IN PEA BREEDING PROGRAMME

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In a previous project a GWAS analysis (Genome-Wide Association Study) of pea (*Pisum* L.) was performed to identify DNA markers/loci associated with selected traits (agronomic traits; seed quality traits; resistance to fusarium wilt – *Fusarium oxysporum* f. sp. *pisi* and resistance to Pea Enation Mosaic Virus). A total of 564 genotypes of the genus *Pisum* covering the variability of selected traits were used for analysis. As a result of the analysis, 376 SNP markers associated with the mentioned traits were obtained. These SNPs were subsequently used to design CAPS (Cleaved Amplified Polymorphic Sequences) markers for easy and fast molecular identification of the reference/alternative alleles in the sample. The next step was to verify the effectiveness of these molecular markers and confirm the association of individual alleles with a specific phenotype on a wider set of samples. Simultaneously with the development of molecular markers, phenotyping of the collection of genetic resources was carried out, targeting the resistance to viral and fungal diseases of pea. Based on association analysis and evaluation of marker segregation and phenotype, the markers are validated and submitted for inclusion in resistance breeding programs. The result of using of the markers are functional samples, pea lines, which are described according to the descriptor and stored in the collection of genetic resources in the Czech GRIN database. These genotypes are intended for breeding of new pea varieties with the desirable traits and with the proven resistance to diseases causing the greatest yield losses.

Keywords: CAPS, breeding, molecular markers, SNP, pea

WHEN THE OFFSPRING OUTSHINES ITS ORIGINS: NONADDITIVE GENE EXPRESSION IMPACTS HETEROSIS IN SYN-1 FABA BEAN

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Yield instability presents a considerable challenge in Faba bean cultivation, primarily due to the unstable nature of cytoplasmic male sterility (CMS), which complicates hybrid breeding. To address this issue, the development of partially heterotic synthetic cultivars has been proposed to exploit heterosis at least partially. Synthetic cultivars are produced by selecting a set of superior inbred lines, which are then grown under open pollination conditions, referred to as the Syn-0 generation. The resulting mixed seed lot, with both homozygous and F1-hybrid offsprings, is harvested and sown as the Syn-1 generation. In our study, we examined 40 Syn-1 families and phenotype them for yield-related traits. Our findings revealed strong positive heterosis for seed weight, flowering time, pod setting, and plant height. To elucidate the molecular mechanisms underlying heterosis in the F1-hybrids of Syn-1 generation, we conducted a transcriptomics study on the same plants. For this purpose, we analysed RNA expression in five Syn-1 families across two tissues—leaf and flower. We identified approximately 900 non-additively expressed genes, exhibiting expression patterns distinct from their mid-parent values across different tissues and time points. We also established a significant association between the number of non-additive genes and mid-parent heterosis. Moreover, we highlighted that non-additive genes enriched in gene co-expression modules are associated with seed weight and flowering time. In summary, this study provides a comprehensive understanding of the nature of non-additive genes and their role in enhancing the performance of F1-hybrids in Syn-1 families and may assist in improving synthetic cultivar breeding.

Keywords: faba bean, heterosis, synthetic cultivars, nonadditive gene expression

VARIABILITY IN SEED STORAGE COMPOUNDS AND MORPHOLOGICAL TRAITS IN PEA AND FABA BEAN PRESENTS OPPORTUNITIES FOR TARGETED BREEDING

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Pea and faba bean are grain legumes that play a central role in the transition toward sustainable plant-based protein systems, supporting global food security and environmentally friendly dietary shifts. These crops are rich in proteins and other valuable storage compounds, such as starch and lipids, offering significant potential for diverse food applications. Alongside nutritional composition, seed size, shape, and colour contribute to consumer acceptance, industrial needs, and functional properties. Together, these seed quality traits represent key targets for breeding programs, and the characterization of available genetic diversity for such traits is therefore of great importance. Two diversity panels were assembled: one consisting of 50 pea accessions and the other of 50 faba bean accessions, capturing broad variation and providing a robust foundation for in-depth phenotypic studies. The diversity panels were assessed for seed nutritional and morphological traits, followed by subsequent correlation analyses. Nutritional profiling included quantification of protein, starch and fat content, as well as determination of protein and starch composition. Seed morphology was characterized by determining geometric and colour traits using Videometer technology. Understanding variation and covariation among seed quality traits can provide critical insights for addressing current challenges in crop improvement programs. Ultimately, integrating these findings with genetic information will enable the identification of key genetic drivers underlying these traits, thereby supporting the development of crops tailored for optimal performance and food industrial applications.

Keywords: *Pisum sativum*, *Vicia faba*, diversity panels, nutritional profiling, morphological characterization

PROFIT-BASED SELECTION INDEX IMPROVES MULTI-TRAIT GENETIC GAIN IN CHICKPEA

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The selection index weights in plant breeding programs are mostly yield focused and not expressed in monetary terms. However, the primary objective of a breeding program should be to improve overall production profitability rather than yield per se. Therefore, we defined the aggregate genotype as profit using absolute economic values (A\$ ha⁻¹) for grain yield (GY), and resistance to the two major chickpea disease constraints in Australia, *Ascochyta* blight (AB), and *Phytophthora* root rot (PRR). Using a simulated chickpea breeding program undergoing recurrent selection, we compared a profit-based index with a yield-focused regression method, where AB and PRR resistance were weighted based on the regression coefficients of GY on both traits. The methods were evaluated over ten selection cycles, assuming favourable and unfavourable genetic correlations between GY and AB or PRR resistance. Across scenarios, the profit-based index achieved gains in GY that were comparable to, or only slightly lower than, the yield-focused method, while delivering superior gains in resistance to AB and PRR and consistently higher profit gains. Trait emphasis and realised-contribution analyses showed that the profit index placed 35-40% of emphasis on GY and 60-65% to disease resistance traits, with these traits contributing proportionately to the realised gain. In contrast, the yield focused method placed a higher emphasis and concentrated over 70% of realised gain on GY, resulting in resistance deterioration and lower profit gains under unfavourable correlations. We concluded that the profit-based index enables long-term economic benefits in chickpea breeding without compromising yield gains.

Keywords: selection index, profit, gains, yield

GENOMIC SIGNATURES OF ARTIFICIAL SELECTION AND GENETIC EROSION IN LENTIL (*LENS CULINARIS* MEDIK.)

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Modern plant breeding programs rely on the exploitation of genetic diversity to sustain yield gains and adapt to various local stresses. Intensive selection, however, narrows the genetic base of elite germplasm. This study quantifies the extent of genetic erosion and characterizes genomic signatures of selection in the Canadian lentil breeding program. We compared an elite breeding collection sequenced with long-read PacBio HiFi technology against a global diversity panel sequenced via exome capture. To enable an unbiased comparison across sequencing technologies, a "coding-only" intersection was generated, yielding 136,957 common SNPs covering ~ 25% of the annotated genes. Population structure analysis revealed a moderate differentiation ($F_{ST} = 0.1$) between the panels, with the elite lines forming a cohesive cluster occupying a subset of the global genetic diversity. Linkage disequilibrium (LD) analysis demonstrated significantly slower decay in the elite germplasm, reflecting the conservation of extensive haplotype blocks driven by breeding history. Genome-wide diversity scans revealed a general trend of genetic erosion, with mean Expected Heterozygosity (H_e) decreasing by 10.1% in the breeding panel. However, this reduction was not uniform. While most chromosomes exhibited diversity losses consistent with population bottlenecks, chromosome 7 emerged as a significant outlier, showing a 26.2% increase in diversity in the elite lines. This unexpected retention of diversity suggests the influence of balancing selection or recent introgression, likely associated with the segregation of high-performance agronomic traits. These findings provide a high-resolution genomic baseline for managing genetic resources and highlight specific genomic regions where selection has effectively reshaped the lentil genome.

Keywords: genetic diversity; lentil; genetic erosion; domestication; selective sweeps

DOMESTICATION-RELATED CHANGES AT PVMYB26 IN COMMON BEAN SHED LIGHT ON THE ORIGINS OF AGRICULTURE IN THE AMERICAS

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Domestication imposed radical selection pressures on plants, transforming them into crops that support global populations today. In this study, we investigate the loss of seed dispersal via pod shattering during common bean (*Phaseolus vulgaris* L.) domestication. We identified PvMYB26 mutations in all three main gene pools of common bean, including an 8 kb deletion in Middle American lines eliminating the gene's transcription start site and promoter, a frameshift/truncation deletion in the independently domesticated Andean population, and another frameshift/truncation insertion in the genetic background of the "undomesticated" debouckii population. Mutants with the 8 kb deletion express PvMYB26 at <1% of the level of wild types and produce 44% less pod lignin. RNA in situ hybridization and fluorescence microscopy showed that PvMYB26 is expressed in the lignified fiber layer of pods, while mutants showed no visible expression and a greatly reduced fiber layer. Sequencing of 327 accessions revealed that the mutation is nearly diagnostic for domestication status among Middle American common bean and identified a 125 kb hard selective sweep, indicating the gene's importance in domestication. The main Andean frameshift mutation was found in 84.5% of Andean domesticates but 0% of wild lines, while the debouckii truncation was identified in six domesticated lines of race Peru, suggesting that a third proto-domestication of common bean may have occurred in Ecuador and/or northern Peru. Wild haplotypes most like Middle American domesticates were found in eastern Jalisco, Mexico, strongly suggesting west-central Mexico as the site of common bean domestication and the rise of agriculture in Middle America.

Keywords: plant domestication, crop evolution, *Phaseolus vulgaris* L., PvMYB26, pod shattering

YIELD AND NUTRITIONAL VALUE OF PORTUGUESE COWPEA GERMPLASM

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Cowpea (*Vigna unguiculata* (L.) Walp) is a drought-tolerant grain legume and an important source of plant-based protein, particularly in Mediterranean diets. This study evaluated the agronomic performance and seed quality of 10 cowpea landraces grown at the INIAV–Elvas experimental fields, Portugal, using a randomized complete block design with three replications. Four agronomic traits were assessed: days to flowering, days to maturity, 100-seed dry weight, and grain yield. Seed nutritional quality was evaluated by determining crude protein content and mineral composition (sodium, potassium, phosphorus, calcium, magnesium, iron, and zinc). Data were analyzed using one-way ANOVA followed by Tukey's test. The results revealed clear genetic variability among landraces for both agronomic and nutritional traits. Days to flowering ranged from 47.7 to 53.5 days, with earlier genotypes showing potential adaptation to shorter growing seasons. The 100-seed weight varied considerably, from 13.1 g ("Cp5940") to 25.3 g ("Cp5814") while grain yield ranged from 1552 to 3113 kg/ha. Accessions "Cp 4906", and "Cp5814" have the largest seeds, which is a desirable commercial trait in many markets. Protein content ranged from 21.5% to 24.8% with accessions showing high levels of potassium, phosphorus, iron, and zinc. Accessions "Cp5024" and "Cp4893" exhibited higher productivity while "Cp4924" and "Cp5940" showed promising nutritional profiles, making them strong candidates for breeding programs focused on improving nutritional quality and promoting sustainable cowpea production systems.

Keywords: agronomic evaluation, landraces, seed quality, *Vigna unguiculata*

COST-EFFICIENT AND ACCURATE LEGUME VARIETY REGISTRATION AND RECOMMENDATION IN EUROPE

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Legumes are essential crops for the agroecological and food transition due to their ability to fix atmospheric nitrogen. They enrich soils with nitrates and diversify crops in rotations, while providing plant-based protein for feed and food. However, legume production in Europe is insufficient due to the low crop competitiveness. WP6 of the European BELIS project aims to improve the registration and recommendation processes for varieties at the EU level, in order to provide farmers with improved, profitable, and well-adapted legume varieties. Task 1 identifies and uses molecular markers to increase cost-efficiency and accuracy of Distinctness, Uniformity, Stability (DUS) testing, for variety registration in pea and lucerne, as well as for seed-lot identity checking in pea, lentil, chickpea, faba bean, and lucerne. Task 2 collects and compares Value for Cultivation and Use (VCU) protocols deployed in EU countries for variety registration of 13 legume species, and tests for an innovative organization of pea and alfalfa VCU field trials at EU agrizone level, to improve and increase efficiency of VCU variety assessment. Task 3 collects information on lists and traits of varieties registered in post-registration networks in EU countries for 14 legume species, and models projected crop yield maps at EU level for alfalfa, to strengthen and adapt varietal recommendations of legume species and varieties in current and future agro-climatic conditions. WP6 will provide methods to better characterize legume varieties and optimize their recommendations for use by farmers, in order to optimize their performance and increase their deployment in Europe.

Keywords: DUS, VCU trials, molecular markers, modeling, forage and grain legumes

PERFORMANCE OF GRASSPEA (*LATHYRUS SATIVUS* L.) GERMPLASM FOR YIELD, LOW ODAP, AND FARMER-FIELD DEMONSTRATION IN NEPAL

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Grasspea (*Lathyrus sativus* L.) is a climate-resilient legume with potential for marginal environments, but its adoption is limited by yield variability and neurotoxin (ODAP) content. This study evaluated diverse grasspea germplasm, including CWR-derived lines and local gene bank accessions, through a two-year, phased approach in Nepal. In 2024, twenty-five genotypes were evaluated at a Grain Legumes Research Program, Khajura, Banke Nepal showing significant genotypic differences ($p < 0.05$) for phenology, plant height, pods per plant, biomass, and seed yield, which ranged from 124 to 2630 kg/ha. Promising genotypes were advanced to 2025 multi-environment testing across Khajura, Doti, and Bara, where combined analysis revealed significant effects of genotype, environment, and genotype $\times$ environment interactions for yield and biomass. Mean yield of NGRC00419 (1281 kg/ha) and Bidhan (1125 kg/ha) exhibited consistently high and stable seed yields, while AF2-2-15/5 (3563 kg/ha) and AF2-8-4/2 (3429 kg/ha) produced superior biomass. In parallel, farmer-field demonstrations of the Ratan variety were conducted over 3.7 ha in four districts, producing 2800 kg of grain, supporting quality seed production and farmer training. All evaluated gene bank accessions were analyzed for ODAP content, with NGRC0439, NGRC0479, NGRC0483, NGRC08873 and Bidhan recording.

Keywords: CWR, demonstration, grasspea, landraces, ODAP

THE IMPORTANCE OF SAINFOIN (*ONOBRYCHIS SATIVA* LAM.) IN THE FODDER BASE IN NORTH MACEDONIA IN THE PAST AND TODAY

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In recent years, the areas under fodder crops in North Macedonia have not been sufficient for intensive livestock farming. In addition, the impact of climate change has created a need to expand and restructure the fodder base. In the past period in North Macedonia, the production of annual fodder crops such as peas, vetch, corn, and turnips has usually been dominant. Among perennial crops, alfalfa is the most widely grown, for the production of which there are excellent conditions. However, in dry regions where irrigation is not possible (Ovche Pole, Tikvesh region) and on hilly, less productive soils (sandy with a shallow arable layer), alfalfa yields and quality are low. Therefore, sainfoin (*Onobrychis sativa* Lam.) is a suitable replacement. It is a forage species that, in the 1970s, 80s, and 90s, was widely spread and included in breeding programs. In 1982, the first Yugoslav variety, Makedonka, was created at the Institute of Field and Horticulture in Skopje. However, in recent decades, it has been gradually disappearing from arable lands. As a perennial fodder crop, sainfoin is a very stable and important source of animal feed, which in the newly emerging climatic conditions of North Macedonia is a suitable substitute for alfalfa. For these reasons, its re-promotion and return to agricultural areas are justified. This review paper aims to compare scientific findings on the characteristics and production technology of sainfoin and to identify the reasons for its withdrawal from arable land in North Macedonia.

Keywords: sainfoin, climatic conditions, fodder base

MULTI-PARENT POPULATIONS (MPP) OF WINTER PEAS FOR ORGANIC AGRICULTURE

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Winter peas are sown in autumn and show stout above-ground growth during winter while developing a strong root system. Once day length exceeds 12 hours in spring, above-ground elongation begins. Winter peas are well adapted to spring droughts and tolerate foot diseases due to the harsh conditions in which they grow. However, like other grain legumes, they exhibit large yield fluctuations. Long-term trials with wheat composite cross populations (CCP) and evolutionary populations have shown that high genetic diversity improves yield stability. This project aims to enhance winter pea performance by further breeding multi-parent populations (MPP) and testing previously developed populations. Populations can originate from single crosses of two parents, double crosses of four parents, or multi-parent crosses. While single-cross populations develop quickly, they combine only a few traits. MPP allow the combination of many genotypes or alleles, which is essential for quantitative traits such as yield and overwintering performance. Agronomic requirements—such as standing ability, uniform maturity, consistent growth height, and similar grain characteristics—must also be met. In this project, winter pea MPP are being tested and further developed through positive and/or negative mass selection to achieve agronomically meaningful uniformity. The populations will be evaluated against initial lines and other winter pea varieties for yield, yield stability, and winter hardiness in mixed cropping with triticale. In addition to serving as populations, the MPP will provide starting material for new line varieties and act as a diversity pool for future winter pea breeding.

Keywords: winter pea, organic, breeding, multi-parent population, composite cross population, diversity

ODAP CHARACTERIZATION OF DIVERSE GRASS PEA ACCESSIONS FROM THREE CONTINENTS BY RP-HPLC METHOD FOR FUTURE BREEDING PROSPECT AND CONSUMABILITY PREDICTION

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Grass pea (*Lathyrus sativus* L) and other species of *Lathyrus* are a lucrative and cheap sources of high nutrition for human and animals of drought-prone areas in Southeast Asia and Africa. The β -ODAP (β -N-oxalyl-L- α , β -diaminopropionic acid) a non-protein amino acid causes "neurolathyrism" for consuming grass pea as a main food at least 3-4 months where nontoxic isomer α -ODAP present in leaves and seeds has positive effect and do not causes "neurolathyrism". The assessment and separation of both ODAP isomers to find less toxic consumable grass pea genotypes that could support breeding activities to improve the grass pea quality and consumability. Methods: RP-HPLC method were used to determine both the α - and β -ODAP isomers from fresh and dry samples of 400 grass pea genotypes representing Asia, Africa and Europe. We determined the ODAP variations and clusters with correlation to predict consumability for safe intake of human and animals. A strong positive correlation between α -and β -ODAP values ranged from 0.854 to 0.996. The average range of α - and β -ODAP of these leaves samples observed 0.165 to 3.298 and 0.622 to 8.161 in fresh, 0.792 to 13.234 and 2.526 to 29.342 mg/g in dry, respectively. High, medium and low β -ODAP content accessions were identified in three continents. Low ODAP content 211 accessions may be considered as a slot product strongly describing native ethnic and cooking habit (harmless for human intake).

Keywords: grass pea; *Lathyrus sativus* L; ODAP content; consumability prediction; RP-HPLC; correlation

REDEFINING SOME AGROTECHNICAL MEASURES AND THEIR IMPACT ON ALFALFA PRODUCTIVITY

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Climate change requires the introduction of new or adaptation of existing methods of growing crops. Alfalfa is a leading forage crop, widespread throughout the world, characterized by high fresh forage and hay yields and a well-balanced nutritional composition. Unfortunately, in the agro-ecological conditions of R. North Macedonia over the last decade, there has been a lack of scientific contributions that would redefine the basic agrotechnical measures for alfalfa cultivation. Due to the mild winters in recent years and the absence of the risk of winterkill in the early stage of the stand establishment, it was of particular interest to examine the alternative autumn sowing and to replace the usual spring sowing of alfalfa in R. North Macedonia. For this purpose, a three-year field experiment (2021 – 2023) was conducted to examine the effect of: seeding rate (8 and 16 kg ha⁻¹), row spacing (20 and 40 cm), and cultivar (NS Jelena, Banat VS, Debarska, and Nijagara) on alfalfa productivity. Comparing the two row spacings, a statistically significantly higher yield of fresh forage (90 t ha⁻¹) and hay (24.7 t ha⁻¹) was obtained with narrow-row sowing (20 cm). The seeding rate has no significant effect on alfalfa productivity. The NS Jelena cultivar recorded the highest yields of fresh forage (82.1 t ha⁻¹) and hay (22.5 t ha⁻¹), due to its best adaptability to the continental-sub-Mediterranean climate of the Skopje region in the R. North Macedonia and its resistance to extreme summer drought during the studied vegetation years.

Keywords: alfalfa, agrotechnical measures, autumn sowing

CULTIVATION OF CHICKPEA ADAPTED TO RAINFALL-RICH REGION OF NORTH MORAVIA

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Chickpea (*Cicer arietinum* L.) is an annual self-pollinating grain legume adapted to dry climate, primarily grown in arid and semi-arid regions. Cultivated chickpeas are classified into two distinct types: desi and kabuli. While chickpea cultivation is generally limited by various abiotic (cold, heat, drought, salinity) and biotic (disease, pest, weed) factors, production in North Moravia faces specific constraints due to relatively high amount of rainfall and subsequent waterlogging during summer months. In the Vykrovice locality, the long-term average annual precipitation is 658.13 mm (with 729.5 mm in 2023, 783.9 mm in 2024 and 645.3 mm in 2025) and a mean temperature of 8,3 °C. Although chickpeas typically thrive in medium rainfall areas with 300–500 mm (desi requires about 350 mm, while kabuli more than 450 mm of annual rainfall), this study demonstrates that cultivation in North Moravia is feasible. The primary constraint is not the total annual precipitation, but rather the intensity and seasonal distribution of rainfall. Our three-year evaluation of 6 desi and 6 kabuli accessions of genetic resources revealed that the desi type exhibit significantly higher tolerance to water stress (including limited supply and waterlogging) regarding yield stability. While excessive moisture increases the frequency of empty pods, desi genotypes maintained a significantly higher ratio of full pods (average 46.30 full pods out of 69.58 total per plant) compared to kabuli genotypes (averagely 23.36 full pods out of 44.19 total per plant). Consequently, the desi type is more resilient and suitable for the high-rainfall conditions of North Moravia.

Keywords: chickpea, waterlogging tolerance, adaptability

EVALUATING COMMON BEAN (*PHASEOLUS VULGARIS*) LANDRACES AND WILD ACCESSIONS FOR INDIRECT EFFECTS OF DOMESTICATION AND SELECTION ON ROOT ARCHITECTURE

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Early selection for domestication efforts in many species have resulted in loss of shattering, reduced photoperiod sensitivity, and seed size variation. With the unique divergent evolutionary history of common bean (*Phaseolus vulgaris*), domestication syndrome has also resulted in these changes and additional distinct shoot and seed morphological changes. While these changes have been extensively studied, possible effects of indirect selection to belowground root architecture remain largely unknown. To characterize domestication effects on root systems, field data were collected for two years on three landraces (Common Red Mexican, Black Turtle Soup, and Robust) and two wild bean accessions (PI535428, W624135) representing the Middle American gene pool. Observed maturity, growth habits, leaf nutritional concentrations, and root system architecture were collected. Exploratory data analysis revealed a distinct separation by year collected. In both years, basal root angle, percent nodulation, and lateral root number contributed to variation in wild bean accessions. MANOVA and pairwise comparisons showed significantly longer total root length, smaller adventitious root distribution, and higher solidity ratios in landraces compared to wild accessions. Nutrient concentrations in the leaves showed significantly higher concentrations of calcium, manganese, iron, copper, and aluminum in wild accessions compared to landraces. Results will inform scientists with knowledge of how early selection efforts indirectly affected the root architectural traits and the dynamics of root to leaf translocation seen in modern day.

Keywords: roots, domestication, indirect selection

EVALUATING YIELD STABILITY OF DRY PEA AND FABA BEAN IN SWEDEN UTILIZING NATIONAL MULTI-ENVIRONMENT TRIAL DATA

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Despite increasing attention to plant-based protein and sustainable cropping systems, grain legumes have consistently occupied less than 2% of Sweden's total arable land. The perception that grain legumes show greater year-to-year yield variability than other crops has been identified as a major factor limiting their adoption. To investigate this, the temporal and spatial yield stability of yellow pea and faba bean in southern Sweden was evaluated using multi-environment variety trial data from 2015-2024. A dataset of 13 faba bean and 8 dry pea cultivars, evaluated across three locations over nine and ten years respectively, was used for stability and genotype x environment (GxE) analyses. Yield stability was assessed using the coefficient of variation (CV) for static stability and BLUPs derived from linear mixed models for dynamic stability. Environmental covariates were incorporated into linear mixed-effect models to quantify their contribution to the environmental and GxE variation. Preliminary results indicate substantial year-to-year yield variation for both crops. Significant GxE was found and was predominantly non-crossover. Weather covariates (average seasonal temperature, accumulated precipitation and growing degree days) accounted for approximately 22 and 27% of the environmental variation for faba bean and pea respectively, with the remainder attributable to other environmental and management-related factors. These findings support the identification of stable cultivars and inform breeding strategies aimed at improving the reliability of domestic grain legume production.

Keywords: genotype x environment interaction, MET, yield stability, *Vicia faba*, *Pisum sativum*

LEGUME GENERATION: THE LUPIN INNOVATION COMMUNITY LUPIC

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Lupins, especially white and narrow-leaf lupins, cultivated in Europe are well adapted to dry, acidic soils and offer strong market potential due to their high protein and oil content. Andean lupin is also of growing interest. Legume Generation provides a breeder-led framework to integrate research resources for long-term lupin improvement. For narrow-leaf lupin, several agronomic traits, including calcareous soil tolerance and increased pod set, are being developed through introgression and pyramiding. Promising lines with good yield and protein content, including segregating F₂ populations and advanced material, are under field evaluation at JKI. In white lupin, genebank accessions and INCREASE project (Intelligent Collections of Food Legumes Genetic Resources for European Agrofood Systems) single-seed-descent (SSD) lines are being multiplied and evaluated for yield, anthracnose resistance or tolerance, and alkaloid content, forming a new breeding programme at ESKUSA.

Keywords: lupins, breeding, genetic resources, adaptation

DEVELOPMENT OF HETEROTIC POOLS AND SYNTHETIC BREEDING RESOURCES IN FABA BEAN

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Faba bean is a key grain legume for sustainable European cropping systems because of its high protein content and biological nitrogen fixation. However, breeding progress is slower than in major crops, partly due to the lack of an efficient hybrid system to exploit heterosis. Developing heterotic pools and synthetic populations may therefore increase genetic gain. We developed a structured breeding population to establish heterotic pools and to create a genetic basis for future synthetic breeding approaches. A total of 120 elite founder lines from an existing breeding program served as the starting germplasm. To investigate combining ability and identify heterotic patterns, testcross progenies were generated by pairing two founder lines in isolation tents. Cross-pollination was facilitated by bumblebees, enabling natural outcrossing between the parental genotypes. The resulting testcross progenies were evaluated in field trials together with the founder lines to assess agronomic performance and heterosis response. In parallel, the founder lines were genotyped to characterize their genetic diversity and to support the formation of two genetically distinct heterotic pools. Based on this classification, new intrapool crosses among founder lines were conducted to generate F1-populations representing the initial pool structure. A second recombination cycle was subsequently performed through intrapool F1×F1 crosses to further broaden the genetic base of each pool. The newly developed plant material will be multiplied and comprehensively phenotyped in multi-environment field trials. These activities will evaluate the breeding potential of heterotic pools and to support the development of synthetic breeding strategies exploiting heterosis in faba bean.

Keywords: *Vicia faba*, heterotic pool development, synthetic breeding, genetic diversity

REACHING EXPERT CONSENSUS ON DEMAND-LED LEGUME BREEDING IN EUROPE: A MULTI-STAGE DELPHI STUDY

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Legume breeding is of great importance for agriculture and food systems in Europe. However, the small legume acreage means that breeding programmes are often not economically viable. Improvements to the system could be initiated by the governance structure of demand-led breeding. Demand-led breeding is an approach in which the needs of stakeholders downstream of agriculture are explicitly considered. This could enable product optimisation, which can lead to a better selling proposition of end products. To evaluate this approach, we conducted a Delphi study with 21 legume breeding experts from across Europe. The experts discussed indirectly to form a group opinion through repeated quantitative ratings of statements on demand-led breeding. The data was analysed using a mix of methods, including qualitative content analysis and inferential statistics with a non-parametric rank variance analysis. Expert consensus emerged that breeding goals should be oriented towards the end use of the products, that demand-led breeding is more likely to become established in Europe in the medium term, and that better alignment with market demand can increase value creation in breeding. However, the complexity of breeding programmes would increase and the added value would only become apparent retrospectively after a time lag. We conclude that demand-led breeding may be implemented within the framework of vertical strategic alliances and development partnerships, but that this business model needs further research. We also recommend that research efforts should focus on establishing an information infrastructure within the legume sector and strengthening the links between breeding and the processing industry.

Keywords: demand-led breeding; breeding system governance; Delphi Study; food systems

GENETIC DIVERSITY AND BREEDING STRATEGIES OF THE "DONGSHENG" FOOD-GRADE SOYBEAN SERIES IN HEILONGJIANG, CHINA

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Soybean is one of the most widely cultivated legume plants globally, primarily due to its high protein content. Originating in China, this typical short-day plant has two recognized centers of origin. However, through extensive cultivation and targeted breeding programs, Heilongjiang Province—a high-latitude region in northeastern China—has become the country's primary soybean production area. In 2023, the total soybean harvested area in China reached 10.47 million hectares, with Heilongjiang Province accounting for 46.7% of this total. Therefore, breeding new varieties adapted to the specific conditions of Heilongjiang Province is crucial for Chinese soybean production. The "Dongsheng" soybean series stands out in this context due to its exceptional yield and premium food-grade quality. This study assembled a panel of 288 soybean varieties registered over the past decade in Heilongjiang province, including 12 from the "Dongsheng" series. A high-throughput functional SNP array was utilized to explore the genetic diversity among these novel varieties. Furthermore, a genome-wide scan was conducted to identify differential functional loci that were specifically selected for by the breeders of the "Dongsheng" series. In summary, this study evaluates the genetic distance among modern soybean varieties in China's main production region and reveals the breeding strategies underlying this highly popular food-use soybean series.

Keywords: soybean, genetic diversity, genome-wide scan

LEVERAGING MULTI-ENVIRONMENT TRIALS AND HAPLOTYPE ANALYSIS TO DELIVER GENOMIC TARGETS FOR HEAT-TOLERANT CHICKPEA

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Heat stress during reproductive development is a primary constraint to chickpea (*Cicer arietinum* L.) productivity globally. This study characterised genotype-by-environment interactions (GEI) and environmental drivers of yield variation in 168 diverse kabuli genotypes from six rainfed field trials conducted across three years (2018-2020) at Narrabri, Australia - a location representative of subtropical growing regions. Two sowing times were implemented each year using optimal (June) and delayed (August) plantings to impose differential heat stress. Delayed sowing exposed germplasm to higher mean maximum temperatures by 3.06 to 5.46°C during the critical reproductive period, contributing to average yield reductions of 0.65 to 0.99 t ha⁻¹. Multi-environment trial analyses using a factor-analytic framework revealed significant GEI for grain yield, with genetic correlations among trials ranging from -0.34 to 0.96. While genotype reranking was observed for grain yield, rankings for 100-seed weight and phenology were highly stable across environments. To investigate the genetic basis of performance, SNP effects were estimated for 8,093 genome-wide markers and subsequently grouped into high-LD blocks to derive haplotype effects. This haplotype-based approach increases detection power, and top yield-associated regions were identified as blocks explaining the largest proportion of genetic variance. This revealed high-value haplotypes for yield and 100-seed weight present in genebank accessions that were absent from commercial Australian cultivars. Through the discovery of novel haplotypes, this study provides chickpea breeding programs with immediately deployable genomic targets to improve performance under heat stress. This is an important resource as reproductive heat stress intensifies across chickpea production systems globally.

Keywords: chickpea, heat stress, delayed sowing

DISSECTING THE GENETIC AND PHYSIOLOGICAL COMPONENTS OF HEAT TOLERANCE IN CHICKPEA

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Chickpea cultivars with tolerance to high temperature are needed for sustainable food production under climate change. This research investigates the physiological and genetic basis of heat tolerance in an interspecific chickpea population to accelerate breeding for climate resilience cultivars. We evaluated 200 inbred lines derived from crosses between an elite kabuli cultivar and wild *Cicer reticulatum* accessions using controlled heatwave (35°C [day]/29°C [night]) simulations during flowering. Selected tolerant and sensitive lines from the initial screening were further tested under more severe conditions (40°C [day]/25°C [night]). Seed yield stability was influenced by both the developmental stage exposed to heat stress (flowering vs. podding) and nighttime recovery temperatures. Heat-tolerant lines were able to maintain productivity under heat stress, while elite checks and sensitive lines experienced major seed yield losses. Detailed physiological characterization was performed on four selected lines (three most tolerant and one sensitive). Most flower loss (~90%) occurred after pollination, with the sensitive line exhibiting poor pollen viability and structural defects based on SEM. Analyses of photosynthetic efficiency, pigment levels, osmoprotectants, membrane stability, and antioxidants revealed distinct heat mitigation strategies among tolerant lines. Genome-wide association analyses revealed yield-related traits under heat stress to genomic regions on chromosome 4, including a SNP within the sugar transporter gene that was strongly associated with the response to heat. This work provides insights of the potential mechanism and practical tools for developing heat-resilient chickpea cultivars for sustainable agriculture.

Keywords: chickpea, tolerance, heat stress, genetics, physiology

BREEDING FABA BEAN FOR RESILIENCE: GENOMIC AND PHYSIOLOGICAL INSIGHTS INTO DROUGHT RESILIENCE

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Faba bean (*Vicia faba* L.) is an important cool-season legume cultivated worldwide for food and feed, valued for its nitrogen-fixing ability and role in sustainable crop rotations. Current climatic pressures, particularly heat and drought, highlight the urgency of developing more resilient varieties. Understanding the genetic basis of drought tolerance is essential to enhance breeding efficiency for stress-adapted faba beans. A panel of 300 Mediterranean faba bean accessions were genotyped using a GBS approach with the restriction enzymes HindIII-FaeI for genetic polymorphism analysis, and evaluated under control well-watered (WW) and water-deficit (WD) conditions. Water deficit was maintained at 50% field capacity, and 27 physiological and morphological parameters were recorded 21 days after germination for GWAS-based dissection of drought tolerance. Linear mixed models revealed significant effects for accession, treatment, and their interaction for all traits. Water-deficit conditions generally reduced gas exchange parameters (CO₂ assimilation, stomatal conductance, transpiration) and biomass, while increasing leaf temperature and the root-to-shoot ratio. Phenotypic data (adjusted means for each trait and water treatment taken separately) were tested for association with the genotyping dataset (304,226 SNP markers) using restricted maximum likelihood. Seventy-one regions (27 under WW, 44 under WD) were found associated to 12 traits, and explaining 2-13% of the phenotypic variance. Seven SNPs were associated with multiple traits, with the strongest associations observed for root biomass under WD and aerial biomass under WW. These genomic regions represent promising targets for developing molecular tools to enhance selection efficiency and accelerate breeding of drought-tolerant, resilient faba bean varieties.

Keywords: drought tolerance, genetic diversity, GWAS, photosynthesis, *Vicia faba*

ECOPHYSIOLOGICAL AND GENETIC ANALYSIS OF ROOT PLASTICITY IN RESPONSE TO WATER DEFICIT IN PEA

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Legumes are important for sustainable cropping systems because they can reduce the need of nitrogen fertilizers thanks to the symbiotic nitrogen fixation (SNF) resulting from their partnership with soil bacteria. Among abiotic stresses, water deficit has the greatest negative impact on plant production. When facing water deficit, plants adapt their root system architecture (RSA) by adjusting root growth, branching and direction to optimize soil exploration and water acquisition. The aim of this work was to evaluate the phenotypic plasticity of RSA using a diversity panel of 150 accessions of pea (*Pisum sativum*). Plants were cultivated in rhizotubes in high-throughput phenotyping platform 4PMI (Dijon, France), without nitrogen input to promote symbiotic association. A two-week water stress period was applied during the vegetative phase, followed by a two-week re-watering period. Time-series phenotypes (imaging) were obtained during the whole duration of the experiment for numerous traits such as tap root length, convex hull and root density and allowed a detailed monitoring of RSA changes during and after stress. Kinetic parameters were therefore obtained and genotypes were compared for their growth rate, recovery, and plant resilience. Moreover, RSA- and nodulation-related traits and calculated plasticity indices were used to perform genome-wide association studies to decipher their genetic architecture. This work contributes to a better understanding of pea resilience, including resistance to water stress and post-stress recovery, with a particular focus on RSA and nodulation. Finally, results should help figure out combination of traits for the design of pea ideotypes more resilient to water deficit.

Keywords: water stress, pea, plasticity, roots

A MULTI-OMICS STUDY OF CHICKPEA'S INTERGENERATIONAL ADAPTIVE STRATEGIES UNDER DROUGHT STRESS

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Drought is a big issue for legumes, especially in arid regions. The present work aims to gain insight into the molecular mechanisms of intergenerational memory to drought stress in chickpea. Significant memory effects were observed at morphological biochemical levels as shown by the significant changes of key parameters such as plant height, leaf specific area, osmolites and activities of drought-related antioxidant enzymes. We provided evidences that the drought stress render offspring more adapted in case of a next event of stress while it might negatively affect filial fitness under optimal irrigation. Reactive oxygen species (ROS) scavenging mechanisms were significantly elevated in offspring of stressed parents (G1S) compared to those of control parents (G1C). In addition, RNA-seq data showed that G1S exhibited upregulation of oxidoreductase related genes, while offspring from well-watered parents (G1C) favoured the expression of genes related to proline biosynthesis. The intergenerational memory also showed to have an effect at DNA methylation level as shown the significant changes of DNA methylations in response to drought stress between filial generations of different type of parents. Metabolic profiling further highlighted a distinct metabolite profile in plants derived by a different parental stress condition. Proline, tryptophan, alanine, fructose were particularly affected by a second stress exposure implying that the memory has also a profound effect at metabolism level. Our findings helped to shed light the potential application of seed and plant priming in breeding programs to develop climate-smart legume varieties through sustainable biotechnological strategies.

Keywords: chickpea, drought, intergenerational stress memories, legume adaptive strategies

LEVERAGING LOCAL ADAPTATIONS IN MUNGBEAN TO COMBAT HEAT STRESS

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Mungbean (*Vigna radiata* L.), the only short duration, high value summer grain legume in Australia, undergoes 35-40% yield loss for every degree of temperature above its optimum range. With climate change, the mungbean cultivating areas in Australia are experiencing high heat, decreasing yield and crop survival. This study aims to identify valuable genetic mechanisms underpinning heat resilience in diverse accessions to improve cultivation in Australia's changing climate. Previous studies show that wild mungbean populations historically underwent local adaptation to high temperature environments, driven by climate-induced genetic changes. Building upon that, we analysed the genome of 300 lines of mungbean mini-core collection from WorldVeg Centre and identified population clusters associated with origins and breeding history. We correlated the genomic data with the environmental variables from previous field trials, including climatic conditions and geographic coordinates through a Genotype Environment Association study to identify environment-associated loci contributing to heat resilience. These environment associated loci were then intersected with yield related phenotypic data from heat stress field evaluations, allowing us to pinpoint variants that not only respond to environmental gradients but also show measurable effects on yield, thereby identifying strong candidates for heat resilience. Going forward, these adaptive alleles will be validated in controlled environment experiments, and their patterns of inheritance will be studied, enabling us to efficiently incorporate them into stacked mungbean varieties. This study uniquely utilises population genomics techniques and existing crop diversity to find potential heat-resilient mungbean lines for future varietal improvement, contributing to the improvement of yield under high-temperature conditions.

Keywords: mungbean, heat stress, local adaptations, landscape genomics

MAPPING GENOMIC REGIONS FOR KEY HEAT-STRESS TOLERANCE TRAITS IN PEA (*PISUM SATIVUM* L.)

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Heat stress during the reproductive stage reduces the yield and quality of pea (*Pisum sativum* L.). To identify genomic regions associated with heat stress tolerance, we conducted a genome wide association study (GWAS) using 100 accessions selected from a pea diversity panel (N = 233). The panel was evaluated in an alpha lattice design across three locations in Saskatchewan over two years (2024–2025), with sowing delayed by three weeks to synchronize flowering with typical July heat waves (>28 °C). Among several measured traits, six key heat relevant traits—canopy temperature (CT), SPAD chlorophyll content, lodging score (plant architecture), grain yield, pods per plant, and seed to ovule ratio—were selected for GWAS analysis using the FarmCPU and BLINK models in GAPIT. A total of 21,317 filtered SNP markers were used for marker–trait association (MTA) analysis, identifying 150 MTAs across 128 unique SNPs. Seventeen MTAs were detected in multiple environments (N ≥ 2) across the six evaluated traits. Repeated association signals were observed on chromosomes Chr1LG6, Chr2LG1, Chr5LG3, and Chr7LG7, consistent with previously reported QTL regions for heat stress tolerance in pea. These genomic hotspots represent promising targets for the simultaneous improvement of multiple traits, including canopy cooling, chlorophyll retention, lodging resistance, and yield stability under heat stress. The identified SNPs provide valuable candidates for marker assisted selection aimed at developing heat tolerant pea cultivars.

Keywords: pea, *Pisum sativum*, heat stress tolerance, GWAS, marker–trait associations (MTAs)

LINKING ROOT AND STOMATAL TRAITS TO DROUGHT TOLERANCE IN FABA BEAN AND PEA

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Faba bean (*Vicia faba*) and pea (*Pisum sativum*) are high-yielding grain legumes well suited to temperate Europe, where biological nitrogen fixation supports sustainable cropping systems. However, both crops are sensitive to transient droughts, particularly during anthesis, when water limitation can cause severe yield losses. To improve yield stability, we investigate the physiological basis of drought tolerance and phenotypic plasticity by integrating below- and above-ground traits. Controlled mesocosm experiments are combined with multi-site field trials to link mechanistic understanding with field performance under varying moisture regimes. Root systems of grain legumes remain understudied, particularly in relation to aboveground traits. We therefore examined root system development, rooting depth, and anatomical traits influencing water uptake alongside stomatal size, density, and regulation that shape transpiration and plant water use. Linking these traits enables identification of drought-tolerance ideotypes and improves understanding of adaptive plasticity. Our results show plastic responses to drought in both root and stomatal traits, including changes in xylem vessel number and stomatal size and density. Faba bean accessed water at 1 m depth, with roots reaching 1.6 m within two weeks post-anthesis. This indicates that deep rooting occurs only after the drought-sensitive anthesis period, suggesting that delayed deep root growth contributes to vulnerability to transient drought. Overall, this work improves prediction of drought performance and supports breeding of climate-resilient grain legumes.

Keywords: drought stress, roots, stomata, plasticity

DROUGHT AND HEAT STRESS IMPACT ON NECTAR PRODUCTION, POLLINATOR BEHAVIOR, AND YIELD IN FABA BEAN (*VICIA FABA* L.)

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Faba bean is sensitive to abiotic stressors such as drought and heat, which adversely affect yield. While insect pollination markedly enhances seed set and yield, the interaction between environmental stress, floral rewards (nectar) and pollinator behavior remains poorly understood. This study examines the effects of combined drought and heat stress on nectar traits and subsequent bee-mediated pollination dynamics and yield. We evaluated 25 spring faba bean genotypes in a field-based rainout shelter experiment under increased temperature and two water regimes: well-irrigated and drought-stressed. During the peak flowering period, we assessed nectar volume and sugar concentration per flower. Concurrently, we conducted pollinator observations, classifying bee species and their specific foraging strategies (pollination vs. nectar robbing). Drought stress significantly altered floral rewards, characterized by a decrease in nectar volume and an increase in sugar concentration. Furthermore, drought stress led to a reduction in both the number of open flowers and the overall bee visitation rate. We will further present data on how stress influences the ratio of legitimate pollination to nectar robbing, the significance of genotype-specific responses, and the cumulative impact of these factors on final grain yield. Our results suggest that abiotic stress not only directly limits plant physiology but also indirectly impairs yield potential by reducing floral attractiveness and pollinator activity.

Keywords: *Vicia faba*, abiotic stress, plant-pollinator interactions, floral rewards, genotypic variation

FABALOUS: FABA BEAN ABIOTIC STRESS TOLERANCE FOR IMPROVED YIELD STABILITY

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Among the cold-adapted legumes, faba bean (*Vicia faba*) has the highest yield potential. However, biotic and abiotic stresses reduce interannual yield stability. Abiotic stress is particularly harmful during reproductive stages expected to occur more often under current climate change prospects, turning faba bean less attractive for farmers. The German consortium initiative FABALOUS unites 15 partners and aims to improve yield stability under combined environmental stresses as an essential trait to turn faba bean into a productive crop for farmers. In the first four year funding period, we focus on understanding combined heat and drought stress responses in the present breeding pool. The project is structured by three main lines of research, and first results show the potential of the expected data set. The first line of research uses a representative set of genetically and physiologically contrasting accessions which are currently analysed for the response of key agronomic and physiological parameters under single and combined abiotic stresses. Paralleled by metabolomics, transcriptomics and epigenomic analyses, the aim is to identify gene candidates for genetic engineering that can improve vegetative and reproductive development under stress using systems biology and genome editing. Second, FABALOUS combines association mapping using both a multi-parent population and a diversity set with genome structure analyses to obtain markers associated with stress resilience under very different environmental conditions. In the third line, FABALOUS benefits from access to a unique, above-ground heat pumping facility under an arable field in Bad Nauheim to study heat stress effects at field scale.

Keywords: combined stress, heat, drought, faba bean

BOLD GRASSPEA: DEVELOPMENT OF CLIMATE RESILIENT GRASSPEA VARIETIES FOR YIELD AND STRESS TOLERANCE IN MARGINAL ENVIRONMENTS

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Grasspea (*Lathyrus sativus* L.) is a protein-rich, climate-resilient, and widely cultivated pulse crop in Bangladesh, ranking among the leading pulses in acreage and production. It serves as a staple food grain and an important forage and feed resource. However, nearly 30% of grasspea-growing areas face waterlogging, and about 1.05 Mha of land is affected by salinity, both of which severely limit productivity. Developing high-yielding varieties adapted to stress-prone environments is therefore essential. Under the BOLD project, target ecologies were identified and local landraces and CWR-derived lines, along with popular varieties, BARI Khesari-3 and BARI Khesari-6 as checks, were evaluated for yield-related traits under waterlogging and salinity stress environments. Waterlogging was imposed 28 days after sowing by maintaining the water level at the soil surface for 21 days, while control pots were irrigated to 80% field capacity. Local landraces were assessed for salinity tolerance under field conditions at Satkhira, with weekly monitoring of soil salinity. Significant genotype × treatment interactions were recorded for grain yield, branches, pods per plant, shoot and root biomass, and root length, indicating diverse responses to waterlogging. BK-2 and BK 2021-002 showed strong tolerance to waterlogging stresses, maintaining higher yields and harvest index. Salinity stress reduced plant height, biological yield, pods per plant, and 100-seed weight in most genotypes. Stress Tolerance Index values identified five salinity-tolerant genotypes. The promising genotypes are being evaluated in preliminary (PYT), regional (RYT) and on farm trials on specific agro-ecologies.

Keywords: grasspea, yield, abiotic stress

EVALUATION OF A DIVERSE COWPEA COLLECTION FOR DROUGHT ADAPTATION AND IDENTIFICATION OF ASSOCIATED LOCI

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Climate change is affecting Spanish agriculture, rising air temperatures, and altering seasonal rainfall patterns and distribution of diseases and pests. Dryland farming is especially vulnerable to climate shocks. In the Spanish region of Castile and Leon, drylands represent over 80% of the cultivated area. Enhancing the resilience of these systems involves adopting practices that conserve water and improve soil health, such as crop diversification and the integration of low-input crops that are well adapted to drought conditions. Cowpea (*Vigna unguiculata*) is a warm-season legume well-known for its good adaptation to drought, heat, and infertile soils. In a current study, we are exploring the potential of cowpea as an alternative crop to increase sustainability of Spanish dryland cropping systems. A total of 315 cultivated accessions belonging to the UCR Minicore have been grown under well-watered and water-stressed conditions at a field site in Leon (Spain) during the summer of 2025. Traits related to drought adaptation including days to flowering (an adaptative mechanism of scape) and grain yield have been scored from each accession and treatment. A general trend towards earlier flowering has been observed under water-stressed conditions, together with a considerable reduction in grain yield. However, germplasm that maintained a consistent performance across treatments have been identified, and will be further tested in replicated trials. Genome-wide association studies (GWAS) are underway to identify genomic regions and candidate genes controlling drought-adaptive traits that can inform future breeding efforts. This work is part of project CNS2023-144668, funded by MCIN/AEI/10.13039/501100011033 and "Next Generation EU/PRTR."

Keywords: cowpea, drought adaptation, dryland agriculture, GWAS, *Vigna unguiculata*

LEVERAGING BREEDING TRIALS AND GLOBAL GENETIC DIVERSITY TO ADDRESS ABIOTIC RESILIENCE IN CHICKPEA

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The increasing frequency of extreme weather events poses a major threat to global chickpea (*Cicer arietinum* L.) production, necessitating the development of cultivars with improved resilience to abiotic stress. Chickpea is particularly vulnerable during the reproductive stage, where abiotic stresses severely disrupt pod set and seed development, leading to substantial yield losses across production regions. This study presents two complementary approaches to identify germplasm and genetic targets valuable for breeding abiotic resilience. The first approach integrates several environmental covariates across the entire growing season into factor analytic linear mixed models (FALMMs) to dissect genotype-by-environment (G×E) interactions using historical yield data from Chickpea Breeding Australia (2018–2023). This framework characterises the environmental drivers of G×E, identifying trial sites experiencing distinct abiotic stress conditions and enabling the potential to identify genotypes that perform best under them. The second approach leverages landscape genomics to identify genetic signatures of local adaptation in 1,331 globally diverse chickpea accessions. By integrating region-specific historical climate data with geographic origin, we identified significant marker-trait associations linked to environmental adaptation, with adaptive alleles displaying clear geographical distribution patterns consistent with local adaptation to specific climatic conditions. Together, these approaches address complementary mechanisms for identifying abiotic resilience, one within existing breeding pipelines, the other uncovering novel adaptive alleles across a broader genetic pool, providing actionable targets for developing climate-resilient chickpea varieties while supporting the utilisation of genetic diversity.

Keywords: abiotic resilience, chickpea breeding, genotype-by-environment interactions, genetic diversity

POTENTIAL APPLICATIONS OF SPECTRAL MEASUREMENTS IN THE SELECTION OF FREEZING-TOLERANT PEA GENOTYPES

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Early detection of freezing stress in winter crops such as pea (*Pisum sativum* L.) is essential for selecting tolerant genotypes under changing climatic conditions. This study evaluated the response of a freezing-tolerant cultivar, a freezing-sensitive cultivar, and ten reciprocal crossing lines to induced freezing stress. Vegetation indices were measured and combined with visual damage assessment as a reference method before treatment and at 24 and 72 hours after freezing. Visual assessment differentiated genotypes as early as 24 hours after stress induction. The sensitive cultivar exhibited damage on most plants (86%), indicating severe injury, whereas the tolerant cultivar showed lower damage (12%). Linear mixed model analysis confirmed that time after freezing, genotype, and their interaction affected damage intensity and most spectral indices ($p < 0.001$), indicating genotype-specific response dynamics. The Chlorophyll Index (CI) showed a strong correlation with visual damage 24 hours after stress ($p = 0.53$), increasing after 72 hours ($p = 0.59$). The Normalized Difference Water Index (NDWI) and Water Band Index (WBI) were correlated with damage at the later stage (72 hours; $p = 0.25$), reflecting changes in plant water status. In contrast, the Photochemical Reflectance Index (PRI) was sensitive to early functional changes in photosynthetic efficiency. Principal component analysis (PCA) identified PC1 (43.1%) integrating pigment and water related indices with visual damage, while PC2 (23.6%) represented functional photosynthetic responses. These results demonstrate that combining the CI, NDWI, and PRI enables early and reliable detection of freezing stress and provides an effective approach for selecting freezing-tolerant pea genotypes.

Keywords: spectral indices, freezing, field pea, selection

ENHANCING DROUGHT RESILIENCE IN LENTILS VIA SILICON-MEDIATED OPTIMISATION OF NODULE METABOLISM

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Drought resilience is critical in sustaining lentil production, particularly in regions prone to water scarcity. While Si supplementation improves above-ground drought tolerance in lentils, its impact on root nodule metabolism remains underexplored. Drought impairs nitrogen (N) fixation by disrupting nodule formation, nitrogenase activity, and carbon (C) supply; this study explores how Si affects C-N dynamics, enzymatic activity, and N fixation efficiency in nodules. A glasshouse experiment tested selected lentil genotypes under moderate (40–45% field capacity;FC) and severe (20–25%;FC) stress and absolute control conditions with and without Si. Biochemical and enzymatic parameters linked to nodule metabolism were analysed. Silicon supplementation enhanced drought resilience by improving C-N metabolism in nodules (Variance 85.74%; $P \leq 0.05$). It boosted the activity of key N assimilating enzymes - nitrogenase, nitrite and nitrate reductase - in stressed plants compared to control ($P \leq 0.001$). Silicon reduced the activity of sucrose synthase and invertase, helping regulate C partitioning and preventing metabolic imbalances under drought. It also facilitated ammonia assimilation by significantly increasing the activity of glutamate synthetase, glutamate synthase, and glutamate dehydrogenase. A strong positive correlation ($r=0.91-0.98$) was observed between nodule number, N fixation efficiency, photosynthetic rate, and harvest index, explaining the broader benefits of Si in lentils. These findings demonstrate that Si enhances drought resilience by optimising nodule function and metabolic balance, making it a valuable strategy for improving crop productivity and sustainability in drought-prone regions.

Keywords: drought, lentil, silicon, nodule, nitrogenase

IDENTIFICATION OF ROOT MICRORNAS INVOLVED IN DROUGHT RESILIENCE IN COWPEA

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Cowpea (*V. unguiculata* L. Walp.) is a promising multipurpose legume crop, well-adapted to high temperatures and drought conditions and is a good model for studying drought tolerance. To date, most research has focused on the aerial tissues to identify cowpea drought-resilient genotypes, while studies on root system are largely underexplored. MicroRNAs (miRNAs) are known to regulate plant stress responses. However, their specific role in cowpea roots under drought stress is not fully understood. The main objective of this study was to optimize a reliable miRNA extraction protocol for cowpea root tissues and to characterize drought-responsive miRNAs. A germination experiment was conducted using four cowpea genotypes (C1 and C2, drought-tolerant, C3 drought-intermediate and C4 drought-sensitive) grown for eight days at 25±1°C under two conditions: control (distilled water) and drought stress (–1.5 bars PEG 6000 solution). Germination, early growth parameters, and lipid peroxidation were recorded to assess drought stress effects. Three miRNA extraction protocols were tested and optimized for root samples to ensure RNA integrity and small RNA recovery. Additionally, the expression of previously described cowpea miRNAs was evaluated in roots using RT-qPCR. Germination rates and seedlings growth differed between genotypes under drought stress. Moreover, differential expression patterns were observed for several miRNAs between treatments, suggesting their modulation of drought stress responses in cowpea roots. Acknowledgments: This work was supported by National Funds by FCT – Portuguese Foundation for Science and Technology, under the projects UID/04033/2025: Centre for the Research and Technology of Agro-Environmental and Biological Sciences (doi.org/10.54499/UID/04033/2025) and LA/P/0126/2020 (doi.org/10.54499/LA/P/0126/2020).

Keywords: *Vigna unguiculata* L., Walp., miRNAs, drought stress, roots, gene expression

CULTIVAR SELECTION AND AGRONOMIC STRATEGIES FOR PRODUCTION OF HIGH YIELD AND HIGH PROTEIN PEAS IN DIVERSE ENVIRONMENTS

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Pea (*Pisum sativum*) protein represents an attractive plant-derived protein for its non-genetically modified, high digestibility, gluten-free, neutral flavor, and low allergenic risk. Great challenges exist in producing high yield and high protein peas in diverse environments. A multi-year and multi-location variety trial was carried out to identify cultivars that can produce high protein content with a stable yield across diverse environments. In addition, agronomic management practices, including inoculation with rhizobium inoculants, applying nitrogen fertilizer, and supplemental irrigation were imposed to the selected cultivars. N15 isotope analysis was also employed to aid understanding the symbiotic nitrogen fixation of different pea cultivars under various experimental treatments. Results identified four distinct pea cultivars in terms of yield and protein production across different environments: 1) cultivar A produced high yield but low protein content across the testing site-years, 2) cultivar B produced high yield and high protein content, 3) cultivar C and D produced medium high yield and protein content. Agronomic studies with these four contrasting cultivars showed that rhizobia inoculant and nitrogen application had minimal impacts on yield and protein content in the fields with previous pea planting history. However, supplemental irrigation in combination with rhizobia inoculant provided optimal environment for selected pea cultivars to accumulate protein and biomass, particularly in late growing season, to boost the protein and yield. Research demonstrates that protein content of field peas is largely controlled by genetics, but environmental conditions and agronomic practices can enhance the cultivar performance to produce high yield and protein peas.

Keywords: field pea, protein, cultivar, agronomy, environment

CLIMATE-SMART GRASSPEA: TRANSFORMING RICE-FALLOW SYSTEMS FOR SUSTAINABILITY

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Grasspea (*Lathyrus sativus* L.) is an important climate-resilient legume cultivated under rice-fallow ecosystems of South Asia, where relay sowing into standing rice enables efficient utilization of residual soil moisture with minimal external inputs. However, increasing climatic variability has intensified two major abiotic constraints in this ecology: transient waterlogging during germination and early seedling establishment, and terminal heat stress during the reproductive stage, both of which adversely affect crop establishment and productivity. Two independent studies were conducted in West Bengal, India, to elucidate the adaptive responses of grasspea to these contrasting stresses and to identify tolerant lines. In the first study, a diverse panel of grasspea genotypes was screened under controlled hypoxic conditions by subjecting seedlings to six days of waterlogging followed by a recovery phase. Significant genotypic variation was observed in germination behaviour, seedling vigour, biomass accumulation, and chlorophyll retention. In a complementary field study, grasspea genotypes were evaluated under normal and late-sown environments to assess responses to terminal heat stress. Heat stress caused marked reductions in phenological duration and yield-attributing traits, indicating substantial stress-induced variability. Tolerant lines displayed improved physiological resilience through higher relative leaf water content and chlorophyll stability, greater membrane integrity, and increased accumulation of osmoprotectants and phenolics. Differential expression of key heat-responsive transcriptional regulators and molecular chaperones and β -ODAP biosynthesis-related genes further elucidated the molecular basis of adaptation. Overall, the identified grasspea genotypes with tolerance to early-stage hypoxia as well as terminal heat stress are suitable for sustainable rice-based relay cropping systems under changing climatic scenarios.

Keywords: transient water logging, hypoxia, terminal heat stress, rice-fallow, grasspea

INTEGRATING PHYSIOLOGICAL AND GENOMIC ANALYSES TO UNRAVEL THE BASIS OF DROUGHT TOLERANCE IN MEDITERRANEAN COMMON VETCH

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Common vetch (*Vicia sativa* L.) is a high-protein legume widely cultivated for animal feed as forage, and as a service crop. This species combines a strong nitrogen-fixing capacity with high drought tolerance, making it a promising but underexploited crop for sustainable feed and cropping systems in Europe. In line with the growing demand for locally produced protein sources and the EU's commitment to more sustainable agricultural systems, its Mediterranean germplasm represents an untapped reservoir of adaptive diversity. To elucidate the genetics and physiological bases of drought tolerance, a panel of 286 Mediterranean common vetch accessions was evaluated under controlled well-watered (WW) and water-deficit (WD) conditions (10-15% field capacity), followed by genome-wide association studies (GWAS). Thirty-five morphological and physiological traits including leaf gas exchange, leaf temperature, chlorophyll content, chlorophyll a fluorescence parameters, biomass-related traits, relative water content and reflectance spectra indices were recorded 21 days after germination. Linear mixed models revealed significant accession effects for 31 traits out of the 35 tested. Phenotypic data were assessed for association using 272,643 high-quality SNPs, identifying 17 significant marker-trait associations under WW, for 9 traits, and 16 under WD, for 8 traits. The proportion of phenotypic variance explained by the associated SNPs ranged from 2.7% to 25.3%. Ongoing is the identification of candidate genes underlying the significant loci. The identified drought tolerance controlling loci, after validation, will be used in the development of molecular selection tools for increasing efficiency and speed of breeding programs targeting climate-resilient, protein-rich vetch crops.

Keywords: breeding strategies, drought tolerance, genetic diversity, GWAS

HIGH-THROUGHPUT PHENOTYPING AND GENOMIC PREDICTION OF FROST RESISTANCE TO SUPPORT AUTUMN SOWING IN PEA

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Autumn sowing can increase pea grain yield in various European regions through a longer crop cycle and/or escape from terminal drought but requires frost-resistant cultivars. Frost resistance may be crucial even in relatively mild winters, as sudden frost events may cause substantial mortality of poorly-acclimated plants. The increasing year-to-year climatic variability complicates the field-based selection for frost resistance, emphasizing the interest of selection under controlled conditions and/or genomic selection. Our study aimed to investigate the genetic variation, genetic architecture, and genomic prediction of frost resistance in a world germplasm collection comprising 136 landrace genotypes and eight cultivars. This material was evaluated at the vegetative stage using a high-throughput phenotyping platform to assess plant mortality and a biomass injury score at -13°C , a temperature that maximized the plant mortality variation of pea in a previous study. The population was genotyped by 38,879 SNP markers issued from genotyping-by-sequencing. Plant mortality ranged from 0% to 92% and was highly correlated with the biomass injury score ($r = 0.90$). Both traits displayed high broad-sense heritability ($H^2 = 0.77\text{--}0.80$). A genome-wide association study indicated a polygenic control of frost resistance and identified six significant markers linked to seven candidate genes. The predictive ability of genomic prediction models reached 0.53 for plant mortality and 0.57 for the biomass injury score. Our large germplasm evaluation of frost resistance highlighted the wide genetic variation, identified untapped landrace genetic resources, and provided an unprecedented assessment of genomic selection for frost resistance improvement.

Keywords: *Pisum sativum*, frost resistance, genetic resource, GWAS, genomic selection

ADAPTING TO ADVERSITY: GRASSPEA'S GENETIC BLUEPRINT FOR THRIVING IN HARSH CONDITIONS

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Abiotic stresses such as heat, drought, and salinity pose significant challenges to global crop production. grasspea has garnered attention for its resilience to various environmental stresses, offering promise for cultivation in marginal environments. This study presents a comprehensive evaluation of 66 diverse grasspea genotypes for their tolerance to heat, drought, and salinity, aiming to elucidate physiological responses and identify tolerant lines. Under controlled and field conditions in Morocco, a series of experiments were carried out from November 2020 to December 2021. Diverse grasspea genotypes were selected based on various phenotypic characteristics, including flowering time, sensitivity to *Orobanche*, drought tolerance, and ODAP content. The experiments were designed to evaluate below and above-ground traits, including root traits, shoot traits, and ODAP concentration using different methods and techniques. The drought study highlighted relationships between root architecture and ODAP content under water-limited conditions. Genotypes IG 114935 and IG 115119 exhibited wide root growth angles, contrasting with IG 64836, IG 64839, and IG 64861 with narrow angles. IG 64868 (*L. cicera*) demonstrated superior drought tolerance with high grain yield and low ODAP content. Combined heat and drought trials identified IG 64977, IG 64868, and IG 65066 as highly tolerant. Salinity tolerance screening revealed substantial genotypic variation; IG 114935 and IG 115119 maintained strong growth even at 100 mM NaCl. This study provides insights into *Lathyrus* genetic diversity and identifies resilient genotypes that contribute to developing stress-tolerant cultivars, advancing sustainable agricultural practices.

Keywords: grasspea, abiotic stress, heat tolerance, drought tolerance, salinity tolerance

HIGH-THROUGHPUT PHENOTYPING REVEALS GENOTYPIC VARIATION IN DROUGHT RESPONSE AND WATER-USE STRATEGIES IN *LATHYRUS* SPP. UNDER PROGRESSIVE SOIL DRYING

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Grass pea (*Lathyrus sativus* L.) is a legume crop with broad adaptation to marginal and stress-prone environments, making it a promising option for climate-resilient agricultural systems. However, its physiological response to soil drying remains poorly characterized. In this study, we assessed the physiological performance of *Lathyrus* spp. diversity panel in response to progressive soil drying (dry-down) at the PHENO-MA high throughput phenotyping platform. Breakpoint (BP) analysis of transpiration declines with decreasing soil moisture (FTSW) revealed significant genotypic variation in drought sensitivity thresholds. The genotypes IG 107703 (*Lathyrus sativus*) IG 114545 (*Lathyrus sativus*) and IG 64990 (*Lathyrus cicera*) exhibited conservative water-use strategies, with BPs at 0.72, 0.70 and 0.64 respectively. Multi-sensor high-throughput phenotyping using RGB, Thermal Infrared, and a spectrometer data showed significant differences between well-watered (WW) and water-stressed (WS) plants across vegetation Indices namely NDVI, PRI, MCARI, red-edge chlorophyll content, and green cover. Ridge plots and heatmaps highlighted distinct genotype-specific responses, particularly under WS conditions. Agronomic and phenological traits (e.g., flowering time, biomass, grain yield) were integrated with sensor-derived data using PCA and TOPSIS-based multivariate analyses. Genotypes were ranked according to drought tolerance, combining physiological resilience with superior yield performance. These results highlight the value of integrating high-throughput phenotyping and physiological screening to identify drought-tolerant candidates for breeding programs targeting water-limited environments.

Keywords: dry down, progressive soil drying, high throughput phenotyping

ASSESSMENT OF GRASS PEA (*LATHYRUS SATIVUS* L.) GENOTYPE PERFORMANCE UNDER CONCURRENT WATERLOGGING AND SOIL COMPACTION STRESS

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Waterlogging is an increasingly prevalent challenge for grass pea (*Lathyrus sativus* L.) production due to shifting climate patterns and poor soil drainage. Grass pea is cultivated generally as relay cropping after monsoon rice, where compacted soil layers and high soil moisture frequently co-occur, creating combined stresses of waterlogging and mechanical impedance. In rice-based cropping systems, soil compaction caused by puddling operations and the use of agricultural machinery for sowing and harvesting further restricts root development and aggravates oxygen deficiency. Therefore, this project aims to examine the combined effects of waterlogging and soil compaction on the phenological, morphological, physiological, bio-chemical, and nutritional performance of contrasting grass pea genotypes, as well as changes in physico-chemical properties of soil. Treatments comprised two waterlogging conditions, two levels of soil compaction (1.4 and 1.6 g cm⁻³, representing moderate and high compaction for the sandy loam under study), and three genotypes. Waterlogging was imposed for 8 days at 20 days after emergence. Photosynthetic performance, water status, membrane stability, and antioxidant enzyme activities elucidate physiological stress-adaptation mechanisms. The quantification of β -ODAP accumulation in leaves and seeds provides an assessment of food safety and nutritional quality. The root–shoot balance and yield contributing traits determine the seed yield performance of genotypes, while examination of essential nutrients and vitamins reveals their nutrient uptake capacity under stress. In addition, the evaluation of root performance, physical and chemical changes of soil properties, shows the root–soil interactions and soil health status under stress conditions.

Keywords: waterlogging, soil compaction, grass pea

PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF CROATIAN COMMON BEAN LANDRACES TO DROUGHT STRESS

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Drought is among the most severe abiotic stresses limiting crop productivity worldwide, and its impact is expected to intensify with ongoing climate change, posing a serious threat to global food security. Local crop landraces represent an important reservoir of genetic diversity and often display diverse adaptive strategies to cope with environmental stresses. In this study, four Croatian common bean (*Phaseolus vulgaris* L.) landraces, belonging to four morphotypes ('Trešnjevac', 'Zelenčec', 'Puter', and 'Biser'), were evaluated for their responses to drought stress. Five plants per landrace were grown under control and drought treatments for 18 days. Soil water content was maintained at 40% volumetric water content (VWC) in control plants and at 20% VWC in drought-stressed plants. Gas exchange was measured using a LI-6400XT Portable Photosynthesis System, and leaf area was determined using a LI-COR Portable Area Meter. Drought stress markedly reduced photosynthesis (A), stomatal conductance (gs), and leaf area, but genotypic differences were pronounced. 'Biser' showed only minor functional impairment under drought (leaf area -36%, gs stable, A -13%), whereas 'Trešnjevac' exhibited severe reductions (leaf area -73%, gs -65%, A -60%). Morphotypes 'Zelenčec' and 'Puter' showed intermediate reductions in photosynthesis (-25% and -39%) and leaf area (-16% and -42%), indicating moderate drought tolerance. These results highlight the considerable adaptive diversity of Croatian common bean landraces, emphasizing their value as genetic resources for improving drought resilience under future climate conditions.

Keywords: physiological response, drought tolerance, photosynthesis, common bean

DYNAMIC WATER RELATIONS AND ALTERNATIVE SPLICING IN COMMON BEAN UNDER PROGRESSIVE SOIL DROUGHT AND RECOVERY

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Alternative splicing (AS), a post-transcriptional regulatory mechanism, plays a pivotal role in regulating plant stress tolerance by diversifying transcriptomes and the resultant proteomes. Despite its well-documented role in model species, the dynamics of drought-induced AS and their functional relevance in common bean (*Phaseolus vulgaris* L.), a globally vital legume crop, remain largely unexplored. To address this knowledge gap, we employed an integrated approach combining advanced physiological phenotyping, high-resolution PacBio Isoform Sequencing (Iso-seq), and Illumina RNA-Seq to systematically characterize water relations and AS landscapes in common bean leaves under progressive soil drought (light, moderate, severe) and subsequent recovery. Our analysis demonstrated that, across all conditions, intron retention (IR) emerges as the predominant AS type, accounting for ~90% of all events, followed by alternative 3' splice sites (A3SS), alternative 5' splice sites (A5SS), and exon skipping (ES). We uncovered 312, 601, and 492 differentially alternative spliced genes (DAGs) responsive to light, moderate, and severe drought stress, respectively. This study not only provides a global landscape of AS regulation in drought-stressed common bean but establishes a foundation for deciphering AS-mediated molecular networks underlying drought adaptation. The integrated dataset serves as a valuable resource for future genomic annotation and precision breeding strategies aimed at enhancing drought resilience in legumes.

Keywords: common bean, alternative splicing, progressive drought, recovery, water relations

PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF DROUGHT TOLERANCE DURING PEA SEED GERMINATION

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Drought represents one of the major abiotic stress factors limiting crop production under ongoing climate change. Local populations (landraces) constitute an important source of genetic variability that may contribute to the identification of mechanisms underlying drought tolerance. The natural variation in drought resilience of 185 pea landraces were evaluated under optimal and osmotic stress conditions induced by polyethylene glycol (PEG). Based on germination rate, genotypes were classified into 5 clusters exhibiting diverse tolerance or sensitivity to PEG-induced stress. A significant negative correlation between seed size and drought tolerance was identified, suggesting a potential trade-off between seed resource allocation and resilience to osmotic stress. Afterward, the representatives from each cluster were characterized biochemically, especially with focus on general indicators of osmotic adjustment, oxidative status, and antioxidant capacity. Tolerant genotypes were associated with enhanced accumulation of protective metabolites and a more efficient activation of antioxidant systems under stress conditions. In contrast, sensitive genotypes showed a higher degree of oxidative damage and a comparatively weaker protective biochemical response, suggesting limited capacity to cope with PEG-induced water deficit. Transcriptomic analysis of representatives of each cluster revealed differential expression of genes associated with drought response, in osmolyte biosynthesis, and regulators of antioxidant defense pathways. To further elucidate relationships contributing to landrace environmental adaptation, modern geoinformatics tools and spatial modelling were applied. Their integration with phenotypic, biochemical, and transcriptomic data enables us to identify molecular mechanisms underlying landrace adaptation to water deficit and highlights their potential as valuable sources of alleles for breeding drought-tolerant legume cultivars.

Keywords: landraces, PEG-induced stress, pea, seeds

ASSESSING EARLY-STAGE DROUGHT TOLERANCE IN PEA: PHYSIOLOGICAL TRAITS ACROSS CONTRASTING GENOTYPES

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Legume crops play a crucial role in sustainable agriculture due to their nutritional value and their ability to enrich soils through biological nitrogen fixation. Yet, their productivity is increasingly threatened by drought conditions driven by ongoing climate variability, making it essential to understand how different genotypes cope with water limitation during early developmental stages. Therefore, we compared the complex physiological responses of hydroponically grown seedlings of several contrasting pea (*Pisum sativum* L.) genotypes (e.g. Trendy, Cameor and JI92) after five days of reduced water availability, which was induced by a 20% polyethylene glycol (PEG6000) solution. The water content and water potential of the leaves and roots was measured, as well as the content of photosynthetic pigments (chlorophylls and carotenoids) and chlorophyll fluorescence parameters that reflect photosynthetic function. We also assessed stomatal conductance, cell membrane thermostability, and malondialdehyde content in the leaves, which indicates the level of lipid peroxidation. While the qualitative changes in the measured parameters were similar across the studied genotypes, the quantitative differences were notable. This indicates varying degrees of drought tolerance in the genotypes during the early growth stage of seedlings. Acknowledgements: Work is supported by TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK and INTER-COST-LUC25023 MEYS CZ projects.

Keywords: photosynthesis, seedlings, PEG-induced stress, drought tolerance

UNCOVERING DROUGHT TOLERANCE AND SEED QUALITY TRAITS IN WHITE AND YELLOW LUPIN: ITQB NOVA'S ROLE IN THE PROSPER PROJECT

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PROSPER - Promoting Resilient Orphan Legumes for Sustainable Agriculture and Food Security - is a Horizon Europe project involving 27 partners from 14 countries, coordinated by the University of Pavia. It aims to develop sustainable, science-based solutions to valorize resilient orphan legumes across Mediterranean, and Central and Northern Europe. Using a multi-actor approach, stakeholders and end-users will contribute to the co-creation of impactful solutions. By integrating expertise in agronomy, plant breeding, agroecology, physiology, genetics, seed and food technology, circular bioeconomy, precision agriculture, artificial intelligence, climate modeling, and science communication, PROSPER fosters cross-sector collaboration. Lupins (*Lupinus* spp.) are annual legume crops valued for their high-protein seeds, low production costs, and strong adaptability to diverse and challenging environmental conditions, particularly acidic areas. Although most Portuguese agricultural soils are acidic, national lupin production remains highly unstable due to variable climatic conditions. At ITQB NOVA, we will evaluate 200 white lupin (*Lupinus albus*) and 200 yellow lupin (*L. luteus*) accessions for drought tolerance under controlled conditions. Photosynthetic and morphological traits will be measured under well-watered and water-deficit regimes. Participatory yield evaluations at farmers farms will complement controlled experiments, focusing on agronomic performance, disease and pest incidence, seed protein and alkaloid content, to identify resilient, high-quality sources for cross breeding. In parallel, both collections will be genotyped, and genome-wide association studies will be used to identify genomic regions associated with stress-tolerance, high-yield, and high-quality lupin varieties, as the first steps on the development of precision breeding approaches to enhance breeding efficiency for more resilient lupins.

Keywords: Lupins, GWAS, PROSPER EU project, drought tolerance, orphan legumes

EFFECT OF ACUTE HEAT STRESS DURING POD FILLING ON SEED END-USER AND NUTRITIONAL TRAITS OF LENTIL

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Lentils are a nutrient-dense grain legume valued for their high protein and dietary fibre content, bioactive compounds, and their contribution to sustainable cropping systems through biological nitrogen fixation. Beyond yield, end-user traits such as milling traits and cooking behaviour are critical determinants of market value in Australia. However, there is limited understanding of how short-term elevated temperatures influence both nutritional composition and processing-related traits. This study evaluated eight lentil genotypes from the Australian breeding program under controlled heat stress (a 3-day heat event) applied during pod filling. End-user traits (dehulling efficiency, splitting yield, footfall recovery, hydration capacity, and cooked seed firmness) and nutritional traits (protein, total starch, phytic acid, total phenolics, flavonoids, condensed tannins, anthocyanins, and total dietary fibre) were quantified to assess genotype-specific responses. Acute heat stress induced consistent yet strongly genotype-dependent changes in end-use and nutritional quality. End-user traits were sensitive to elevated temperature, with dehulling efficiency and splitting yield declining alongside reductions in seed size. Hydration capacity generally increased, while cooked seed firmness decreased, indicating modifications to cooking behaviour. Nutritionally, protein concentration increased, and total starch declined, reflecting concentration effects associated with smaller seeds. Phytic acid and several phenolic-related compounds increased in most genotypes, whereas anthocyanin content declined uniformly. Total dietary fibre showed no consistent directional response. Overall, heat stress during pod filling compromises processing quality and modifies nutritional composition, highlighting the importance of breeding heat-resilient genotypes to maintain both end-use functional and nutritional quality under increasingly variable climates.

Keywords: lentil, heat stress, seed quality, end-use quality, nutritional quality

COMMON BEAN BIOFORTIFIED EXPERIMENTAL AND COMMERCIAL ACCESSIONS ANALYSIS TO PROMOTE FOOD SECURITY IN THE CONTEXT OF CLIMATE VARIATION: MORPHO-AGRONOMIC OVERVIEW AND WATER DEFICIENCY RESPONSE

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Climate change is posing a severe threat to global food security, particularly for legume crops such as common beans (*Phaseolus vulgaris*). The seeds, appreciated for their organoleptic qualities (crucial sources of dietary protein and essential nutrients), represent a strategic food in sustainable diets. Drought is one of the main causes of significant yield losses and can also modify seed composition, with serious consequences for food security. Our study aimed to characterize resilience and nutritional quality of 14 common bean accessions through an integrative approach that combined genetic selection, morphological and agronomical analysis in field trials, water stress experiment, qualitative, nutritional and bioavailability analysis on raw and cooked seeds and recipe formulations for diets. We report here the results observed in field trials in Italy and South Africa on nine main morphological traits. Most of these traits are genotype-dependent and strongly influenced by the environment, while some traits are affected only by genotype. The commercial lines showed the greatest yield in field, although the African commercial lines had strong sensitivity to environmental conditions. The low phytic acid lines showed a lower yield than the commercial ones, but they were the most stable genotypes in all the environments analyzed. The results of water stress applied to assess the effect of water shortage on seed production and nutritional quality highlighted the susceptibility of some genotypes to high temperatures, but none were statistically resistant/susceptible to drought. Our data showed that some of the genotypes analysed combine agronomic potential, nutritional characteristics and drought resilience.

Keywords: common bean, morphological and agronomical traits, water deficient experiments

ADAPTATION OF SPRING FABA BEAN TO HEAT AND DROUGHT STRESS: PHYSIOLOGICAL AND ROOT TRAIT RESPONSES

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In recent years, faba bean (*Vicia faba* L.) has gained renewed attention in Europe due to its high protein content and its potential as a locally produced alternative to soybean. However, rising temperatures and more frequent drought events pose major challenges to its production. Faba bean is particularly sensitive to heat and drought stress during the reproductive stage, which can negatively affect flowering and pollination and ultimately impair seed yield. To better understand stress responses, we evaluated the physiological and morphological performance of twelve contrasting spring faba bean accessions under four conditions: heat stress, drought stress, combined heat and drought stress, and control. The results showed strong genotype-specific responses. Under heat stress, physiological parameters showed mixed relationships with yield, while under combined stress, stomatal conductance and photosynthetic efficiency were negatively associated with yield components. Pollen production and viability declined significantly under heat stress compared to drought stress alone. We found that some genotypes developed larger and more highly branched root systems with greater biomass under combined stress, indicating adaptive strategies to cope with both stresses. Despite clear physiological stress effects, several genotypes showed compensatory growth and yield recovery after stress release, reflecting the indeterminate growth habit of faba bean. These findings highlight substantial genetic variation and identify promising genotypes for breeding climate-resilient cultivars.

Keywords: genotypic variation, combined stress, root architecture, pollen viability, photosynthetic efficiency

PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSE OF FABA BEAN (*VICIA FABA* L.) GENOTYPES TO TERMINAL HEAT STRESS DURING FLORAL DEVELOPMENT

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Faba bean (*Vicia faba* L.) is a cool-season legume valued for its high protein content for both food and feed purposes. However, its cultivation remains limited due to yield instability observed under abiotic stress. Instability is driven in part by floral abortion—the premature shedding of floral organs. While floral abortion is a natural process, it is exacerbated under stress conditions such as heat. Predicted increase in global temperature therefore poses a further threat to the yield of faba bean, and its potential for wider adoption in human nutrition. Stabilizing yield in faba bean is therefore a critical breeding objective. To investigate this trait, we initially developed a floral count protocol to reliably capture total and raceme-wise flower production and assessed replication levels for robust estimation of floral abortion. This protocol was then used to assess the morphological and physiological responses of 22 spring faba bean genotypes grown under control conditions (25/15C) to capture genotypic variation in floral abortion rate. The same trial will be repeated under heat stress (35C) to capture genotypic variation in response to abiotic stress. Likewise, we assess morphological traits including plant height, total flower number, and pod production as well as physiological responses by measuring stomatal conductance, transpiration, leaf temperature and effective quantum efficiency of Photosystem II. In addition, lipid peroxidation and ROS enzyme activity are measured to characterize oxidative stress responses. This study lays an important foundation to uncover the genetic base of floral abortion.

Keywords: faba bean, abiotic stress, heat stress, floral abortion

DROUGHT ALTERS JASMONATE-DEPENDENT REGULATION OF ANTHER DEVELOPMENT IN YELLOW LUPIN (*LUPINUS LUTEUS* L.)

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Drought is a major factor limiting plant sexual reproduction and yield. Post-meiotic, late anther development involves a tightly regulated sequence of events culminating in pollen chamber opening (dehiscence), followed by tissue degeneration (senescence). Precise coordination of these processes is essential for male fertility and is governed by phytohormones, including jasmonates (JAs). Our morphological and histological analyses revealed that anthers of yellow lupin (*Lupinus luteus* L.) exposed to drought display a delayed dehiscence phenotype. These abnormalities were associated with altered jasmonic acid (JA) homeostasis, assessed by LC–MS/MS, immunohistochemical, and real-time PCR methods. The concentrations of JA during individual developmental phases of stressed anthers were disrupted: JA levels decreased when they were high under optimal conditions, and conversely, increased under drought when they would typically decline in well-watered plants. These fluctuations corresponded with altered JA localization during stress conditions: in earlier stages, a reduced JA signal was observed mainly in the epidermis, including the stomium, and in other specific cells of the anther wall; whereas at later stages, drought-treated anthers showed strong and widespread JA accumulation within anther wall cells, localized both at cell peripheries and in the cytoplasm. Consistently, expression patterns of genes involved in JA biosynthesis and perception were modified in a stage-dependent manner. These results demonstrate that drought disrupts phytohormonal regulation of late anther development in *L. luteus*, leading to impaired dehiscence and compromising reproductive success under water-limited conditions. This work was supported by the National Science Centre of Poland [grant number 2021/05/X/NZ9/00700].

Keywords: anther development, drought stress, jasmonates, legumes, yellow lupin (*Lupinus luteus* L.)

THE EFFECT OF MOISTURE DEFICIT ON FIELD BEAN (*VICIA FABA* L.)

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Climate change is leading to more frequent extreme weather conditions, with drought being one of the most critical factors affecting crop production. Field bean (*Vicia faba* L.) requires sufficient moisture for optimal growth; however, moisture deficit, especially during flowering and pod formation, significantly reduces yield. To identify varieties better adapted to drought conditions, a field experiment was established in 2025 at the Centre of Estonian Rural Research and Knowledge (METK) in Jõgeva. Eighteen field bean varieties of different origins were grown under controlled soil moisture conditions. The experiment included two treatments: natural precipitation (control) and drought stress. The results showed that moisture deficiency during flowering and pod formation (BBCH 61–67) had a significant negative effect on yield. The average seed yield under drought conditions was 2198 kg ha⁻¹, with the highest yield recorded for the variety 'LG Stego' (2902 kg ha⁻¹). Under control conditions, the average yield was 3348 kg ha⁻¹, again with 'LG Stego' producing the highest yield (3899 kg ha⁻¹), resulting in a yield reduction of 997 kg ha⁻¹. The smallest yield loss under drought was observed in the variety 'Sampo' (131 kg ha⁻¹). The average protein content decreased significantly under drought stress; however, in the breeding line 'V-257', protein content remained stable at 31.4% in both treatments. This research was supported by: (1) Project FabaNova (Climate-ready faba beans for the Nordic and Baltic Region); (2) the Centre of Excellence AgroCropFuture "Agroecology and new crops in future climates", financed by the Ministry of Education and Research, Estonia.

Keywords: field bean, drought, yield

MOTHER OF FT AND TFL1 – A REGULATOR OF SEED GERMINATION UNDER ADVERSE ENVIRONMENTAL CONDITIONS IN *MEDICAGO TRUNCATULA*

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The transition from seed to seedling is a key developmental phase that requires tight regulation to ensure survival of the young plant under variable environmental conditions. This is achieved through adaptive traits such as dormancy, which prevents out-of-season germination, and delayed germination in response to abiotic stresses. The decision to germinate is controlled by the interplay of abscisic acid (ABA) and gibberellin (GA) pathways. One of the components involved in these pathways is the MOTHER OF FT AND TFL1 (MFT) protein, acting as a transcriptional regulator. We have shown that MFT from *Medicago truncatula* is involved in seed development, dormancy maintenance, and inhibition of germination under unfavourable conditions, demonstrating its functional versatility and significance in seed biology. Specifically, we have shown that MtMFT is preferentially expressed in the endosperm during seed filling and in the embryonic roots of developed imbibed seeds. Transcript accumulation of MtMFT depends on seed dormancy status, and phenotypic analyses of the *mtmft* mutant reveal its role in dormancy maintenance. Furthermore, in non-dormant seeds, MtMFT expression is upregulated by exogenous ABA and NaCl treatment, with the strongest induction observed under high temperature, indicating its prominent involvement in thermoinhibition. Nuclear localization of MtMFT, together with our crystallographic data, supports its potential role in transcriptional regulation. We therefore propose that MtMFT integrates internal and external cues to control seed germination under various conditions, possibly through the formation of diverse transcriptional complexes that regulate downstream gene expression. National Science Centre supports this work: OPUS 2021/43/B/NZ3/00672

Keywords: MFT, *Medicago truncatula*, seed dormancy, germination, phytohormones

FOLIAR SILICON APPLICATION ENHANCES DROUGHT RESILIENCE IN LENTILS

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Legume crops are crucial sources of protein and play an essential role in food and nutrition security. However, increasing drought conditions adversely affect these crops' life cycles, impacting production. Silicon (Si) is recognised for enhancing plant tolerance to environmental stresses. While Si's role as a stress alleviator is well-documented in high Si-accumulating crops like cereals, further study of its effects on low Si-accumulating crops like legumes is needed. This study examines Si's drought-mitigating effects in lentils. Glasshouse experiments were conducted comparing responses in lentils, a low Si-accumulator to wheat, a high Si-accumulator. Foliar Si was applied at various concentrations, and drought conditions were maintained at 35% field capacity for 21 days. Results indicate that foliar Si application at 2 and 4 mM significantly mitigates drought stress in both plants by enhancing photosynthetic efficiency, reducing yield loss from 77% to 31% in lentils and from 47% to 15% in wheat. Foliar Si application proved effective in augmenting Si effects, particularly in lentils, which have lower root Si absorption capacity. Studies demonstrate that plant response is influenced by Si concentration rather than total Si application rate, emphasising the importance of appropriate concentrations. The study showed that foliar Si application enhances drought tolerance in lentils similar to wheat at optimal concentrations of 2-4 mM. Therefore, foliar Si supplementation could be a viable strategy for enhancing drought tolerance in legume crops and promoting environmental sustainability.

Keywords: drought tolerance, foliar application, lentils, silicon, yield loss

IN VIVO VISUALIZATION OF ROS FORMATION IN PEA SEEDS DURING IMBIBITION

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Imbibition is an important phase in the life of a plant and reactive oxygen species (ROS) play a key role in imbibition. We managed to visualise the formation of ROS under in vivo conditions via ultra-weak photon emission (UPE). UPE is a phenomenon caused by ROS oxidizing biomolecules which leads to the formation of electronically excited species which subsequently emit photons in the visible range. With the usage of highly sensitive CCD camera, we are able to detect the light and see the temporal and spatial distribution of ROS formed within individual seeds during the imbibition phase. We compared three contrasting pea (*Pisum sativum* L.) genotypes (Trendy, Arvika, JI92) under control conditions and reduced water availability induced by a 15% polyethylene glycol (PEG-6000). Significant differences in both the intensity and kinetics of ROS formation not only between individual genotypes, but also between seeds of the same genotype were found under both normal and reduced water availability conditions. Under water limitation conditions overall ROS concentration was lower compared to control conditions with postponed kinetics. JI92 genotype was showing highest ROS formation and fastest kinetics under both conditions, while Trendy genotype showed the lowest ROS formation and slowest kinetics. Those data are in a good correlation with data from germination experiments. Acknowledgements: Work is supported by TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK and INTER-COST-LUC25023 MEYS CZ projects.

Keywords: reactive oxygen species, seeds, PEG-induced stress

MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF COLD STRESS ON FABA BEAN

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In our food production system, faba bean is a significant source of nutritional protein along with its importance as animal feed and in biological nitrogen fixation. Majorly grown spring bean are highly prone to heat and drought. Winter cultivation of faba beans has the advantage to evade dry and hot phases in the summer and results in higher yield compared to summer cultivation. Low availability of cold tolerant cultivars imposes restricts growing faba beans in temperate areas. We established a protocol for winter screening of juvenile faba plants and elucidating the physiological and morphological effect of cold stress on identified cold tolerant and sensitive genotypes. For morphological quantification of severity of damage in these genotypes, we defined a Frost Damage Scoring (FDS) parameter which varies from 0-9, where 0 stands for no damage at all and 9 shows complete death. Assessment of a set of winter lines and spring lines in decreasing temperature for pre-acclimation, acclimation and stress phases determines the effect of each temperature on the targeted traits. Acclimation had a clear positive effect on survival and regrowth of all the genotypes and was more pronounced in winter lines, indicating presence of cold responsive gene networks in winter varieties. We aim to identify underlying regulatory factors and cold response gene networks with Differential gene expression (DEG) analysis. Varied responses of freezing sensitive and tolerant genotypes already indicate genotypic variability towards cold tolerance and compliance of the protocol for cold screening in faba bean.

Keywords: faba bean, cold tolerance, DEG, acclimation, FDS

IMPROVING LEGUME STRESS RESILIENCE: FUNCTIONAL ANALYSIS OF THE AUXIN INFLUX CARRIER MTLAX3 UNDER SALT AND DROUGHT CONDITIONS

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Abiotic stresses are one of the main limiting factors affecting the quality and yield of agricultural crops. Recent studies have shown that some auxin transporter genes are involved in the response to abiotic stress, such as drought and salinity. In the model legume plant *Medicago truncatula*, the redistribution of the phytohormone auxin is a primary mechanism for coping with these environmental challenges. The MTLAX3 gene, which facilitates cellular uptake of auxin, has emerged as a key regulator in this adaptive process. The study aimed to reveal the role of MTLAX3 to the systemic stress response and adaptive capacity of *M. truncatula* when subjected to salinity and drought conditions. Transgenic lines overexpressing MTLAX3 were used as a platform for functional analyses, in parallel with their wild type. The expression pattern of MTLAX3, along with morphological, physiological and biochemical changes in the growth and development of the studied plants were monitored following the induction of salt (150 mM NaCl) and drought (5% PEG) stress. Our results demonstrate that overexpression of MTLAX3 confers an advantage in the morphological development, allowing transformants to overcome induced stress more effectively compared to the wild type. Understanding the function of MTLAX3 gene will provide valuable knowledge for the improvement of stress resilience in economically significant legume crops, such as soybean, peas and lentils, and their adaptation to adverse environmental conditions.

Keywords: MTLAX3, *Medicago truncatula*, drought, salinity, stress resilience, functional analysis

STRENGTHENING THE EUROPEAN COMMON BEAN VARIETAL PORTFOLIO: MULTI-ENVIRONMENT ANALYSIS OF YIELD PERFORMANCE AND STABILITY

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Common bean (*Phaseolus vulgaris* L.) is a strategic legume for European agroecosystems, contributing to crop diversification, biological nitrogen fixation, and reduced external inputs. However, the limited renewal of the European varietal portfolio highlights the need for coordinated breeding actions to enhance productivity, stability, and resilience under increasingly variable climatic conditions. Within this context, the Legume Generation project aims to strengthen the European breeding base of common bean through multi-environment evaluations. In 2025 two breeding panels, BBS1 (dry bean) and BBS2 (snap bean), each comprising approximately 200 genotypes, were assessed in Southern Italy (Paterno; 40°16'20.352" N, 15°54'37.905"E) within a Multi-Location Field Trial (MLFT) framework. The same panels were simultaneously cultivated across three European environments characterized by contrasting agroclimatic conditions, enabling a robust assessment of genotype x environment interactions. Trials were conducted using a Randomized Complete Block Design with repeated checks; BBS1 included three replicates and BBS2 two replicates, with plots consisting of single rows of 14 plants. A total of 35 traits were recorded, including 19 qualitative and 16 quantitative descriptors covering phenology, morphology, and yield components. Priority was assigned to key productivity traits, namely number of seeds per pod, total dry seed mass per plot, and 100 seed weight. The MLFT dataset will provide a robust assessment of genotype adaptation and yield stability across contrasting European agro-environments, facilitating the selection of superior lines characterized by consistent performance and reliable expression of yield components.

Keywords: common bean; multi environment field trial; genotype × environment interaction; yield stability; plant phenotyping

PRIMING OF PEA SEEDS WITH H₂O₂ AND NO IMPROVES GERMINATION AND PLANT GROWTH

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Understanding the mechanisms that enable plants to adapt to adverse environmental conditions is increasingly important, particularly in the context of water deficit, one of the most severe stress factors limiting crop growth and yield. Drought induces a number of biochemical and physiological changes in plants, among which the balance between the formation of free radicals and their neutralization by antioxidant systems plays a central role. Stress priming, based on pre-treatment of seeds or plants with specific compounds to enhance subsequent stress tolerance, represents a promising strategy for improving plant resilience. In this study, we investigated the effect of (*Pisum sativum* L.) seed priming on key biochemical parameters associated with drought-induced oxidative stress. We assessed changes in peroxidase activity, total antioxidant capacity, lipid peroxidation, and expression of heat shock protein genes, which are important for the stress response of plants. The seeds were primed with water, hydrogen peroxide (H₂O₂), and nitric oxide (NO) donor sodium nitroprusside, and subsequently germinated and cultivated under conditions of varying water availability simulated by polyethylene glycol. Priming with the signaling molecules H₂O₂ and NO significantly enhanced germination and early seedling growth and modulated antioxidant responses, as reflected by altered peroxidase activity and lipid peroxidation levels. These results provide new insights into the physiological and molecular mechanisms underlying priming-induced drought tolerance in pea seeds and young plants and highlight the potential of redox-based priming strategies for improving crop performance under limited water availability. Acknowledgement: This work is supported by TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK.

Keywords: germination, seed priming, drought, oxidative stress, pea

PEA FROST TOLERANCE : COMPARISON OF FIELD AND CONTROLLED CONDITIONS EVALUATIONS

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Growing autumn-sown grain legumes would enable higher and more stable yields thanks to a longer development cycle and to the possibility to escape terminal drought. In the context of climate change, breeding for frost tolerant winter varieties remains a major concern because milder autums are responsible for a limited plant cold acclimation. At the same time, large and unpredictable climatic variations across years and locations reduce the efficiency and repeatability of field evaluation for frost tolerance. In this context, screening tests in controlled conditions may provide an additional phenotyping tool for breeding. We compared and discussed the frost tolerance scorings of a representative range of pea accessions evaluated in i) field conditions, in ii) controlled conditions with observation of frost damages on whole plantlets and in iii) controlled conditions with measurements of electrolytes leakage on frozen sampled tissues.

Keywords: *Pisum sativum*, frost tolerance, evaluation methods

EFFECTS OF REDUCED WATER AVAILABILITY ON IMBIBITION AND GERMINATION IN DIFFERENT PEA GENOTYPES ASSESSED BY SEED WATER POTENTIAL AND CHLOROPHYLL FLUORESCENCE

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Global climate change is associated with more frequent and severe drought events, posing a serious threat to agricultural production, including production of legumes. It is therefore desirable to study how plants respond to drought and what mechanisms they use to cope with it, as well as to search for and breed plants with higher drought tolerance. Seed germination and early seedling growth are critically sensitive to water limitation. The water uptake during imbibition depends on various factors, including the difference in water potential between the seed and its environment. We compared the imbibition and germination of three contrasting pea (*Pisum sativum* L.) genotypes (Trendy, Arvika, JI92) under conditions of reduced water availability, induced by a 15% polyethylene glycol (PEG-6000) solution. Changes in seed water potential (Ψ) and seedling chlorophyll fluorescence, reflecting photosynthetic activity, were compared. For all genotypes, the Ψ increase during imbibition was slower in the stressed variant than in the control, which reflects slower water uptake. In both variants, Ψ increased most rapidly in JI92 and most slowly in Trendy. Reduced water availability slowed seedling growth in all genotypes. Chlorophyll fluorescence measurements revealed a slight decline in photosynthetic efficiency accompanied by increased non photochemical quenching, suggesting enhanced regulatory dissipation of excess energy under stress. In terms of imbibition, JI92 appeared to be the most tolerant to limited water availability, but in terms of seedling photosynthesis, the response of all genotypes was comparable. Acknowledgements: Work is supported by TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK and INTER-COST-LUC25023 MEYS CZ projects.

Keywords: chlorophyll fluorescence, PEG-induced stress, seedlings, seeds, water potential

EFFECTS OF ELEVATED CO₂ COMBINED WITH WATER DEFICIT ON CARBON ALLOCATION BETWEEN ROOTS AND NODULES IN PEA (*PISUM SATIVUM* L.)

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In future climate scenarios, elevated CO₂ (eCO₂) could partially mitigate the effects of water deficit by increasing carbon assimilation efficiency, despite reduced stomatal conductance. In legumes, nitrogen fixing nodules act as strong carbon sinks, competing directly with roots for carbon. How eCO₂ modulates this trade off under water deficit remains poorly understood. This study investigated the effects of eCO₂ combined with water deficit on biomass allocation in pea (cv. Kayanne), using wheat (cv. Extase) as a non-fixing reference species to assess the role of symbiotic nodulation. Plants were grown in pots and RhizoTubes® in controlled environment chambers under two CO₂ levels (ambient, 420 ppm; elevated, 800 ppm) during the vegetative growth period (35 days). Within each CO₂ treatment, half of the plants received optimal water and nutrient supply, while the other half was subjected to water withholding from day 8 after sowing, inducing a progressive and moderate water deficit. Shoot and root architectures were monitored weekly through imaging. Stomatal conductance and chlorophyll fluorescence were measured throughout the experiment. At harvest, net photosynthesis was measured and dry biomass was quantified for each compartment (shoots, roots, nodules). We hypothesize that eCO₂ partially compensates for the negative effects of water deficit on carbon assimilation and that the additional carbon availability alleviates the competition for carbon between roots and nodules observed under ambient CO₂. Results on biomass allocation, architectural dynamics of shoots and roots, and physiological responses will be presented and discussed, with perspectives for functional-structural modelling of legume responses to combined stresses.

Keywords: elevated CO₂, water deficit, carbon sink competition, nodulation, legume resilience

TEMPORAL TRANSCRIPTOMICS DETERMINES GRAIN YIELD AND PROTEIN CONCENTRATION UNDER INDEPENDENT AND COMBINED HEAT AND DROUGHT IN *VICIA FABA*

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Vicia faba (*Faba bean*) is a good candidate to help meet the growing demand for sustainable protein due to its high yield potential, protein concentration and established role in global cropping systems. However, grain yield is sensitive to heat and drought, and protein concentration is unpredictable across environments and varieties. As growing region temperatures rise and rainfall drops with climate change, the need for controlled environment studies investigating legumes under mild, prolonged, abiotic stress grows. Current results from this study identify significant yield loss through reduced biomass and pod abortion, and consistent protein concentration under independent and combined heat and drought conditions. Therefore, the aim of this paper is to investigate the transcriptional regulation of leaf and grain development that determines this pod set and grain filling phenotype. This was done through a single commercial variety grown under control, mild drought, heat or combined stressors from first flower to physiological maturity in controlled environment rooms on DroughtSpotter™ platforms to ensure consistent stress conditions at The Plant Accelerator at the Australian Plant Phenomics Network, Adelaide, Australia. Leaf and whole grain samples were collected at five developmental stages and processed for mRNA sequencing to 50M read depth. Results will provide molecular depth to the understanding of faba bean yield and protein determination under temperate climate change conditions. Physiological and transcriptional mechanisms will be highlighted for targeting in the development of stress tolerant, high-yielding, high-protein faba, supporting its role in meeting growing sustainable protein demand.

Keywords: abiotic, yield, protein, faba bean, transcriptomics

A CLE11B-CLE16 SIGNALING RELAY MEDIATES ROOT-SHOOT-ROOT CROSSTALK FOR DROUGHT ADAPTATION IN COMMON BEAN

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Under soil drought conditions, plant roots sense stress and transmit signals to the shoots, leading to coordinated whole-plant responses to the stress. While the existence and significance of shoot-to root signals in this process remain unclear. Here, we identify a two-step, CLE peptide-mediated root-shoot-root signaling relay underpinning drought adaptation in common bean. We show that PvCLE16, a gene predominantly expressed in leaves under well-watered conditions, was specifically upregulated in leaves, but not roots, under moderate drought. This spatially restricted transcriptional activation was driven by the leaf-preferentially expressed transcription factor PvTCP10. Accumulated PvCLE16 in leaves promoted stomatal closure and also translocated to the roots, where it suppressed primary root elongation and stimulated lateral root development, adaptations that collectively enhance drought resilience. PvBAM3 was identified as the primary receptor for PvCLE16. Upstream of this module, we found that drought-induced expression of PvCLE16 in leaves requires PvCLE11b, a root-derived CLE peptide that moved acropetally under drought. Together, our findings reveal a novel root-shoot-root signaling relay, wherein root-derived PvCLE11b functions as the upward signal to induce PvCLE16 in leaves, which subsequently acts both locally and systemically by translocating to the roots to coordinate whole-plant drought adaptation responses.

Keywords: drought, CLE, signaling relay, crosstalk

CROSS-SPECIES TRANSCRIPTOMICS UNRAVELS ROOT DROUGHT RESPONSES IN FABA BEAN AND MAIZE

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During soil drying, roots located in shallow versus deeper soil layers encounter substantially different hydraulic environments. However, the molecular reprogramming that accompanies and potentially drives depth-specific changes in root water uptake capacity remains poorly characterized. We addressed this gap by integrating depth-resolved RNA-seq data with previously measured hydraulic conductance profiles in faba bean (*Vicia faba*) and maize (*Zea mays*) roots. Samples were obtained from both shallow and deeper zones, either under optimal watering conditions or after four days of soil drying. Differential expression analysis revealed strongly shallow region - dominated transcriptional responses in both species, consistent with the steeper hydraulic challenge in drying upper soil layers. In *V. faba*, drought-induced downregulation of PIP-type aquaporins and upregulation of class III peroxidases were confined to shallow roots, providing a molecular basis for the observed reduction in root conductance. For cross-species comparison, we generated orthologous gene grouping with OrthoFinder and identified hundreds of drought-responsive orthogroups. Interestingly, only a minor portion of these were found shared between the two species, despite broadly convergent gene ontology (GO) enrichment profiles. Phylogenetic tracing showed that majority of *V. faba*-specific and *Z. mays*-specific drought-responsive orthogroups are conserved across monocot and dicot lineages, suggesting that species-specific drought transcriptional reprogramming arise primarily through differential recruitment of shared ancestral gene families. Altogether, these findings define a molecular framework linking root hydraulic architecture to gene regulation under drought and identify conserved transcriptional regulatory hubs as candidate targets for broad-spectrum abiotic stress improvement in both faba bean and maize.

Keywords: drought, roots, cross-species, transcriptomics

PHYSIOLOGICAL RESPONSES OF FABA BEAN (*VICIA FABA* L.) TO ELEVATED TEMPERATURE

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Faba bean (*Vicia faba* L.) is a protein-rich legume with high agronomic and ecological importance in sustainable cropping systems. Climate projections for Germany indicate that temperatures of 28–30°C during critical growth phases will become more frequent. Plants are sensitive to high temperatures, which can alter development, reduce cell expansion, affect photosynthesis, and modify nutrient uptake. It is important to understand how this species responds to moderate warming. To investigate temperature-induced responses, a greenhouse experiment was conducted with 25 diverse faba bean accessions grown under control (22–25°C) and elevated temperature (28–30°C) conditions. Traits analyzed included plant height, internode length, node and leaf numbers, shoot and root biomass, chlorophyll content, photosynthetic activity, chlorophyll α fluorescence, leaf temperature with thermal camera, and leaf macro- and micronutrient concentrations. Results revealed an architectural shift starting at the fourth node, with reduced internode length producing shorter, more compact plants, while node number increased. Despite reduced shoot elongation, shoot fresh and dry weight, as well as root dry weight, increased under elevated temperature. Elevated temperature didn't alter photosynthetic activity significantly, supporting biomass accumulation. These results indicate a developmental and physiological plasticity of faba beans under moderate warming. The elevated temperature induced a compact architecture with significantly reduced plant height and internode length, while biomass accumulation was maintained or increased. The reduced stem elongation suggests temperature-sensitive regulation of cell expansion, potentially mediated by altered gibberellin (GA) biosynthesis or distribution.

Keywords: faba bean, elevated temperature, physiological plasticity, biomass accumulation, internode length

COMPARATIVE TRANSCRIPTOMIC ANALYSIS IN DEVELOPING SEEDS OF CULTIVATED AND WILD LENTILS (*LENS* SP.) SUBJECTED TO WATER STRESS

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Lentils are among the most widely consumed legumes worldwide and constitute a key dietary component in low-income regions due to their affordability and nutritional value. Lentil production is severely affected by drought, which represents the most severe abiotic constraint, with yield losses reaching up to 78% under water-deficit conditions. Nevertheless, certain genotypes exhibit enhanced drought tolerance, highlighting the genetic basis of this trait. In addition, wild relatives constitute a valuable reservoir of adaptive traits associated with environmental resilience. At the molecular level, transcriptomic analyses enable the identification of differentially expressed genes (DEGs), regulatory networks (e.g., abscisic acid signaling and cuticular wax biosynthesis), and key mechanisms underlying plant responses to water limitation. In this context, comparative transcriptomic analyses between drought tolerant and susceptible genotypes—particularly between cultivated and wild accessions—offer a powerful approach to uncover genes and pathways involved in drought adaptation. This study investigates the differences at the transcriptome level between the cultivated lentil *Lens culinaris* Alpo (susceptible to drought) and the wild relative *Lens odemensis* ILWL235 (tolerant). The focus was on developing seeds, a critical stage highly sensitive to water deficits, impacting both yield and seed quality. Results are expected to provide novel insights into the genetic and molecular basis of drought tolerance in lentils, facilitating the identification of candidate genes and molecular markers for marker-assisted selection, and ultimately supporting the development of improved, drought-resilient cultivars.

Keywords: drought tolerant, transcriptome, water deficit, abiotic stress, gene expression

INTERACTION BETWEEN NITROGEN NUTRITION MODE AND SOIL WATER AVAILABILITY SHAPES AQUAPORIN EXPRESSION AND ROOT WATER UPTAKE IN PEA

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Abstract: Nitrogen and water acquisition are tightly interconnected processes in legumes. Pea (*Pisum sativum* L.) exhibits strong inhibition of symbiotic nitrogen fixation under drought stress, largely due to altered root water relations and carbon allocation within the nodulated root system. Aquaporins (AQPs), which facilitate transmembrane water transport, play a central role in regulating root hydraulic conductivity. However, how nitrogen nutrition mode interacts with soil water availability to modulate AQP expression and thereby regulate plant water transport remains insufficiently understood. To address this question, two pea genotypes differing in root architecture (Rondo and the highly branched mutant BRT) were grown under contrasting water (well-watered vs. water deficit) and nitrogen nutrition modes. Nitrogen treatments consisted of 14 mM nitrate, supplied to inhibit symbiotic N₂ fixation, or 0 mM nitrate, inducing full reliance on symbiotic fixation. Root tissues were sampled from three functional zones—young shallow root tips, mature shallow roots, and deep root tips—for AQP gene expression analysis. In parallel, root and shoot architectural traits, biomass allocation, tissue carbon and nitrogen concentrations, leaf water potential and whole-plant evapotranspiration were assessed. By integrating hydraulic regulation with carbon and nitrogen allocation patterns and plant water fluxes, this study aims to determine whether spatial AQP expression is coordinated with root architecture and nitrogen nutrition mode. This integrative framework provides mechanistic insight into how nitrogen nutrition mode and soil water availability interact to regulate plant water transport in structurally contrasting genotypes.

Keywords: aquaporins(AQPs), nitrogen nutrition, drought, root architecture, plant water consumption

IDENTIFICATION AND CHARACTERISATION OF TWO EFFECTORS OF ASCOCHYTA RABIEI AND THEIR ROLE IN PATHOGENICITY

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Chickpeas (*Cicer arietinum*) are hampered globally by Ascochyta blight disease, caused by *Ascochyta rabiei*. The pathogen produces a set of pathogenicity proteins that interact with the chickpea host to cause disease. Here we present the findings for two effectors. The first, ArToxB was identified using a biparental fungal mapping population with isolates with low and high pathogenicity on chickpea accession ICC3996. The genomes of the parents were assembled using Oxford Nanopore sequencing and the offspring shallow sequenced and the variants called. QTL mapping identified a region at the end of chromosome 4 that was strongly associated with pathogenicity on ICC3996. This region contained a candidate effector with sequence identity to ToxB of *Pyrenophora tritici-repentis* and amino acid differences between the parental isolates. A gene knock-out of ArToxB in reference isolate ME14 showed reduced pathogenicity compared to the wild type. The second effector was identified as a candidate following the annotation of the reference genome and the use of various effector prediction tools. The effector is expressed at low levels and suppressed by an upstream transposable element. Deletion of this transposable element leads to elevated levels of effector expression and importantly recognition by several wild and domesticated chickpea genotypes resulting in an inability to cause disease. Pre-treatment of susceptible chickpea with an overexpressing strain significantly reduced disease symptoms upon subsequent wild type infection in seedling assays. These findings raise interesting questions about *A. rabiei*'s evolutionary history, suggesting that transposon-mediated silencing of an effector may have altered its interaction with the host.

Keywords: Ascochyta blight, chickpea, biotic stress

COMPARATIVE GENOMICS PROVIDE INSIGHT INTO THE EVOLUTION OF EUROPEAN *APHANOMYCES EUTEICHES* STRAINS

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Aphanomyces euteiches is an oomycete pathogen causing root rot in pea and other legumes, leading to devastating yield losses in pea production. Previously, three genetic groups of *A. euteiches* were identified in Europe based on simple sequence repeat markers. In this study, we determined the genome sequences of 68 European *A. euteiches* strains and performed a comparative genomic analysis with the aim to investigate the population genetic structure of *A. euteiches*, to delineate species boundaries and to identify gene families evolving under selection for gene gains or losses. Population genetic analysis based on genome-wide single nucleotide polymorphisms identified three genetic groups in Europe, where strains from Italy formed a distinct and genetically isolated group. Genealogical concordance phylogenetic species recognition analysis indicated that strains from Italy may represent a cryptic species. *Aphanomyces* strains differed in virulence on susceptible and partially resistant pea host genotypes, but virulence did not correlate with genetic groups. Analysis of virulence-related gene family evolution revealed a significant expansion of gene families acting in the detoxification of plant-derived secondary metabolites in plant pathogenic *Aphanomyces* species. Moreover, the carbohydrate esterase family 1 (CE1) was significantly expanded in *A. euteiches*, but not in the cryptic species represented by Italian strains. Modular structure analyses revealed exclusive presence of feruloyl esterase domains in CE1 homologs of plant pathogenic *Aphanomyces* species, indicating a role in plant cell wall degradation.

Keywords: *Aphanomyces euteiches*, comparative genomics, pea, phylogenetic analysis, root rot

RESOLVING A DECADES-OLD MYSTERY: MOLECULAR IDENTIFICATION OF THE FUSARIUM WILT RESISTANCE LOCUS FW IN PEA

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Pea (*Pisum sativum*) is a protein-rich grain legume valued for its nutritional quality, nitrogen-fixing ability, and low environmental footprint. However, its productivity is severely threatened by *Fusarium oxysporum* f. sp. *pisi* (Fop), a soil-borne vascular wilt pathogen causing substantial yield losses in pea-growing regions worldwide. Despite the widespread and durable deployment of the single dominant resistance locus Fw for decades, its molecular identity and underlying mechanism of resistance have remained elusive. Previous studies have made progress in mapping resistance loci, yet the gene responsible for Fw-mediated resistance has not been resolved, limiting opportunities for rational breeding and mechanistic understanding. This study aimed to identify and functionally validate the genetic basis of Fop resistance. We first developed a reliable, high-throughput phenotyping platform using the cut-root vermiculite method, enabling reproducible and large-scale scoring of disease severity across a diverse pea germplasm collection. Using pangenome based genome-wide association studies (GWAS), we confirmed the genomic location of Fw on chromosome 5 and identified a striking presence/absence variation (PAV) distinguishing resistant from susceptible accessions. Integrated transcriptomic and genomic analyses revealed that the Fw locus encodes a novel class of resistance gene not previously described in pea. Functional validation through both hairy roots and stable whole-plant transformation confirmed that Fw alone is sufficient to confer systemic resistance to Fop. These findings resolve a long-standing question in pea disease biology, reveal an unexpected mechanism of durable wilt resistance, and lay the foundation for precision breeding of Fop-resistant varieties.

Keywords: *Pisum sativum*, root disease, *Fusarium*, pangenome, GWAS

GENETIC APPROACHES TO IDENTIFY THE LENTIL RECEPTOR FOR *ASCOCHYTA LENTIS* ALAVR1 EFFECTOR

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Pulses are an important source of plant-based proteins with a low environmental footprint, making them climate-smart crops. However, lentils like other pulses remain vulnerable to fungal diseases such as *Ascochyta* blight (AB), which severely affects yield and seed quality. We previously identified the effector AlAvr1 from *Ascochyta lentis*, with allelic variants, AlAvr1-1 and AlAvr1-2 that led to the assignment of Australian isolates into two pathotypes. AlAvr1-1 acts as an avirulence effector that triggers a hypersensitive response in PBA Hurricane XT and PBA Bolt, while AlAvr1-2 contributes to susceptibility in PBA Hurricane XT. Identification of AlAvr1 effector opened the door for cloning the corresponding R gene/s in lentil. To dissect the genetic basis of AB resistance, a recombinant inbred line population developed from PBA Bolt x Nipper was evaluated with both pathotypes under controlled and field conditions. A significant QTL for pathotype 1 was identified on chromosome 2 with LOD scores of 4.9 (controlled) and 12.3 (field). Notably, this QTL disappeared when inoculated with Pathotype 1 isolate lacking AlAvr1-1, suggesting this locus interacts with AlAvr1-1. The QTL region spans 432 genes, including 88 disease-resistance-associated genes with two NBS-LRRs. One of the primers segregates with the resistance phenotype and resides in an NBS-LRR gene. PCR screening of lentil cultivars with this marker correlates with necrosis response in historical lentil cultivars agroinfiltrated with AlAvr1-1, making this a promising candidate as the receptor of the AlAvr1-1 effector. These findings provide molecular markers to support the efficient deployment and stacking of resistance genes in lentil breeding programs.

Keywords: lentil, *Ascochyta* blight, QTL, resistance, avirulence, effector

DIGGING DEEPER INTO PULSE CROP ROOT ROTS: THE COMPLEXITY OF *FUSARIUM* SPECIES COMPLEXES

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Fusarium species are important contributors to the root rot complex affecting pulse crops in many countries including Canada. Among several *Fusarium* spp. detected on the roots of the large-acreage crops pea and lentil, *F. solani*, *F. avenaceum* and *F. oxysporum* are the most common species in western Canada. While often perceived as distinct species by plant pathologists and plant breeders, these three species are in fact species complexes comprising several phylopecies. The involvement of species complexes in root rot of pulse crops has limited the accuracy and reliability of pathogen identification, particularly with molecular probes developed prior to the recognition of these “species” as species complexes. They also complicate resistance breeding, which requires exact species identification and evaluation of their virulence. We explored the phylogenetic and pathogenic diversity of isolates of the *F. solani* complex (FSSC) and *F. oxysporum* species complex (FOSC) among a collection of isolates from a wide range of pulse crops. Three, including one novel phylospecies were identified among 44 isolates of the FSSC, and five, also including one novel phylospecies were discerned among 66 isolates of the FOSC. Pathogenicity testing of FSSC isolates revealed that isolates of all three species caused symptoms on pulse crop roots, albeit of variable severity among plant varieties and host species. This was also the case for isolates of the FOSC, where differences in virulence among the pathogen species were, however, more pronounced.

Keywords: *Fusarium solani*, *Fusarium oxysporum*, phylospecies, virulence

WHEN CROP ROTATION DOESN'T WORK: IMPLICATIONS FOR MANAGING THE BROAD SPECTRUM PATHOGEN, *FUSARIUM AENACEUM*, IN PULSE CROPPING SYSTEMS

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Fusarium avenaceum is a common generalist pathogen in the Canadian prairies associated with root rot of pulses and oilseeds, and Fusarium head blight of cereals. *F. avenaceum* has the genetic machinery to produce a wide variety of secondary metabolites and mycotoxins. *F. avenaceum* isolates collected from diseased pea, wheat and lentil, were assessed for their ability to cause root rot in pea and lentil, and head blight in durum wheat. Isolates collected from all three hosts were pathogenic on all hosts, but when comparing overall isolate aggressiveness, isolates recovered from pea were more aggressive on all three hosts. Studies of colonization patterns on pea, lentil, durum wheat and canola in field and greenhouse trials showed that *F. avenaceum* mainly colonizes roots and lower stem nodes, with the latter being the primary above-ground colonization site across multiple host crops. These studies demonstrate that crop rotation, an essential tool for disrupting the disease cycle, has limited utility to manage pulse root rots caused by *F. avenaceum*. In order to develop novel management tools, studies to determine the role of secondary metabolites in virulence and host range are underway. Mutant isolates have been generated by knocking-out key biosynthetic and growth regulator genes and are currently being assessed for their pathogenicity and virulence on pea and lentil.

Keywords: *Fusarium avenaceum*, mycotoxins, virulence factors, pea, lentil

STUDY ON PSBMV ADAPTATION IN DIFFERENT LEGUMES AND ITS IMPACT ON THE HOST TRANSCRIPTOMIC RESPONSE

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Pea seed borne mosaic virus (PSbMV) is a seed and aphid transmitted, single stranded, positive sense RNA virus in the genus *Potyvirus* (*Potyvirus pisumsemenportati*). It infects a wide range of pulse and forage crops worldwide, causing significant yield and quality losses. To understand how the virus adapts across legumes, how this adaptation alters viral genomes, and how it affects virulence and disease severity, PSbMV was serially passaged in pea, chickpea, and faba bean over six generations. These adapted virus strains were then used to inoculate pea, chickpea, and faba bean plants, and leaf samples collected 2 weeks post-inoculation were used for transcriptomic analysis by next-generation sequencing. Transcriptomic data from pea, chickpea, and faba infected with each virus strain were analyzed for differential gene expression, and full genome sequences of these virus strains were also generated. In general, PSbMV induces distinct transcriptomic changes in chickpea and pea, followed by faba, primarily affecting genes involved in protein binding, RNA binding, and host translation. Our results showed no significant differences in disease severity among strains, but each adapted strain exhibited a few mutations in its genome compared with the original virus strain. Interestingly, each virus strain induces distinct transcriptomic changes in pea, chickpea and faba, suggesting important roles for these strain-specific mutations. This study highlights regions of the PSbMV genome that are susceptible to change during adaptation and their potential effects on the host defence mechanism. It also provides a better understanding of legume-PSbMV interactions and insight into the diversity and evolutionary dynamics of the PSbMV isolates.

Keywords: pea seed-borne mosaic virus, transcriptomic analysis, pea, faba bean, chickpea

UNTANGLING PLANT EPIDEMICS: A MODELING APPROACH TO SEPARATE WITHIN-LEAF PATHOGEN GROWTH FROM WHOLE-PLANT PATHOGEN DISPERSAL – A CASE STUDY ON PEA FUNGAL DISEASES ANTHRACNOSIS AND ASCOCHYTA BLIGHT

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Describing and predicting epidemic progress is critical for effective reduction of crop losses due to infectious diseases. Disease progression is commonly characterized using quantitative or semi-quantitative indicators of severity, influenced both by pathogen growth at the organ scale and its spread at the plant scale. While these two processes cannot be disentangled from raw data alone, we developed a mechanistic model that, for the first time, distinguishes disease growth within organs from dispersal between organs. We fitted this model to a set of experimental data about the severity of pea infection by the anthracnose foliar disease caused by a *Colletotrichum* complex on one hand and *Ascochyta* blight on the other hand assessed on four susceptible cultivars (Flambo, Hamino, Smiley, Spencer) at five time points in plots grown either sole or intercropped with wheat under natural infection conditions. Disease spread and growth over time (degree days) and space (leaf levels) was modeled by a two-variable logistic function, derived from an approximation of the traveling wave Fisher–KPP model solution. Results showed that the apparent diffusion speed of the disease varied across cultivars, years and cropping systems, and was consistently reduced under intercropping. By interpreting model parameters biologically, we identified apparent diffusion as a novel quantitative trait to characterize pathogen strategies and epidemic speed. This new metric offers plant pathologists and breeders a useful tool to better describe within-plant disease progression across environments and genotypes.

Keywords: foliar disease, modelling, intercrop, legumes, diffusion

FIELD RESISTANCE TO *OROBANCHE CRENATA* IN PEA (*PISUM SATIVUM* L.): BEYOND STRIGOLACTONES

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Orobanchaceae are parasitic weeds causing substantial yield losses in many crops, including pea (*Pisum sativum* L.). Within host species, genotypes that display enhanced resistance to *Orobanchaceae* often exude low levels of strigolactones (SLs) from their roots, in line with evidence that SLs stimulate the germination of *Orobanchaceae*. However, to which extent genetically determined low SL exudation contributes to field resistance to *Orobanchaceae* was poorly investigated. Here, we studied the relation between SL exudation and field response to *Orobanche crenata* Forsk. (Oc) in pea. The screening of a germplasm panel identified three novel sources of field resistance to Oc and revealed an association between field resistance and low SL exudation. Although the SL-deficient mutants rms1 and rms5 were more resistant than their wild-type backgrounds, they still suffered substantial Oc parasitization. Genetic analysis and RNA-seq of recombinant inbred lines uncovered both SL-dependent and SL-independent mechanisms contributing to the near-complete resistance to Oc previously reported in the breeding line ROR12, and identified candidate genes possibly underlying resistance loci. Besides identifying novel sources of resistance to Oc, our study indicates that reduced or absent SL exudation alone is not sufficient to confer complete field resistance to Oc in pea. This suggests the necessity of exploring SL-independent resistance mechanisms for breeding purposes. Further investigations are needed to clarify whether a similar scenario applies to other crops affected by *Orobanchaceae*, and to characterize genes causally related to Oc resistance.

Keywords: *Orobanchaceae*, pea, *Orobanche crenata*, resistance, strigolactones

INTEGRATING GWAS AND APHID BIOASSAYS TO REVEAL RESISTANCE STRATEGIES IN GRASS PEA

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Grass pea (*Lathyrus sativus* L.) is a resilient legume with potential for sustainable agriculture in marginal environments but suffers severe yield losses from pea aphid (*Acyrtosiphon pisum* H.) infestation through phloem-feeding and virus transmission. Increasing aphid outbreaks driven by climate change, combined with stricter EU regulations on pesticide use, highlight the urgent need for resistant varieties. However, the genetic basis and mechanisms of aphid resistance in grass pea remain poorly understood, constraining breeding progress. This study aimed to identify aphid-resistant accessions within a diverse worldwide collection of 160 grass pea accessions, elucidate the genetic basis of resistance through genome-wide association studies (GWAS), and characterize underlying resistance mechanisms. Resistance was quantified under controlled conditions using infested area (%IA) at 7 days and plant damage (Dam) at 10 days after infestation. Substantial phenotypic variation was observed, with %IA ranging from 0 to 20 and Dam from 0 to 93. GWAS with 208,317 SNPs and four models (MLM, MLMM, BLINK, and FarmCPU) revealed 6 SNPs significantly associated with resistance traits, with MLMM and BLINK providing the best model fit. Candidate genes were identified based on physical proximity and linkage disequilibrium. Choice and no-choice assays using contrasting accessions indicated that both antibiosis and antixenosis contribute to resistance, indicating the presence of multiple defence mechanisms. The identified loci and phenotypically validated accessions constitute valuable resources for grass pea breeding and provide a foundation for comparative genomics with pea, facilitating the development of durable aphid-resistant varieties in both crops.

Keywords: partial resistance, genome-wide association study, plant-insect interactions, antibiosis, antixenosis

INTERACTIONS BETWEEN FABA BEAN AND ITS *BOTRYTIS* PATHOGENS

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Chocolate spot, caused by *Botrytis* species, is the major disease of faba bean worldwide. Use of fungicide is the only way to manage the disease, and genetic resistance would be a more sustainable. The disease-causing efficacy of 21 isolates of six *Botrytis* species was investigated. The isolates were grown for 14 days on half-strength PDA media treated with NUV light to induce sporulation. Spores were suspended to 4×10^6 ml⁻¹ and inoculated onto detached, expanded leaves of genotypes 'Kontu', 'Mélodie/2' and 'ILB 938/2'. *B. fabae* 19B053-4 was the first isolate to cause water-soaked black necrosis and produced the largest lesions, followed by *B. fabiopsis* 19B175. Kontu was the most susceptible one, followed by Mélodie/2, then ILB 938/2. The most virulent isolate of each of the four main species was chosen for further work. An existing, genotyped population of 165 RILs from the cross ILB 938/2 × Mélodie/2 was used to identify QTLs for response to the four isolates, using the detached-leaf method. Six QTLs associated with the infection responses to the four *Botrytis* isolates were detected across Chr1, 4, and 6. The strongest signals for all four *Botrytis* species were located on LG1, where q-BF1 (*B. fabae*), q-BC1 (*B. cinerea*), q-BPC1 (*B. pseudocinerea*), and q-BFsis1 (*B. fabiopsis*) with LOD scores ranging from 3.66 to 4.79 and R² values 8.5% and 10.8%. Two additional QTLs for *B. fabae* response were identified on LG4 and LG6. Furthermore, NEP marker analysis displayed two main clusters and four distinct subclusters of the *Botrytis* species.

Keywords: chocolate spot, *Botrytis species*, QTL, gene mapping, candidate gene, disease resistance

DEVELOPMENT OF ADVANCED LENTIL LINES WITH PARTIAL RESISTANCE AGAINST *COLLETOTRICHUM LENTIS* (RACE 0) CAUSING ANTHRACNOSE

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Colletotrichum lentis Damm is a hemibiotrophic fungal pathogen that causes anthracnose, which is the primary foliar disease of lentil (*Lens culinaris*) in Canada, reported from 74% to 92% of surveyed lentil fields (2018 – 2019). Under favourable conditions, anthracnose can cause up to 100% yield loss, particularly in regions with short crop rotations, high rainfall, and warm temperatures. The virulent race 0 dominates the pathogen population in Canada, and high partial resistance to *C. lentis* race 0 was identified in the wild relative *L. ervoides* but not *L. culinaris*. Efforts were previously made to transfer this resistance into *L. culinaris*, but were met with limited success due to linkage drag. In a renewed effort to transfer five resistance QTLs from two *L. ervoides* lines to *L. culinaris*, interspecific crosses were made, and embryo rescue procedures were adapted and improved. Crossing with elite lentil lines is ongoing, which will be followed by backcrossing complemented with foreground selection for the five anthracnose resistance QTLs and extensive background selection for favourable *L. culinaris* loci. To explore whether other anthracnose resistance loci are present in the *L. ervoides* gene pool, 130 *L. ervoides* lines with phenotypic data were tested for the presence of the five known resistance QTLs and will be further assessed through a Genome-Wide Association Study. The identification of additional anthracnose resistance loci is desirable to ensure the durability of resistance through the pyramiding of resistance loci into elite *L. culinaris* lines.

Keywords: interspecific hybrids, *Lens ervoides*, GWAs, molecular markers

INTEGRATING FIELD MANAGEMENT AND PHENOTYPIC INSIGHTS TO ENHANCE RESILIENCE AND PRODUCTIVITY IN MEDITERRANEAN LEGUMES

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Grain legumes are central to sustainable Mediterranean cropping systems, yet their productivity is constrained by weed competition and environmental stress. We conducted field experiments during two growing seasons to evaluate weed management strategies and competitive ability in four grain legume crops — vetch, pea, faba bean, and white lupine — using a randomized complete block design with three replicates. Nine selective (PRE) and (POST) herbicide treatments (PRE: aclonifen, pendimethalin + clomazone, metribuzin + clomazone, benfluralin, terbuthylazine + pendimethalin, S metolachlor + pendimethalin, flumioxazin; POST: pyridate, imazamox) were applied alongside untreated controls to assess effects on crop phytotoxicity, dry matter accumulation, and yield. Results demonstrated differential herbicide efficacy and crop responses across species. Flumioxazin provided the highest overall weed control and lowest weed biomass, whereas benfluralin consistently resulted in the highest grain yields across all four crops in both seasons. Some treatments caused low to moderate transient phytotoxicity (notably in vetch and peas). Competitive responses varied by crop: vetch exhibited strong early competitive ability and lower relative yield loss, followed by faba bean and lupine, with peas showing the greatest yield reduction under weed pressure. These findings provide practical guidance for integrated weed management. Building on these field insights, ongoing controlled environment studies in diverse *Lathyrus* spp. genotypes are assessing root architecture and stress physiology under salt and drought to begin linking mechanistic tolerance traits with field performance. This work underscores a continuum from field management to physiological phenotyping, advancing the understanding to support resilient legume cropping systems.

Keywords: salt tolerance, drought tolerance, minor legumes

ELUSIVE YET REDUNDANT: NEW DEVELOPMENTS TOWARD IDENTIFICATION OF RESISTANCE GENES TO *PHYTOPHTHORA SOJAE* (RPS) IN SOYBEAN

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Exploiting genetic resistance to reduce yield losses due to diseases offers a formidable opportunity to improve the sustainability of our agriculture. Most plant species have developed an arsenal of resistance genes against hemi- and biotrophic pathogens that can be exploited for that purpose. The relationship between *Phytophthora sojae* and its host, soybean, is a typical example of the gene-for-gene model where resistance genes to *P. sojae* (Rps) allow recognition of effector proteins (Avr) from the pathogen. In recent years, field surveys conducted around the world have exposed a loss of efficacy from the few commercially available Rps genes. Indeed, the two most deployed genes, Rps1a and Rps1c, are practically circumvented wherever soybean is grown. This situation could hamper efforts to protect soybean production against *P. sojae*. Given that as many as 45 Rps genes have been reported, one could argue for the abundance of resistance sources, but only two of these genes have been functionally validated while the identity and practical use of the others remain obscure. In this research, we have successfully characterized soybean resistance genes to *P. sojae* with state-of-the-art NLR gene capture, cell death assay and root transformation. We have also uncovered unsuspected redundancy in the reported Rps genes, which indicates that there may be fewer sources of resistance than assumed and should instill caution in using current Rps genes to ensure sustainable management of *Phytophthora* root rot.

Keywords: soybean, *Phytophthora sojae*, Rps, Avr, NLR

UNCOVERING IN-FIELD DYNAMICS, FITNESS AND HOST-MEDIATED ADAPTATION OF A CLONAL *ASCOCHYTA RABIEI* FIELD POPULATION

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Ascochyta rabiei is a major fungal disease of chickpea (*Cicer arietinum*), which imposes a significant biotic constraint to global production. The Australian *A. rabiei* population is clonal with extremely low genetic diversity, composed of a single mating type. Despite this, aggressive isolates have become more frequent, rapidly overcoming existing host resistances. However, the interactions of the isolates with host cultivars, and their fitness and adaptation dynamics and persistence remain largely unstudied. To address this, a field-based micro-evolution/adaptation experiment was conducted, where a community of isolates comprised of six *A. rabiei* genotypes with varying pathogenicity levels was released then re-captured after three years of continuous cropping on a range of chickpea cultivars of varying resistance/susceptibility. After three cropping seasons, 304 re-captured isolates were sequenced and classified into nine distinct multi-locus haplotypes (MLH) using discriminant analysis of principal components (DAPC). Six MLH 'founder groups' aligned with the initially released experimental population in addition to three "wild-type" introductions. Recaptured isolates were then assessed and compared for relative fitness, adaptation indices and genomic mutational hotspots, identifying host-mediated adaptation and selection, with no clear correlation between pathogenicity level and host resistance rating. These findings provide new insights into the field dynamics, fitness and adaptation potential of clonal *A. rabiei* and present a novel workflow for detecting adaptation in low diversity, clonal fungal populations where other genetic markers are unknown or lacking sufficient resolution. This highlights the necessity of maintaining pathogen population surveillance and collaboration between chickpea breeders and pathologists to maintain durability in commercial cultivars.

Keywords: *Ascochyta*, chickpea, adaptation, genomics

GENETIC DISSECTION OF RESISTANCE TO THE PEA APHID IN PISUM SPP.: IDENTIFICATION OF NOVEL RESISTANCE SOURCES AND ASSOCIATED GENOMIC REGIONS

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Pea (*Pisum sativum* L.) production is seriously constrained by biotic stresses such as the pea aphid (*Acyrtosiphon pisum*). Given the economic and environmental impacts associated with insecticide use, identifying sources of genetic resistance has become a priority. Attempts have been undertaken to evaluate the natural diversity of aphid resistance in pea; however, its effective utilization in breeding remains constrained because limited resistance accessions have been released, and little is known so far about the genetic architecture controlling *A. pisum* resistance. To overcome this limitation, resistance was evaluated in a collection of 322 *Pisum* spp. accessions under field conditions across four agricultural seasons (2021-2025) and under controlled conditions. Infestation severity traits and agronomic traits were recorded, and BLUPs (best linear unbiased predictors) of severity relative to the control were estimated using linear mixed models to integrate information across environments. Accessions with consistently low infestation levels were identified, including also some pea wild relatives. A genome-wide association study (GWAS) was conducted: a number of significant trait–marker associations were identified using 35,790 polymorphic markers, of which 24,279 were SilicoDART and 11,511 were SNPs. Candidate genes were identified based on physical proximity to significant markers (± 20 -30 kb windows) and/or linkage disequilibrium ($r^2 > 0.5$), and included genes associated with key processes in the plant-aphid interaction, such as protein turnover and regulation (proteasome and RING/F-box E3 ligases), transcriptional regulation (MYB domains), solute transport, cell-wall remodelling, and stress/detoxification responses, including glutathione S-transferases (GST). This study establishes a basis for future functional validation and genomic selection methodologies.

Keywords: *Pisum*, pea aphid, resistance characterization, GWAS analysis

PLANT AND APHID GENOTYPES MODULATE LEGUME RHIZOBIUM-INDUCED DEFENSE AGAINST APHIDS

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This work examines how genetic variation in plants and aphids influences the protective effects of nitrogen-fixing symbiosis (NFS) against herbivores. Using two genotypes of *Medicago truncatula* and different clonal lines of the pea aphid (*Acyrtosiphon pisum*), researchers compared plants inoculated with *Sinorhizobium meliloti* to those supplied with nitrate. Results show that aphid performance depends on both plant and aphid genotypes and is further affected by rhizobial inoculation. Plant defence responses—particularly those linked to the jasmonic acid (JA) and salicylic acid (SA) pathways—varied according to specific plant–aphid genotype combinations and were significantly influenced by the presence of rhizobia. Both defence pathways appear to regulate aphid growth, likely through distinct or complementary mechanisms. Overall, the findings demonstrate that interactions between plant genotype, aphid genotype, and rhizobial symbiosis ($G \times G \times R$) play a key role in shaping plant–aphid dynamics. This highlights the importance of genetic context and beneficial microbes in multitrophic interactions and suggests that optimizing plant–microbe associations could enhance pest resistance in sustainable agriculture.

Keywords: pea aphid (*Acyrtosiphon pisum*), *Medicago truncatula*, genotype, symbiosis, rhizobium, nitrogen fixation, plant defence

MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH *APHANOMYCES EUTEICHES* AND *FUSARIUM AVENACEUM* ROOT ROT RESISTANCE IN AN INTERSPECIFIC *LENS ORIENTALIS* AND *LENS CULINARIS* MULTI-PARENT POPULATION

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Root rot is a serious issue for lentil (*Lens culinaris*) production in Canada, causing an estimated loss of \$1.5 billion a year. The two most common causal pathogens are *Aphanomyces euteiches* and *Fusarium* spp., including *F. avenaceum*. There are currently limited options for farmers as there are no effective seed treatments or resistant cultivars available. Lentil growers are instead encouraged to forgo lentils for 10 years for fields significantly infected with *A. euteiches*. Breeding has proven difficult due to the multi-genic nature of the trait. Current studies have had limited success in identifying resistance in *L. culinaris*; however, resistance has been noted in the closely related *Lens orientalis*. A multi-parent advanced generation intercross (MAGIC) population was created for this study, which includes four *L. orientalis* *Aphanomyces* root rot or *Fusarium avenaceum* root rot- resistant parents and four *L. culinaris* susceptible parents. The MAGIC population is being assessed for both diseases in a growth chamber. After phenotyping, quantitative trait loci (QTL) mapping analyses and genome wide association studies (GWAS) will be completed to identify significant QTLs associated with *Fusarium avenaceum* and *Aphanomyces* root rot resistance. Using these identified QTLs, PCR-markers will be designed. A second MAGIC population is under development and was created using four different *L. orientalis* lines as resistance sources. This population will be used to validate the effectiveness of the PCR-markers. These markers will be the foundation for a marker-assisted breeding platform for root rot resistance breeding in lentil.

Keywords: lentil, resistance, MAGIC, root-rot

CHARACTERIZATION OF THE LENTIL DIVERSITY PANEL FOR RESISTANCE TO FUSARIUM ROOT ROT

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Lentil (*Lens culinaris*) is a major pulse crop grown in Saskatchewan, but its production is increasingly threatened by root rot, primarily caused by *Aphanomyces euteiches* and numerous *Fusarium* species (e.g. *F. avenaceum*, *F. solani*, *F. oxysporum*). These pathogens can lead to significant yield losses by harming root development, resulting in poor emergence, stunted growth, and premature plant death. Currently, the main management practice involves extended crop rotations (6-8 years) to reduce pathogen buildup. However, this limits the frequency of lentil cultivation and presents economic challenges within the industry. This study investigated the genetic basis of the resistance to *Fusarium* root rot using a diverse lentil germplasm and explored the association of root system architecture traits with this resistance. The Lentil Diversity Panel was evaluated for *Fusarium* root rot resistance in field trials and root system architecture was evaluated in growth pouches under controlled conditions. Variation in *Fusarium* root rot resistance was observed among genotypes. A broad range of architectural differences was also observed among these individuals. Analysis of data of both traits will clarify any associations between the two and identify genomic regions linked to both root system architecture and *Fusarium* root rot resistance. These findings will contribute to breeding efforts aimed at developing resistant cultivars, ultimately improving yield, and sustainability in the Saskatchewan pulse industry.

Keywords: root system architecture

TRANSCRIPTOMIC AND METABOLOMIC ANALYSIS REVEALS THAT NITROGEN-FIXING SYMBIOSIS INDUCES VARIABLE DEFENCE AGAINST APHIDS IN DIFFERENT PEA GENOTYPES

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In the context of reducing chemical inputs, enhancing beneficial biological interactions to sustain crop production is a cornerstone of agroecological strategies. The legume–rhizobium symbiosis enables plants to acquire atmospheric nitrogen and contributes to the nitrogen cycle without synthetic fertilizers. Beyond its established role in nitrogen fixation, this interaction may also activate systemic resistance responses, allowing legumes to mount faster and more efficient defenses against pests, a phenomenon referred to as symbiotic priming of plant defenses. Our project aims to analyze and optimize this ecological service in spring and winter pea (*Pisum sativum*) genotypes associated with *Rhizobium leguminosarum symbiovar viciae* (Rlv) in response to pea aphid (*Acyrtosiphon pisum*) infestation. Transcriptomic and metabolomic analyses were conducted to identify molecular and metabolic markers linked to defense activation in this tripartite interaction, by comparing symbiotic and non-symbiotic plants, either infested or not with aphids. Among the 1,500 metabolites analyzed, 50 accumulated significantly in inoculated plants compared with non-inoculated controls, in spring peas, winter peas, or both. These included precursors of phenylpropanoids, alkaloids, and phytoalexins, such as tryptophan and glutamic acid. Secondary metabolites were also enhanced, including phenylpropanoids, flavonoids, alkaloids, coumarins, and iridoids, compounds directly or indirectly involved in anti-herbivore defense and broader stress responses. The results reveal genotype-dependent variation in metabolomic responses and show that Rlv-induced defenses correlate with reduced aphid performance, affecting growth and fertility. Ongoing transcriptomic analyses will further clarify the regulatory mechanisms underlying this symbiosis-driven priming effect.

Keywords: defence priming, rhizobial symbiosis, aphids, metabolomics, transcriptomics, agroecology.

GENOME-WIDE ASSOCIATION STUDIES IDENTIFY DISTINCT CBA BEAN LOCI CONTRIBUTING TO RESISTANCE TO *ACYRTHOSIPHON PISUM* AND *APHIS FABAE*

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Faba bean (*Vicia faba* L) is a legume crop crucial for the transition towards a more sustainable agriculture through lower nitrogen input and more diversified crop rotations. However, its cultivation remains limited by yield instability and insect pests, of which, aphids are among the most damaging. They provoke direct damage through feeding and indirect damage through transmission of plant viruses. As *Acyrtosiphon pisum* and *Aphis fabae* are frequently encountered on faba bean across Europe, this study aimed to identify sources of resistance to *A. pisum* and *A. fabae* and investigate their genetic determinism, as well as comparing the common and distinct genomic regions involved resistance mechanisms against the two aphid species. A panel of 248 faba bean genotypes was screened using aphid fecundity as a proxy for resistance. Partial resistance was observed against both aphid species; however, no correlation was found between resistance levels to the two aphids, suggesting distinct genetic determinisms. Genome-wide association studies (GWAS) using faba bean exome-capture sequencing data identified four quantitative trait loci (QTL) associated with resistance to *A. pisum* and four distinct QTL associated with resistance to *A. fabae*. For several loci, linkage disequilibrium blocks significantly associated with resistance were defined. Finally, candidate genes underlying each QTL were identified. This study lays the foundation for the functional characterization of genes involved in faba bean defense against aphid species and represents a step toward the development of aphid-resistant cultivars.

Keywords: *Vicia faba*, *Acyrtosiphon pisum*, *Aphis fabae*, GWAS, resistance

REFERENCE GENOME ASSEMBLY FOR *ASCOCHYTA LENTIS* VAR. *LATHYRI*, THE *ASCOCHYTA* BLIGHT PATHOGEN OF *LATHYRUS*

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Lathyrus or Grass pea (*Lathyrus sativus*) is a temperate crop that has the potential to address global food security and environmental sustainability worldwide. It is used as a sustainable rotational break crop for controlling cereal diseases. However, grass pea can be affected by fungal diseases such as Ascochyta blight (AB). The primary causal agent of Ascochyta blight in grass pea, *Ascochyta lentis* var. *lathyri*, infects all above-ground parts of the plant, resulting in reduced grain quality and yield. Genomic resources for different *Ascochyta* species are available; however, no reference genome has previously been reported for *A. lentis* var. *lathyri*. Here, we present a high quality, near complete genome assembly of *A. lentis* var. *lathyri* (isolate ER1819) generated using Oxford Nanopore long read sequencing. The assembly comprises 23 telomere to telomere chromosomes, a complete mitochondrial genome, and a linear mitochondrial plasmid, providing a contiguous reference suitable for comparative and structural analyses. The reference genome is 42 MB in size and encodes 11,486 predicted genes. Comparative analysis with published *A. lentis* genomes reveals overall synteny, while also identifying structural rearrangements, including translocations, that distinguish *A. lentis* var. *lathyri* from closely related taxa. Notably, the avirulence gene *AlAvr1*, which is conserved across all examined *A. lentis* isolates, is absent from this genome, suggesting potential evidence for the differences in host–pathogen interactions between the two pathogens. Together, this genome provides a foundational resource for evolutionary, pathogenicity, and host specificity studies in the *Ascochyta lentis* species complex.

Keywords: *Lathyrus*, Ascochyta blight, reference genome

DECIPHERING GENETIC ELEMENTS INVOLVED IN PATHOGENICITY AND VIRULENCE OF *FUSARIUM* STRAINS ON PEA USING COMPARATIVE GENOMICS AND PHENOTYPIC SCREENINGS

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Pea root rot and wilt disease caused by members of the *Fusarium oxysporum* and *Neocosmospora solani* species complexes are devastating diseases in pea that have the potential to cause complete yield losses. Due to an increasing demand for peas and pea-based proteins there is growing interest in identifying and understanding disease resistances in pea. To investigate genetic and phenotypic diversity of pea-infecting *Fusarium* spp., 48 strains were collected from northern European countries, of which 43 were whole-genome sequenced using Illumina short read technology. We used this sequencing data to create de novo genome assemblies and annotations. Phylogenetic analyses of the *tef1* and *rpb2* genes identified 34 strains to be *F. oxysporum*, 7 as *N. solani* and 3 as *Fusarium* spp. Currently, more detailed analyses of the genetic structure and gene content in the *F. oxysporum* strains are underway. Additionally, the strain collection was phenotypically characterized for growth rate and virulence on the susceptible pea variety Linnea under greenhouse conditions. The results show significant ($P < 0.01$) differences in virulence between strains. Combining data on genetic structure with phenotypic differences enable us to address questions regarding potential formae speciales and identification of virulence factors. Strains of *F. oxysporum* with high vs. low virulence were identified and will be used to screen at least 100 pea genotypes where genome-wide single nucleotide polymorphism datasets are available. This will allow us to identify genetic markers segregating for disease resistance against fusarium wilt in pea that can be used for future marker-assisted selection programmes.

Keywords: pea, root rot, *Fusarium*

ECTOPARASITIC NEMATODES: IMPACTS AND REPRODUCTION ON CHICKPEA

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Chickpea are an important pulse crop in many parts of the world. This crop is known to be impacted by endoparasitic nematodes that internally colonize chickpea roots, leading to cysts, galls or lesions. However, the effects of ectoparasitic nematodes, which live in the soil and feed on the root tissue, are less well known. Beginning in 2019, symptoms of unknown cause(s) have been documented in chickpea in Saskatchewan. This issue is sometimes referred to as the chickpea emerging health issue. Two genera of nematodes that are generally considered ectoparasitic – *Paratylenchus* (pin nematodes) and *Helicotylenchus* (spiral nematodes) – may contribute to the chickpea emerging health issue. To explore the chickpea-ectoparasitic nematode relationship, field surveys as well as greenhouse and lab experiments have been undertaken. A wide range of abundance and proportions of pin and spiral nematodes have been detected in surveys of soils in which chickpea have been grown in Saskatchewan, Canada, from 2020 to 2025. In greenhouse trials with nematode-infested field soil, pin nematodes have been shown to reproduce on multiple chickpea varieties, as well as crops frequently grown in rotation with chickpea: mustard, wheat, pea and lentil. Spiral nematodes impact the chickpea metabolome and may increase chickpea susceptibility to drought. Neither spiral nor pin nematodes consistently induce the symptoms the chickpea emerging health issue, but may contribute to it, in conjunction with drought or other factors. Assessment of the reproduction of spiral nematodes on multiple chickpea varieties is ongoing.

Keywords: chickpea, nematodes, field surveys, drought, metabolomics

ASSESSING RED CLOVER TOLERANCE TO INSECT HERBIVORY: LINKING BIOTIC STRESS AND YIELD STABILITY IN A PERENNIAL LEGUME

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Red clover (*Trifolium pratense* L.) is an important perennial legume that supports soil fertility, nitrogen fixation, and pollinator communities in northern temperate agroecosystems. Despite these benefits, its seed production is increasingly threatened by biotic stress, particularly herbivory by the lesser clover leaf weevil (*Hypera nigrirostris*). Larval feeding on vegetative and reproductive tissues reduces plant vigour, delays flowering, and diminishes seed yield. As seed formation is entirely dependent on insect pollination, damage to floral structures not only reduces seed set directly but may also deter pollinator visitation, compounding yield loss. Chemical insecticides are presently the primary control option; however, their repeated use poses risks to pollinators essential for red clover reproduction and may contribute to the development of insecticide resistance. To address these challenges, two years of field trials were conducted across multiple sites in Saskatchewan, Canada. Larval density-yield relationships were used to establish economic injury levels and thresholds, representing a functional measure of the crop's tolerance to herbivory. By quantifying red clover's ability to withstand pest pressure while maintaining yield and pollination services, this work advances understanding of legume tolerance to biotic stress and supports the development of integrated management strategies that protect both productivity and ecological resilience.

Keywords: *Trifolium pratense*, biotic stress, *Hypera nigrirostris*, integrated pest management, yield loss

INTEGRATED APPROACH TO STUDY LEGUME FATIGUE IN PEA: PATHOGENS, SOIL MICROBIOME, AND RESISTANCE SOURCES

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Legume fatigue is considered one of the main causes of yield instability and reduced profitability in grain legume production and is becoming increasingly relevant with the expansion of legume-based cropping systems. It is caused by a complex interaction of biotic and abiotic factors, with soil-borne fungal pathogens such as *Fusarium* spp., *Aphanomyces* spp., *Didymella* spp., and *Phoma* spp. identified as the primary causal agents. To support breeding efforts, a standardized method for testing susceptibility to the major legume fatigue pathogens was developed. The assay simulates natural infection conditions while minimizing confounding environmental factors, such as a complex soil microbiome or waterlogging, thereby enabling robust and reproducible phenotyping. Initial screening of more than 90 accessions with *Fusarium oxysporum* resulted in the identification of 15 genotypes with promising levels of tolerance, which are currently being further evaluated to determine their resistance spectrum against additional pathogens (*Fusarium solani* and *Aphanomyces* spp.). In parallel, field provocation trials for legume fatigue are being established over a three-year period to evaluate selected genotypes under field conditions. Disease development will be assessed using conventional phenotyping approaches and by drone-based multispectral reflectance measurements. Pathogen complexes will be characterized using DNA-based sequencing (ITS) of root samples, while repeated soil sampling will be used to analysis the soil microbiome and to identify additional factors influencing disease severity. The project aims to elucidate the pathogen-driven mechanisms underlying legume fatigue and to identify stable sources of tolerance or resistance, providing a foundation for the breeding of more resilient pea cultivars.

Keywords: legume fatigue, resistance, soil-borne pathogens

PATHOTYPE SHIFTS IN *ASCOCHYTA LENTIS* AND IMPLICATIONS FOR LENTIL RESISTANCE IN SOUTH AUSTRALIA

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Ascochyta lentis causes ascochyta blight (AB) of lentil, a disease of global significance to lentil production. In South Australia (SA), widespread adoption of AB-resistant cultivars over the past two decades has imposed strong selection pressure on pathogen populations, contributing to shifts in aggressiveness and pathotype structure. Historically, isolates virulent on Nipper were classified as Pathotype -1, while those virulent on PBA Hurricane XT were designated Pathotype-2. The increased adoption of GIA Thunder (released in 2022) has coincided with elevated AB severity in commercial paddocks. To investigate potential changes in pathogen virulence, a differential cultivar set was inoculated with 20 isolates collected from multiple SA cropping regions in 2025. All isolates caused substantial disease on PBA Hurricane XT confirming the dominance of Pathotype-2 in the region since its first detection in 2016. Furthermore, all isolates tested also exhibited virulence on Nipper, demonstrating dual virulence. This contrasts with historical isolates, which were typically virulent on either Nipper or PBA Hurricane XT, but not both. These results indicate a shift in the evolutionary dynamics of *A. lentis* populations in SA. The re-emergence of Nipper virulence suggests that genetic recombination between compatible mating types of Pathotypes-1 and 2, rather than contemporary selection pressure alone given GIA Thunders resistance to AB is derivative of PBA Hurricane XT but not from Nipper. The detection of dual-virulence isolates highlights the pathogen's capacity to accumulate multiple virulence genes, reinforcing the need for resistance gene pyramiding, continued pathotype surveillance, and strategic varietal diversification to support durable AB management.

Keywords: ascochyta blight, pathogen virulence, resistance, population dynamics

ASSESSING VIRULENCE AND PATHOGENICITY OF MACROPHOMINA PHASEOLINA ISOLATES ON DRY BEAN (*PHASEOLUS VULGARIS*) UNDER CONTROLLED CONDITIONS IN WESTERN CANADA

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Charcoal rot, caused by *Macrophomina phaseolina*, is an emerging threat to dry bean production in Manitoba, Canada. Six *M. phaseolina* isolates from a 2025 dry bean disease survey were evaluated for pathogenicity and relative virulence on the popular navy bean cultivar T9905. Isolate identities were confirmed based on morphology and DNA sequencing of the rDNA internal transcribed spacer (ITS) region and partial calmodulin (CAL) and translation elongation factor 1-alpha (TEF-1α) genes. Five seeds were sown per pot, and four pots per treatment were used for inoculated (approximately 3×10^5 microsclerotia per L of potting mix) and mock-inoculated control treatments, with four replications. Pots were maintained in a walk-in growth cabinet at 27/20°C (day/night) with a 16 h photoperiod. Disease impact was assessed using emergence and stand establishment at 7 and 15 days after seeding, respectively. At early flowering, plant growth parameters (shoot length, root length, shoot dry weight, and root dry weight) were recorded, and root disease severity was rated on a 0-9 scale (0 = no disease; 9 = plant death). All isolates caused reductions in early growth and stand parameters compared with the control, including up to 66% lower 7-day emergence. Differences in virulence were observed among isolates recovered from regions with varying dry bean production histories. Trials are planned for 2026 to validate isolate virulence under Manitoba field conditions. Understanding virulence diversity in *M. phaseolina* populations will contribute to development of integrated management strategies for charcoal rot in Manitoba, including disease screening of dry bean varieties.

Keywords: charcoal rot, dry edible bean, virulence

GENETIC MAPPING OF RESISTANCE TO CERCOSPORA LEAF SPOT IN FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.)

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Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume grown on approximately 3000 acres in Saskatchewan. Historically, the plant was used for spice, medicine, and animal feed. Interest in fenugreek as another pulse crop option in Western Canada has increased recently due to the plant's tolerance of dry conditions and for being a non-host of *Aphanomyces* root rot which plagues other pulse crops. *Cercospora* leaf spot caused by the fungal pathogen *Cercospora traversiana* is the major foliar disease affecting fenugreek production. The pathogen causes foliar lesions that can rapidly spread between plants resulting in defoliation and decreases in seed yield and quality. The identification and subsequent development of a resistance marker is of high importance within the context of breeding efforts. This marker will allow breeders to rapidly screen early generation material for resistance without the need for disease nurseries, saving time and resources. To identify a resistance marker, a genome wide association study (GWAS) will be conducted using 180 fenugreek accessions located at Plant Gene Resources of Canada. This collection contains seed collected from the Mediterranean, Middle East, Northern Africa, and North America. The accessions will be sequenced using Illumina sequencing. To obtain phenotypic data, each accession will be inoculated with *Cercospora traversiana* indoors and scored for its susceptibility. GWAS will be conducted on the collected genotypic and phenotypic data to identify regions of the genome that confer disease resistance. These identified regions will provide initial markers for prebreeding efforts to integrate *Cercospora* leaf spot resistance into future fenugreek varieties.

Keywords: fenugreek, legume, pathology, GWAS

DIVERSITY FOR RESISTANCE TO THE BLACK BEAN APHID IN OLD AND RECENT FABA BEAN VARIETIES

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Faba bean (*Vicia faba*) has great potential to contribute to more sustainable agriculture and food systems. However, this species is vulnerable to pests, in particular aphids. In the past, aphids were effectively controlled by pesticides, and the resistance level of faba beans to aphids was not examined. The recent ban on certain pesticides in Europe has increased the demand for faba bean varieties with high aphid resistance. We examined the level of resistance vs. susceptibility of recent faba bean varieties, compared to older varieties, against *Aphis fabae*, the black bean aphid. This generalist pest preferentially attacks legumes and can cause significant yield losses in faba bean crops. We established a panel of 40 faba bean genotypes, comprising 27 French and/or European varieties registered over the last 50 years and 13 control genotypes selected for their resistance to various stresses. A screening was conducted in growth chamber to evaluate the fecundity of *A. fabae* on these genotypes. One-day-old larvae were installed on faba bean, with 15 replicates of one plant per genotype, and their offspring counted. Results revealed a continuum of faba bean responses to infection ranging from partial resistance to high susceptibility. Recent varieties showed no greater resistance than older varieties. Control genotypes exhibited varying levels of resistance or susceptibility. These results suggest that resistance to *A. fabae* has not been a breeding target in recent decades. Further research will be needed to identify new sources and genetic determinants of resistance, for breeding resistant varieties to the black bean aphid.

Keywords: *Vicia faba*, *Aphis fabae*, resistance

INTER- AND INTRASPECIFIC CROSSES FOR THE CHARACTERIZATION OF THE GENETIC DETERMINANTS OF PEA APHID RESISTANCE IN WILD PEAS

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To foster the agroecological transition, legumes, and pea (*Pisum sativum* ssp. *sativum*) in particular, are key species to reduce mineral fertilization and diversify crop rotations. However, cultivation of pea is impaired by insect pests among which the pea aphid (*Acyrtosiphon pisum*) is the most problematic. Wild pea relatives (*Pisum fulvum*, *P. sativum* ssp. *elatius*) exhibit higher levels of pea aphid resistance compared to cultivated pea. Therefore, a better characterization of the genetic determinisms of aphid resistance in wild pea relatives is crucial to develop more resilient pea varieties. We have produced eight interspecific F1 populations between four partially resistant wild pea relatives donors and two sensitive or partially resistant pea cultivar receivers. In addition, four intra- and interspecific F1 populations between *P. fulvum* and *P. elatius* were created. Interspecific F1 plants often showed a slender development, a mosaic foliage and sterile flowers. Post-zygotic incompatibility was also observed with one of the two pea receivers. One interspecific F2 population is currently being described morphologically. Additional wild pea relatives are being evaluated for their level of aphid resistance and potentially find new and diversified resistance alleles. Future work will focus on the creation, by single seed descent, of F6 populations of recombinant inbred lines, in order to explore the genetic and metabolic determinisms of aphid resistance from wild pea relatives. In the long term, introgression of wild pea resistance alleles into cultivated pea should improve the level of resistance to the pea aphid.

Keywords: *Acyrtosiphon pisum*, wild pea relatives, *Pisum sativum*, quantitative resistance

DISCOVERY AND FUNCTIONAL CHARACTERISATION OF NOVEL EFFECTOR GENES IN THE CHICKPEA BLIGHT PATHOGEN *ASCOCHYTA RABIEI*

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Chickpea (*Cicer arietinum*) production is significantly constrained by Ascochyta blight, a major biotic stress caused by the fungus *Ascochyta rabiei*. The emergence of highly pathogenic isolates in Australia has challenged resistance durability and highlights the need to understand the molecular basis of pathogenicity variation within the Australian population. Comparative transcriptomic analysis between high and low pathogenic isolates during early the infection stage identified candidate effector genes associated with host colonisation, immune suppression, and infection structure development. Expression profiling of selected candidates across a broader panel of isolates demonstrated consistent upregulation of PDA1 and FGL1/AGLIP1 in high pathogenic isolates. Four genes were subsequently prioritised for functional association with pathogenicity: PDA1 (EKO05_0001368), FGL1/AGLIP1 (EKO05_0004170), LysM/CDIP1 (EKO05_0010718), and Tom1 (EKO05_0008530). For this, targeted gene disruption constructs were generated using Gibson Assembly and introduced into the highly pathogenic isolate AR0231 via *Agrobacterium tumefaciens*-mediated transformation. Mutants were confirmed by molecular screening and evaluated using detached leaf assays, whole-plant infection assays, and histopathological analyses. Disruption of PDA1 and FGL1/AGLIP1 significantly reduced lesion development and disease severity compared with the wild type, confirming their major contribution to pathogenicity. In contrast, LysM/CDIP1 and Tom1 mutants showed milder or stage-dependent effects on pathogenicity. This study established a functional genomics pipeline in *A. rabiei* and provides direct evidence linking transcriptome-derived candidates to pathogenicity phenotypes, advancing understanding of biotic stress mechanisms in chickpea and informing resistance breeding strategies.

Keywords: chickpea, *Ascochyta rabiei*, pathogenicity, effector genes, functional validation

ELUCIDATING THE MOLECULAR BASIS OF RESISTANCE AGAINST CHOCOLATE SPOT IN FABA BEANS

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Chocolate spot, primarily caused by the necrotrophic fungus *Botrytis fabae*, is the most economically important disease affecting faba beans in Western Canada, particularly under cool and humid conditions. Although quantitative resistance to this pathogen has been reported, further characterization, including functional validation of the associated candidate resistance genes, remains limited. Most commercially grown faba bean cultivars remain susceptible, resulting in continued reliance on chemical disease management. To identify and characterize faba bean candidate resistance genes and defense pathways, association mapping and imaging were performed. A faba bean diversity panel comprising 441 lines collected from 32 countries was phenotyped for resistance to BF-5, a moderately virulent isolate collected in central Saskatchewan. Whole-genome sequencing was also performed on the entire association panel to identify SNPs linked to resistance to *B. fabae* through genome-wide association analysis. Initial phenotyping results show variation in resistance to chocolate spot disease across the panel. Disease severity at 2 days post-inoculation ranged from 30% (low disease severity) to 90% (high disease severity), with the intermediate-resistant check IL938/2 exhibiting the lowest mean disease severity of 30%. In addition, a GFP-expressing BF-5 strain was generated to elucidate early stages of infection in the intermediate-resistant ILB938/2, the moderately susceptible Melodie/2, and the highly susceptible Kontu genotype. Microscopy and staining are to be used to detect early infection events, including differences in fungal penetration, host colonization, and defense-related responses.

Keywords: *Botrytis fabae*, chocolate spot, faba bean, *Vicia faba*, disease resistance

RECENT SHIFTS IN FOLIAR DISEASE PATTERNS IN WINTER PEA IN FRANCE

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Foliar diseases are among the major limiting factors for winter pea production in France. Over the past few years, the epidemiological context of winter pea has changed, with variations in disease incidence and severity, as well as the emergence of new pathogens. Until recently, ascochyta blight was the predominant foliar disease affecting winter pea. Bacterial blight, caused by *Pseudomonas syringae*, which had almost disappeared since the late 1980s, re-emerged in 2016 and caused severe outbreaks. The disease has been observed annually since then. In 2020, a *Colletotrichum* species was reported for the first time on winter pea. In 2023 and 2024, foliar diseases caused major yield losses in winter pea crops. A field monitoring network revealed that the main pathogens involved were *Colletotrichum* sp. and *Pseudomonas syringae*. Whether associated with the causal agents of ascochyta blight or not, these pathogens appear to form a highly damaging disease complex.

Keywords: *Pisum sativum*, foliar disease, emerging pathogen, epidemiology

EFFECTS OF MIXED VIRUS INFECTION ON GROWTH, NODULATION, AND VECTOR TRANSMISSION IN PEA (*PISUM SATIVUM*)

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Legumes play a crucial role in sustainable agriculture through biological nitrogen fixation and their contribution to cropping systems. In western Canada, pulse legumes are important in rotations, for the pulse industry, and as an export crop. However, biotic stresses can limit both yield and other functions. The present study investigated the effect of single and mixed-virus infections as a biotic stress to examine their effect on pea (*Pisum sativum* L., cv. CDC Meadow) with respect to the plants' growth and development. In addition to the transmission behavior of the insect vector of those viruses (pea aphid, *Acyrtosiphon pisum*). Plant performance was measured by assessing main characteristics of legume growth and nitrogen uptake, including shoot and root length, dry biomass, plant height, and root nodulation. RT-PCR and RT-qPCR were used to confirm and quantify the presence and accumulation of the viruses. Single-virus infections significantly reduced plant growth compared to non-inoculated and buffer inoculated controls ($p < 0.05$), and infected plants also displayed shorter internodes, deformed leaves and tendrils, decreased biomass, and poor nodulation. Mixed virus infections (PSbMV+ PSV and PSbMV+ PEMV) had more severe symptoms, with significantly lower shoot and root length, stunting, poor nodulation, and a significant decrease in biomass in comparison to control and single-virus infected plants (Tukey HSD, $p < 0.05$). The aphid transmission assay showed effective acquisition and transmission for single and mixed infections. The present study suggests considering complex biotic interactions while making management strategies for improving legume health and performance in a sustainable cropping system.

Keywords: legumes, mixed-virus infection, legume nodulation, plant growth suppression, aphid-mediated transmission

PINPOINTING CANDIDATE GENES IN A QUANTITATIVE TRAIT LOCUS ASSOCIATED WITH RESISTANCE TO BACTERIAL BROWN SPOT IN ADZUKI BEAN

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Bacterial brown spot (BBS) caused by *Pseudomonas syringae* pv. *syringae* van Hall can cause up to 40 % yield loss in the adzuki bean crop. Genetic resistance might be the most effective strategy for managing this disease; however, there are no resistant commercial adzuki varieties. Elucidating the genetic basis for resistance to BBS will accelerate the breeding of commercial resistant cultivars. In this study, a 6 Mbp QTL region associated with BBS resistance was identified on a linkage map constructed from 2,911 filtered SNPs from 105 RILs from a cross between Erimo and a Chinese variety (CV). One hundred and eighty candidate genes were identified within the QTL. Comparative genomics and pathogenicity screening on Erimo and CV were carried out using *P. syringae* pv. *syringae* (Pss27), *P. syringae* pv. *aceris*, and *P. syringae* pv. *dysoxyl*i. Erimo showed more resistance ($p < 0.0001$). Unique virulence factors (VF) were identified in these strains. Pss27 was the most aggressive strain in adzuki and possessed 10 unique VFs. Two of the unique VFs (hopAB1 and hopL1) had a total of seven cognate target genes within the QTL. Of all the target genes, only Ethylene-responsive transcription factor (ERF024) showed polymorphisms between Erimo and CV. Copy number variation polymorphism was observed between Erimo and CV, with two extra copies of ERF024 present in tandem in Erimo. ERF024 was located 20 kbp from a peak QTL marker S02_48822524 and was also the closest candidate gene to S02_48822524. The higher ERF024 copy number is associated with improved resistance in Erimo.

Keywords: adzuki bean, bacterial brown spot, resistance, plant-pathogen interactions, virulence factors

CHARACTERISATION OF GENOTYPIC VARIABILITY OF FABA BEAN RESISTANCE TO BRUCHUS RUFIMANUS BY STUDYING THE NUMBER OF EGGS ON PODS, THE EGG-TO-ADULT RATIO, AND THE SEED INFESTATION RATE

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Faba bean (*Vicia faba*) is an important legume species for cool-season cropping systems due to its high seed protein content (~30 %). The use of faba bean seeds for human consumption is constrained by the damage caused by a seminivorous coleoptera, *Bruchus rufimanus*. Insecticide efficacy is limited, and, in the EU, the range of authorised active substances is shrinking. Genotypic variability of seed infestation has already been described, suggesting potential effects of faba bean genotype on the attractiveness for the pest and on larval mortality in the plant tissues. In this study, a panel of 21 genotypes representative of this variability has been cultivated in different environments in France, over four years, to dissect variation for egg number, egg-to-adult ratio and seed infestation. The number of eggs laid by *B. rufimanus* on the pods and the number of infested seeds have been carefully phenotyped on thirty pods per genotype in each environment. The egg-to-adult ratio brought insightful information on genotype response to the pest. Genotype-by-environment interactions were characterised for the first time for *B. rufimanus* egg number per pod and egg-to-adult ratio. Our findings show that the environmental factor was preponderant in explaining egg number variability while egg-to-adult ratio was mostly explained by the genotypic factor, highlighting the importance of plant genotype effect on larval mortality. The perspective of this work is now to identify the genetic determinants responsible for the resistance to *B. rufimanus*, and this information is expected to be directly used to develop resistant varieties.

Keywords: resistance, genotype-by-environment interaction, seed weevil, *Vicia faba*

GENOTYPE- AND TIME-DEPENDENT TRANSCRIPTOMIC RESPONSE OF WHITE LUPIN (*LUPINUS ALBUS* L.) IN RESPONSE TO COLLETOTRICHUM LUPINI INFECTION

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White lupin (*Lupinus albus* L.) is a promising grain legume, both as a green manure and for its highly nutritious seeds. Nevertheless, challenges in its production hinders the crop reaching its potential. Arguably, the biggest issue is its susceptibility to the ascomycete *Colletotrichum lupini*, the causal agent of anthracnose disease. Following a short biotrophic phase, the pathogen transitions to necrotrophy, and subsequently reproduces via asexual conidia, forming as early as six days post-inoculation. This enables rapid re-infection, potentially resulting in complete yield loss in susceptible cultivars under unfavourable conditions. Previous studies report that anthracnose resistance in white lupin is polygenically controlled, and sources of quantitative resistance are rare within the global germplasm. We hypothesize that in these limited resistant accessions, unique transcriptional reprogramming events occur at critical stages of the rapid infection process. By total RNA sequencing, we performed differential gene expression profiling of white lupin accessions following inoculation with *Colletotrichum lupini*. We compared three accessions representing distinct sources of resistance with two susceptible cultivars and non-inoculated controls across 11 timepoints covering the complete fungal life cycle (from 1 hour to 9 days post-inoculation) under controlled conditions. Pairwise comparisons revealed key differentially expressed genes in specific timepoints and specific resistant accessions consistent with the success of the defence response. In parallel, linear mixed models were applied to identify genes which were constitutively differentially expressed in specific resistant accessions. Funding: National Science Centre, Poland, project OPUS 23 (ID: 2022/45/B/NZ9/01397) and EU Horizon Europe project LiveSeeding (ID: 101059872)

Keywords: white lupin, anthracnose, transcriptomics

DEVELOPMENT AND VALIDATION OF KASP MARKER TO IDENTIFY PEA SEED-BORNE MOSAIC VIRUS RESISTANCE IN LENTIL

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Lentil is a crop of economic importance across the North American Prairies. Infestations of pea aphid, the primary vector of Pea Seed-borne mosaic virus (PSbMV), are predicted to increase in the future across the Canadian Prairies and US Pacific Northwest, intensifying the risk of disease spread and threat to lentil production. Identifying host resistance represents the most practical management strategy for PSbMV. In other legumes, resistance to potyviruses is linked to the host factor eukaryotic translation initiation factor 4E (eIF4E), but in lentil, this mechanism has not been studied to date. This study aims to identify lentil lines with resistance to PSbMV and to develop a molecular marker to support future genotyping and selection. A lentil diversity panel of 324 lines established at the University of Saskatchewan was used for virus inoculation experiments, followed by disease scoring and quantitative PCR assays to quantify viral titers. Inoculation experiments identified several lentil lines exhibiting resistance against the virus, which were then used for Sanger sequencing for the full eIF4E coding region. Analysis of the nucleotide and amino acid sequences of the eIF4E identified SNPs between the susceptible and resistant lines similar to those observed in susceptible and resistant pea lines against PSbMV. A Kompetitive Allele Specific PCR marker was developed by targeting one of the SNPs. Genotyping and phenotypic screening of 80 lines predicted resistance and susceptibility around 80% of the time. Approximately 10% of lines carried the susceptible allele yet remained resistant, suggesting involvement of additional factors contributing to PSbMV resistance.

Keywords: eIF4E; KASP marker, lentil; pea seed-borne mosaic virus

TRANSCRIPTOMIC ANALYSIS OF *PHASEOLUS VULGARIS* L RESPONSES TO *XANTHOMONAS PHASEOLI* PV. *PHASEOLI* INFECTION

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Phaseolus vulgaris L. (common bean) is a crop of significant nutritional and economic value worldwide, yet its productivity is limited by common bacterial blight (CBB), primarily caused by pathogenic strains of *Xanthomonas phaseoli* pv. *phaseoli* (Xpp). A detailed understanding of the early molecular events that differentiate successful pathogenic infections from benign or non-pathogenic interactions is critical for guiding breeding strategies to enhance disease resistance. This study utilized comprehensive RNA-sequencing approaches, including standard RNA-seq to examine transcriptomic reprogramming in *P. vulgaris* following inoculation with either a pathogenic or a non-pathogenic Xpp strain. Global gene expression profiling revealed extensive transcriptional changes in defense perception, immune activation, and cellular stress adaptation. Inoculation with the pathogenic strain led to pronounced modulation of defense-associated gene networks, including those involved in pattern-triggered immunity, signalling cascades, oxidative burst regulation, and secondary metabolism. In contrast, the non-pathogenic strain elicited a markedly weaker transcriptomic response, indicating distinct recognition mechanisms or a lack of effective virulence factor activity. Comparative analyses identified key biological processes and pathways, such as Gene Ontology categories related to immune signalling, hormone-mediated responses, and metabolic reconfiguration, as well as KEGG pathways associated with plant–pathogen interactions. Together, these findings offer an integrated overview of early immune-related transcriptional dynamics in *P. vulgaris* and identify candidate genes and regulatory pathways relevant to CBB resistance. This research provides foundational knowledge to inform the development of disease-resistant bean cultivars and advances understanding of host–microbe interactions in legumes.

Keywords: plant-pathogen interaction, transcriptomics common bean, Common Bacterial Blight

EVALUATION OF A COWPEA MINICORE COLLECTION FOR BRUCHID (*CALLOSOBRUCHUS MACULATUS*) RESISTANCE

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Bruchid resistance is a key trait for cowpea farmers in sub-Saharan Africa. Post-harvest losses caused by the cowpea weevil (*Callosobruchus maculatus*) can reach up to 100 % of the seeds within the first months of storage. This is a main limitation to cowpea production in these regions. While several studies have reportedly identified cowpea germplasm with varying degrees of bruchid resistance, there has been no deployment of the trait in breeding programs. Reasons for this could include the lack of consistency between results from different studies as well the lack of knowledge regarding the genetic control of bruchid resistance, which prevents the use of markers to assist with introgression. The objectives of this work are: (1) to develop an efficient protocol to evaluate bruchid resistance in cowpea; (2) to evaluate a diverse cowpea germplasm collection using the developed protocol; and (3) to identify the loci underlying the variation of the trait as well as the germplasm carrying the beneficial alleles. Here we present the first results obtained from screening a subset of the 368 cowpea accessions belonging to UCR Minicore collection using an optimized protocol. Several cowpea accessions are showing a reduced oviposition on the seeds, while in others, larval or adult development is inhibited within the seeds. The relative frequency of each phenotype will determine the approach used to identify the loci associated to these responses. This project is funded by The Kirkhouse Trust SCIO.

Keywords: cowpea, bruchid resistance, *Callosobruchus maculatus*, post-harvest losses

UNDERSTANDING AND IMPROVING RESISTANCE OF FABA BEANS TO LEGUME FATIGUE PATHOGENS

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Faba bean (*Vicia faba*) is one of the most well adapted legume species to the temperate climates of Central and Northern Europe, however, its yield stability is limited in practice. Legume fatigue, a complex phenomenon typically caused by repeated legume cultivation, leads to accumulation of soil-borne pathogens that cause root and foot diseases. Apart from crop rotation management, there are currently no effective control strategies available. Therefore, enhancing genetic resistance is a sustainable way to improve yield stability. This project aims to identify sources of resistance against legume fatigue and elucidate their genetic determinants to support breeding of more resilient varieties. Genetically diverse faba bean material is assessed under controlled conditions to evaluate its response to soil-borne pathogens. Root system architecture is analyzed using WinRHIZO, to measure traits such as total root length, average root diameter, root surface area, and root volume. Additionally, disease severity is evaluated based on the proportion of necrotic roots, with higher fractions indicating greater root damage. A screening assay has been developed to enable the rapid evaluation of resistance, revealing clear variation among genotypes in their responses to infection. Of 150 accessions examined, differences in disease severity and root morphology were observed and 25 accessions showed substantial symptom reduction. They represent promising candidates for the establishment of segregating populations for genetic analysis of resistance. Systematic evaluation of diverse genetic material will support the development of molecular markers and facilitate breeding of more resilient faba bean varieties, thereby promoting sustainable legume production in temperate agricultural systems.

Keywords: faba beans, resistance, legume fatigue, soil-borne pathogens

APHANOMYCES EUTEICHES CAUSES DISEASE IN *LATHYRUS SATIVUS* WITH A GLOBALLY DIVERSE, POLYGENIC RESISTANCE LANDSCAPE

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Aphanomyces euteiches is a major concern in legume crops with limited efficient methods to its management. Although it has been isolated from a diversity of legume species like pea, lentil, vetch, clover, faba bean, snap bean, and alfalfa, its impact on grass pea (*Lathyrus sativus*) remains unreported. This study aimed to characterize the response of grass pea to *A. euteiches* and to identify genomic regions associated with any potential resistance. A diverse panel of 169 grass pea accessions was inoculated with the *A. euteiches* RB84 strain. Twenty days after inoculation, foliar and root symptoms were evaluated. A wide range of symptoms was observed within the panel, ranging from susceptibility to partial or even complete resistance. Along with these phenotypic data, a 12,974 single nucleotide polymorphism (SNP) dataset was used to perform a genome-wide association study. The association study identified 20 resistance associated SNPs, across five of the seven grass pea chromosomes, suggesting a polygenic nature. In silico analysis identified seven putative candidate genes associated with resistance involved in vesicle trafficking, signal transduction, nucleotide metabolism, fatty-acid metabolism, cell wall synthesis and ethylene signalling. This study reveals for the first time the compatible grass pea x *A. euteiches* interaction, identifying nevertheless sources of resistance and proposing resistance-associated loci with co-located candidate genes for future precision breeding purposes.

Keywords: *Aphanomyces* root rot, grass pea, GWAS, partial and complete resistance

BULKED SEGREGANT ANALYSIS IDENTIFIES A MAJOR GENOMIC REGION FOR ROOT-KNOT NEMATODE RESISTANCE IN COMMON BEAN

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Root-knot nematodes (RKN), particularly *Meloidogyne incognita*, represent a biotic stress limiting common bean (*Phaseolus vulgaris* L.) productivity. Although genetic sources of resistance are available, their deployment is hindered by the quantitative nature of resistance and incomplete characterization of the underlying genomic regions. In this work, we combined phenotyping with a bulked segregant analysis coupled to next-generation sequencing (BSA/QTL-seq) to explore genomic regions associated with resistance to RKN in common bean. An F₂ population derived from a cross between a susceptible Helda-type snap bean breeding line and the resistant cultivar Ouro Negro was evaluated under inoculation conditions. Resistance was assessed using traits related to nematode reproduction, allowing the selection of individuals with contrasting responses. Genome-wide comparisons of allele frequency patterns between resistant and susceptible bulks revealed genomic regions displaying allele-frequency shifts, with a major signal detected on chromosome Pv10. These regions encompass genomic intervals that may be involved in host responses to nematode infection. Preliminary annotation of polymorphic sites within these intervals highlighted genes with roles in plant immunity, including resistance gene analogs (RGAs), although further work will be required to refine candidate genes and validate their contribution to the observed phenotypes. Overall, these results provide a genomic framework for the study of nematode resistance in common bean and illustrate the usefulness of BSA-based approaches for dissecting complex biotic stress traits, while highlighting the need for additional genetic and functional analyses. This work was funded by PID2023-147984OB-I00 and PID2023-151867OB-C31, with technical and scientific support from GENE CROP (IN607A 2024/02; Xunta de Galicia).

Keywords: biotic stress, bulked segregant analysis, QTL-seq, nematode resistance, *Phaseolus vulgaris*

IDENTIFYING GENOMIC DIVERSITY AMONGST *SCLEROTINIA SCLEROTIORUM* ISOLATES: A STEPPING STONE TOWARDS IMPROVED WHITE MOLD BREEDING

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Sclerotinia sclerotiorum (Lib.) de Bary (Ss) causes white mold and can result in near total yield loss in common bean (*Phaseolus vulgaris* L.). Substantial variation in isolate virulence complicates screening, yet most breeding programs rely on a single local isolate without knowledge of its relative virulence. To address this gap, we sequenced the whole genomes of 285 Ss isolates collected from multiple host species across Canada and the northern United States. All isolates were sequenced with short reads and are being phenotyped for virulence on three common bean lines using the seedling straw test. Evolutionary distance and admixture analyses identified six subpopulations. Among the first 100 isolates screened, subpopulation significantly affected virulence: K2 isolates were less virulent (Δ SST = -0.42), whereas K5 isolates were more virulent (Δ SST = +0.71). A strong isolate by bean line interaction further highlighted the complexity of host-pathogen relationships. A preliminary GWAS identified one locus significantly associated with higher virulence (Δ SST = +0.88), and a cluster of near significant SNPs overlapping a gene dense region containing small, effector like hypothetical proteins. A final GWAS will be completed once phenotyping is finished. These results suggest breeders should inoculate with at least one isolate from subpopulations K2 and K5, along with one additional isolate representing the remaining subpopulations, to ensure robust screening for tolerance. Future work includes a multi trait GWAS in common bean to resolve host loci underlying tolerance to each Ss subpopulation. This research will support more effective breeding for white mold resistance, reducing fungicide reliance and mitigating yield losses.

Keywords: *Sclerotinia sclerotiorum*, common bean, genomics, breeding

MULTI-SENSOR PHENOTYPING ENABLES DISCRIMINATION OF DROUGHT RESPONSE STRATEGIES IN COMMON BEAN

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Drought is a major constraint on common bean (*Phaseolus vulgaris* L.) productivity, which is inherently less drought-resistant than other warm season legumes such as cowpea and mungbean. The objective was to characterize physiological and morphological responses to drought using integrated high-throughput phenotyping (HTP) approaches. A panel of 192 bush-type common bean accessions were grown under controlled conditions and exposed to well-watered control and drought. For each genotype, six plants per treatment were evaluated. Phenotyping was performed three times during drought progression (5, 7, and 9 days after treatment initiation), representing different drought severities. Plants were analysed using chlorophyll fluorescence imaging, multispectral imaging and 3D multispectral scanning. Statistical analyses included correlations, exploratory data analysis and multivariate approaches. Differences between control and drought increased with time, indicating progressive stress development. Furthermore, drought progression was accompanied by a shift from morphological adjustments to stronger photochemical responses, including PSII efficiency and energy dissipation. Correlation analysis revealed clustering of chlorophyll fluorescence parameters and multispectral indices, while morphological traits showed weaker associations. Divergent response analysis identified two contrasting response patterns among accessions, representing different physiological strategies under drought. The most discriminating traits were related to PSII functioning and energy dissipation (NPQ, qL, Fq'/Fm' and rETR). Linear discriminant analysis confirmed that separation among response groups increased as drought progressed, with misclassification decreasing from 44.8% at the first, 32.8% at the second and 11.5% at the final measurement. These results demonstrate that HTP enables efficient detection of drought-induced physiological responses and provides a framework for screening large collections of common bean germplasm for drought adaptation.

Keywords: common bean, drought stress, high-throughput phenotyping, chlorophyll fluorescence, multispectral imaging

AN AERIAL VIEW OF NITROGEN UPTAKE AND DEPOSITION IN LENTILS

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The theoretical “nitrogen credit” towards soil nitrogen reserves left by legume crops like Lentil is the result of nitrogen-rich residue left behind post-harvest. The quantity of nitrogen carried over to subsequent crops is therefore determined by the quantity of nitrogen in the residue. Nitrogen taken up by lentils, however, has two potential destinations: residue (where it may be incorporated into the soil) or seed (where it contributes to the quantity of protein). In order to provide maximum benefit to the grower a lentil cultivar should provide sufficient nitrogen to the seed for high protein content while still leaving a sizeable nitrogen credit for use by the following crop. Optimizing nitrogen deposition should therefore be a priority for lentil breeders, but direct selection for this trait is hindered by the costs and labour of tissue analysis. In this presentation we evaluate the relationships between the concentrations of nitrogen in lentil straw and seed as (assessed through tissue analysis) and metrics derived from multispectral unmanned aerial vehicle (UAV) images. Such metrics could be exploited by breeders to select for optimized nitrogen deposition between seed and residue in future cultivars.

Keywords: UAV imaging, digital phenotyping, nitrogen

APPLICATIONS OF DRONE-BASED HIGH-THROUGHPUT PHENOTYPING IN SOYBEAN VARIETY EVALUATION

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The global demand for plant-based proteins is increasing, making the selection of crop varieties a critical decision for both farmers and processors. Variety choice influences yield quantity and quality, input requirements, and environmental impacts. Consequently, variety testing plays a key role in improving legume productivity, although conventional approaches are time-consuming and costly. Recent advances in digital phenotyping aim to improve the efficiency, objectivity, and precision of variety evaluation. Unmanned aerial vehicles (UAVs) enable rapid assessment of large field experiments with high spatial and temporal resolution. In this study, we evaluated protocols for acquiring and analyzing RGB, multispectral, and thermal UAV imagery in official soybean variety trials conducted over three growing seasons (2023–2025) in Switzerland. Traits assessed included plant density, early vigor, leaf area index, canopy temperature as an indicator of drought and heat stress tolerance, plant height, maturity, and grain yield. Plant density, plant height, soil cover, and canopy temperature were estimated accurately and consistently, whereas leaf area and biomass remained more challenging. Based on these results, we developed robust UAV-based protocols for high-throughput data acquisition applicable to variety testing, breeding programs, and field crop research.

Keywords: phenotyping, soybean, RGB, multispectral, thermal

UTILIZING HYPERSPECTRAL IMAGES AND MACHINE LEARNING TO DIFFERENTIATE HYBRID PROGENY IN FABA BEAN

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Though currently a minor crop, faba beans are a promising source of plant-based protein as global diets shift towards more plant-based nutrition. To realise this potential, advances in breeding and cultivation are crucial. To exploit heterosis, faba bean breeding frequently utilises synthetic cultivars, which involves open pollination of inbred lines to produce a mixture of F1 hybrids and self-pollinated offspring. Pure F1 hybrid cultivars are currently unavailable due to unavailability of suitable cytoplasmic male sterility (CMS) systems. Identifying F1 hybrids via seed characteristics, known as the Xenia effect, could change this. The Xenia effect refers to the influence of paternal pollen on seed traits, in faba beans for seed weight and cotyledon cells. In our study, we leveraged the Xenia effect to develop machine learning models that can distinguish between parental and F1 hybrid seeds of biparental synthetic mixtures using hyperspectral imaging. Various algorithms were applied, incorporating Savitzky–Golay filtering and Bayesian hyperparameter optimisation. The models achieved up to 99% accuracy in separating parental components. When hybrid components were included, the F1 score reached 62%, indicating the ability to correctly identify and classify 62% of all hybrid components. While this approach does not yet enable the development of full hybrid cultivars, it paves the way for hybrid-enriched cultivars. These could increase yields, by increasing the proportion of F1 hybrid seeds in synthetic cultivar mixtures. Our study extends understanding of the Xenia effect in faba beans and provides a basis for further research aimed at enhancing breeding methods and productivity.

Keywords: synthetic cultivars, Xenia, breeding

FROM PLATES TO REAL-TIME DATA: A FASTER WORKFLOW FOR SCREENING PLANT-DERIVED ANTIBACTERIAL COMPOUNDS

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Traditional plate counting is a well established method for assessing bacterial growth inhibition, yet it is slow, labour intensive, costly, and generates substantial biological waste. These limitations hinder high throughput screening of natural plant compounds intended to inhibit unwanted bacterial growth. To address this, we examined whether IFC, an impedance flow cytometry–based approach capable of delivering direct bacterial cell counts within minutes, could function as a rapid microbial alternative. IFC provides true cell based growth curves and correlates strongly with plate counts, offering a faster and more reliable readout than turbidity or fluorescence based assays. Method development focused on establishing a screening workflow for plant derived inhibitors. Five shake flask cultures were prepared: one positive control containing only bacteria, and four cultures supplemented with different plant materials. All flasks received identical bacterial inocula and were incubated under the same conditions. IFC measurements were collected every 30 minutes, enabling real time tracking of growth dynamics and comparison of inhibitory effects across plant materials. Because the plant materials contained insoluble particles, they produced considerable signal artefacts that overlapped with bacterial events. This required adjustments to inoculum levels, diluent composition, and gating parameters to distinguish bacterial populations from plant derived interference. Additionally, dose–response experiments were performed on selected plant materials to evaluate concentration dependent inhibitory effects. Preliminary findings reveal clear dose dependent inhibition patterns, positioning IFC as a fast, safe, and cost efficient alternative to traditional plate counting for screening plant based bacterial growth inhibitors.

Keywords: impedance flow cytometry, bacteria growth monitoring, plant based bacterial inhibition, rapid microbial methods

BOOSTING INNOVATION IN EUROPEAN SOYBEAN BREEDING

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Innovation means taking risks. Building on the European Soya Improvement Network led by BOKU, our Soybean Innovation Community (IC) addresses these risks by connecting European researchers with European breeders within the EU-funded Legume Generation project, creating a sustainable framework for pre-competitive, breeder-led research on crop genetic improvement. While soybean yields in Europe are in line with the global average, European cereal yields significantly exceed the global level, leading to a preference for cereal production and the well-known protein deficit. To correct this imbalance and strengthen soybean competitiveness in Europe, targeted public investment in soybean breeding is essential. Our strategic goal is to coordinate breeding activities to optimize both yield and quality, improving soybean competitiveness in European cropping systems. To achieve this goal, breeder and institute data are analyzed comprehensively. Members benefit from joint genotyping and specialized phenotyping trials that provide valuable information about breeding material. We conduct multi-year field trials with modern breeding lines across multiple locations, measuring responses to drought and heat stress, water use efficiency, yield, and protein and oil content. The harvested crop is also examined for parameters relevant to the food industry. We use molecular techniques such as SNP arrays to study genetic diversity. The project also includes innovative phenotyping approaches for legumes, including semi-4D phenotyping, hyperspectral field analysis, and multispectral and thermal drone imaging. These advanced methods are evaluated to support and further advance legume breeding research.

Keywords: soybean; breeding; phenotyping; genotyping

EGYPTIAN FABA BEAN VARIETIES SHOW SUPERIOR ROOT ARCHITECTURE: A NOVEL RHIZOTRON DROUGHT STRESS PROTOCOL WITH AUTOMATED IMAGE ANALYSIS

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Faba bean (*Vicia faba*) faces increasing drought stress under climate change, yet standardized protocols for root phenotyping under progressive water deficit are lacking. Existing rhizotron approaches using pre-dried substrate fail to mimic natural top-down soil drying. We developed an improved rhizotron protocol by installing drainage systems and controlled irrigation to induce gradual drought (~40% field capacity) over four weeks, combined with machine learning-based image analysis for automated root trait extraction. Three Egyptian cultivars (Giza716, Giza843, Sakha4) with documented drought tolerance and three German elite lines (Allison, Tiffany, Trumpet) were compared under control (~60% field capacity) and drought conditions, with weekly field capacity monitoring and non-destructive imaging three times a week. The protocol successfully induced progressive drought, confirmed through reduced biomass, nodulation, and stomatal conductance. Egyptian varieties showed greater aboveground trait reduction under drought but maintained root system architecture—particularly high branching density within stable spatial extent. German varieties exhibited balanced reduction across organs with substantial root contraction under drought. Giza716 demonstrated exceptional performance, maintaining superior root traits under both conditions and exceeding well-watered German cultivars when drought stressed. This validated phenotyping workflow enables high-throughput evaluation of drought-responsive root traits, with Egyptian germplasm offering valuable genetic resources for improving drought resilience in European faba bean breeding programs.

Keywords: abiotic stress, drought, faba bean, image analysis, roots

PHENO-MA HIGH THROUGHPUT PHENOTYPING PLATFORM FOR ADVANCING LEGUME BREEDING UNDER CLIMATE CHANGE

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Climate change is increasing the frequency and intensity of extreme weather events, leading to erratic rainfall, rising temperatures, and growing resource limitations. These challenges highlight the urgent need for innovative agricultural technologies to enhance crop resilience, sustain yields, and support climate adaptation and mitigation strategies. Advances in plant science, combined with cutting-edge diagnostic tools such as high-throughput phenotyping, are accelerating crop improvement and germplasm development pipeline and transforming plant characterization in response to environmental and nutritional stresses. Digital phenotyping approaches enable real-time monitoring of plant performance, helping to unravel complex relationships between genotype, environment, and yield. These tools support faster testing, selection, and development of resilient germplasm adapted to major stresses, including drought, heat, high VPD, pests, diseases, and nutrient deficiencies. Despite these advances, Africa has long lacked large-scale phenotyping infrastructures. The University Mohammed VI Polytechnic (UM6P, Morocco) has established PHENO-MA, a state-of-the-art high-throughput plant phenotyping platform. PHENO-MA enables precise assessment of plant responses under controlled and close-to-field conditions. It integrates (i) an outdoor system combining a fully automated lysimetric system with 1,400 mini-plots for water dynamics and drought simulation, and a mobile phenotyping robot (Phenomobile.v2+) equipped with advanced sensors (LiDAR, RGB, IR, spectrometer), that enable advanced plant measurements, that allow us to assess plant's water budget in response to abiotic stress and nutrients' application, and (ii) an indoor system (PhenoMerzouga.v2) a small-scale lysimetric system for controlled environment experiments and climate scenario simulation. The PHENO-MA platform provides a powerful infrastructure to accelerate crop improvement and develop climate-resilient agricultural solutions.

Keywords: high throughput phenotyping, drought stress, high VPD, PHENO-MA, legumes

COMBINING LIGHT-INDUCED FLUORESCENCE TRANSIENTS, CANOPY TEMPERATURE AND MULTISPECTRAL REFLECTANCE TIME SERIES WITH GENETIC MARKER DATA TO IDENTIFY PHOTOSYNTHETICALLY EFFICIENT PEA GENOTYPES AND ASSOCIATED CAUSAL ALLELES

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Photosynthesis provides the energy basis for crop yield formation and symbiotic processes such as biological nitrogen fixation in legumes. Whereas plant breeders have selected grain legume and cereal varieties that absorb as much as 90% of incoming photosynthetically active radiation (PAR) and partition around 60% of assimilates into grains, whole-season photosynthetic efficiency still rarely exceeds one third of its theoretical maximum. A growing body of evidence suggests that this discrepancy is not caused by a lack of genetic variance associated with photosynthetic efficiency, but rather by the highly dynamic nature of the trait in response to fluctuating environmental covariates such as light intensity and canopy temperature, which makes it exceptionally difficult to phenotype using well-established, but low-throughput gas exchange or pulse amplitude modulation (PAM) chlorophyll fluorescence methods. As we have recently demonstrated, phenotyping throughput can be drastically increased using a light-induced fluorescence transient (LIFT) sensor mounted on an autonomous field robot. However, even this approach does not provide full-season / full-day data coverage, which may be needed to identify photosynthetically efficient genotypes and causal alleles. To achieve this, we combine LIFT-derived photosynthetic efficiency measurements from a two-year field trial with around 200 pea (*Pisum sativum* L.) genotypes with full-season / full-day photosynthetic photon flux rate (PPFR), canopy temperature and multispectral reflectance time series. First, we determine the response of photosynthetic efficiency to PPFR and canopy temperature. Then, we estimate the full-season photosynthetic performance for each genotype and perform a genome-wide association study (GWAS) to identify the underlying alleles.

Keywords: photosynthesis, LIFT, thermal imaging, multispectral imaging, GWAS

NODULENS: A COMPUTER-VISION PIPELINE FOR NODULE DETECTION IN LENTILS

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Nodules are specialized root organs that host the symbiosis between legumes (like lentils) and nitrogen-fixing bacteria. The nitrogen supplied to the legume from the nodules fuels productivity within the growing season and can be incorporated into the soil reserves for subsequent seasons as the legume residue deteriorates. This process represents one of few means of reducing our reliance upon the largest source of greenhouse gas emissions in Canadian agriculture: synthetic nitrogen fertilizer. The number of nodules a genotype is capable of producing is therefore an important metric for evaluating a legume's capacity for nitrogen fixation. While counting nodules manually is effective, it is laborious. Computer-vision methods, which use an AI model to detect and count nodules from images, are far less time and labour intensive. Such methods have been developed for several legume species, but to date none have been developed specifically for lentil. In this poster we detail a computer-vision pipeline for the detection of lentil nodules using YOLOv5 which we are calling "NoduLens". The resulting model exhibited superior performance in counting lentil nodules than the Soybean Nodule Acquisition Pipeline, demonstrating the value of species-specific pipelines.

Keywords: lentil, computer-vision, nodule

QUANTIFICATION OF ROOT BIOMASS IN LUPIN-OAT MIXED INTERCROPPING SYSTEMS BY FTIR SPECTROSCOPY

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Mixed intercropping of legumes and cereals enhances plant protein production and agroecosystem biodiversity, yet belowground interactions, specifically vertical niche differentiation, remain poorly understood. This knowledge gap stems largely from the difficulty of taxonomically classifying fine roots in deeper soil horizons, even after excavation and root washing, as morphological distinctions are insufficient. Fourier transform infrared (FTIR) spectroscopy, combined with chemometric modelling, provides a high-throughput tool, leveraging the ‘fingerprint region’ (600 - 1500 cm⁻¹) to identify and quantify species-specific root proportions based on biochemical composition in root-mixture samples. While successful in pots, FTIR-based quantification is rarely applied in legume-cereal field experiments. In 2025, field trials with pure stands and mixed intercropping of narrow-leaved lupin (*Lupinus angustifolius*) and oat (*Avena sativa*) were established in Bottelare, Belgium and Eselborn, Luxembourg. Cylindrical soil cores were collected to 75 cm depth and subjected to mineral N analysis, flatbed root scanning and FTIR analysis (400 – 4000 cm⁻¹). After cluster analysis of roots from pure stands, two models are compared to quantify species proportions in mixtures: one calibrated on Belgian data and validated against the Eselborn trial, and another using combined data from both sites for calibration and validation. Preliminary results indicate similar total root length density and specific root length for lupin in pure stands and intercropped systems. This suggests that while total investment in root length may be similar, differences in species-specific spatial distribution—to be further clarified by ongoing FTIR analysis—will be key to understanding the vertical niche differentiation.

Keywords: root biomass, mixed intercropping, FTIR spectroscopy, field trials

EFFECTS OF SEED DETERIORATION ON THE PHENOTYPE OF COMMON BEAN SEEDLINGS

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Seed quality is a key factor influencing germination and early plant development. This study examined the effects of long-term storage and visual and physical deterioration of seeds on germination and seedling phenotypic traits of 'Zelenčec', a Croatian common bean (*Phaseolus vulgaris* L.) landrace. The experiment included seven seed quality categories: weevil-damaged, prolonged storage (> 5 years), small-sized, deformed, spotted, small and deformed, and a control group (newly harvested seeds). Each category included 20 seeds. Prior to sowing, seed morphometric traits (length and width) were analysed, followed by assessment of germination performance, including the proportion of normal and abnormal seedlings. Seedlings were analysed using a multispectral 3D scanner to monitor their development, morphology, and physiological status. All reduced-quality seed categories produced weaker seedlings than the control. Germination decreased from 95% in the control to 50% in weevil-damaged seeds, which also exhibited the highest proportion of abnormal seedlings. Reduced seed quality resulted in substantial growth suppression, with decreases of up to approximately 50% in plant height, 46% in leaf area, and 72% in digital biomass relative to control seedlings. Despite reduced growth, seedlings from all seed categories maintained stable NDVI and greenness values, indicating preserved physiological functionality. These findings demonstrate that seed deterioration strongly limits early growth, while multispectral phenotyping provides a sensitive tool for detecting seed quality effects on early plant performance. Ensuring high seed quality is essential for uniform crop establishment and early vigour.

Keywords: germination, landraces, leaf area, vegetation index

AI-DRIVEN HIGH THROUGHPUT NODULE PHENOTYPING TO ACCELERATE IMPROVEMENT OF SYMBIOTIC NITROGEN FIXATION IN MUNGBEAN

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Mungbean (*Vigna radiata*) is an important summer pulse crop in Australia and other countries. Like other legumes, it receives much of its Nitrogen (N) for protein production and growth from symbiotic nitrogen fixation (SNF). SNF takes places in specialized organs called root nodules that accommodate bacteria called rhizobia, which convert atmospheric N₂ to ammonia for plant uptake [1]. SNF in agricultural systems reduces reliance on synthetic N-fertilizers, which account for 1-2% of global energy consumption and contribute to greenhouse gas emissions and environmental pollution [2]. As part of an initiative to improve SNF in Australian pulses, we screened a diverse panel of 316 mungbean genotypes and found that counting nodules is very time-consuming, but a potentially important indicator of SNF potential. To address this bottleneck, we implemented an Artificial Intelligence (AI)-driven approach for high-throughput nodule phenotyping [3]. Pilot experiments were first conducted to optimize growth substrate and root washing to capture high-quality images. Clean roots were scanned, and machine learning algorithms were trained to automatically detect and quantify nodules. A large-scale trial on the mungbean germplasm is underway to generate a comprehensive dataset for AI-driven nodule phenotyping. This data will be used to determine the relationship between AI driven nodule phenotypes and SNF. Furthermore, Genome Wide Association Studies will be carried out to identify genomic regions and optimal haplotypes that control nodulation and SNF. The developed AI-based phenotyping platform presents a scalable and transferable approach applicable to other legume species, accelerating breeding for improved nitrogen fixation and sustainable crop production.

Keywords: symbiotic nitrogen fixation, high throughput nodule phenotyping, artificial Intelligence(AI), mungbean

EFFECT OF MAXIMUM CANOPY COVER DURATION DERIVED FROM UAV IMAGING ON SOYBEAN YIELD

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In the modern era, digital technologies such as unmanned aerial vehicles (UAVs) equipped with powerful sensors offer significant potential for high-throughput phenotyping (HTPP). Various remote sensing tools and techniques have been successfully utilized for trait assessment in numerous crops, providing rapid, accurate, and non-destructive data collection with high spatial and temporal resolution. The aim of this study was to investigate the effect of the duration of maximum canopy cover (CC_{max}), derived from UAV images, on soybean yield. The experiment was conducted in 2020 and 2021 on 206 soybean genotypes grown under both drought-simulated and control conditions. Genotypes were classified as early or late according to their maturity group. In both years, the trials were photographed at identical time points using a multispectral camera mounted on a quadcopter UAV. Canopy cover for each genotype was extracted from the UAV images using vegetation indices (VIs) and a masking procedure. The CC data from both seasons were used to calculate the CC_{max} (number of days with CC above 90%) for each plot. At full maturity, soybean genotypes were harvested, and grain yield was adjusted to 14% moisture content. The relationship between soybean yield and CC_{max} was assessed using a linear mixed model (LMM). Results indicated a positive relationship between the CC_{max} and soybean productivity since genotypes with longer CC_{max} achieved higher yields. This research was supported by the Science Fund of the Republic of Serbia (SoyPredict, No. 6788) and the EU Horizon Europe project BELIS (No. 101081878).

Keywords: soybean, UAV, canopy cover duration, yield, linear mixed model

BELOWGROUND DIVERSITY IN PEA: BRIDGING ROOT PHENOTYPES AND DROUGHT TOLERANCE

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Pea (*Pisum sativum* L) is one the most grown grain legumes but is vulnerable to drought stress. Roots play a key role in water acquisition efficiency, yet below ground diversity remains largely underutilized in breeding programs. This study aimed to phenotype root traits of four modern cultivars and four landraces with known differences in drought tolerance. Plants were grown in Rhizotubes® (4PMI Inrae, Dijon) under well-watered condition and progressive water withdrawal for 4 weeks. We found significant genotypic differences and genotype × condition interaction for most root traits. While some landraces exhibited higher root growth rates under favourable conditions, this pattern was reversed under drought stress. Cross-over interactions occurred for root depth, average diameter, and convex hull area. Modern lines, compared to landraces, showed physiological adaptations to drought stress by maintaining gas exchange and protecting PSII integrity. One landrace from Afghanistan, categorized as drought resistant in the field, showed outstanding root development under drought, developing a deep root system with a rapid vertical descent rate. This genotype displayed a great plasticity in root diameter, a key modulator of hydraulic conductivity. Among modern germplasm, one line developed a large and deep root system and was less impacted by drought stress possibly capturing all the available water. Lines featuring a small root system and a reduced shoot mass were less penalized by drought possibly due to a parsimonious water use during growth. Drought-tolerant genotypes may represent a promising reservoir of beneficial root traits for drought tolerance introgression.

Keywords: pea, roots, drought stress, phenotyping, breeding

EXPLORING THE RELATIONSHIP BETWEEN ENVIRONMENTAL ORIGIN, PHYLOGENETIC RELATEDNESS AND ROOT SYSTEM ARCHITECTURE (RSA) IN WILD LENTIL SPECIES

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Root System Architecture (RSA) regulates water and nutrient uptake and is central to crop resilience under increasing environmental variability, nutrient limitation, and water scarcity. We screened cultivated and wild lentil (*Lens* spp.) using the Rhizoscope (CIRAD), a high-throughput rhizobox phenotyping platform. Forty-two wild accessions and eight advanced breeding lines were quantified for RSA traits at 30 days after sowing. Cultivated lentil displayed higher root mass, diameter, volume, root angle (RA) and maximum root depth (MRD), indicating greater resource acquisition capacity. In contrast, wild accessions showed higher root:shoot ratios and longer Collar–First Ramification Length (CRL), consistent with adaptation to resource-limited environments. To test environmental control of RSA, we linked each accession to ecological variables from its origin (aridity index, soil type, bedrock depth) and analyzed patterns using Linear Discriminant Analysis (LDA) and Classification and Regression Trees (CART). Variation in MRD, RA and CRL aligned more strongly with environmental conditions than with species classification. Deeper rooting was associated with arid regions and deep bedrock, whereas wider RA and shorter CRL were typical of compacted or shallow soils. These findings indicate that RSA in lentil wild relatives is shaped primarily by local environmental selection rather than taxonomic identity. Integrating ecological provenance with phenotypic screening improves identification of adaptive root traits and supports targeted use of wild germplasm for breeding lentil cultivars resilient to drought and edaphic stress.

Keywords: root system architecture, wild lentil species, high-throughput phenotyping, environmental adaptation

USING UAV-DERIVED CANOPY VOLUME TO DIGITIZE HARVEST INDEX IN COMMON BEAN

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Harvest index is an important trait of interest in common bean (*Phaseolus vulgaris*), especially when considering drought resistance. Manually collecting canopy biomass samples to calculate harvest index is time-consuming, labour-intensive, destructive, and impractical when studying large numbers of plots. Timely and efficient collection of canopy biomass samples is also crucial in obtaining accurate harvest index measurements. Unoccupied aerial vehicle (UAV) digital phenotyping offers a high-throughput, non-destructive, and less labour-intensive approach to data collection and can efficiently cover large areas or many plots, providing an opportunity to estimate canopy volume as a proxy for canopy biomass. The objective of this study is to evaluate the relationship between UAV-derived canopy volume and manually collected canopy biomass estimates in both micro and large, yield size common bean plots and to determine the feasibility of using canopy volume as a proxy for canopy biomass in a digital harvest index calculation. Full plot canopy biomass samples were collected at maturity and used to calculate harvest index. UAV flights occurred bi-weekly and immediately preceding canopy biomass collections. Canopy volume was calculated from UAV-derived data. Harvest index measurements were most strongly correlated with digital harvest index estimates calculated using canopy volume from flights conducted near maturity. Additionally, digital harvest index values from microplots showed predictive power for larger plots, highlighting the potential for microplots to serve as a reliable proxy in high-throughput selection. This approach has the potential to streamline harvest index evaluation and enhance selection efficiency in common bean breeding programs.

Keywords: harvest index, digital phenotyping, UAV, plant breeding

FROM PIXELS TO TRAITS – MAPPING LENTIL SEED COAT COLORS WITH DIGITAL PHENOTYPING

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Seed coat color is an economically important trait in lentil that can affect lentil market class, milling quality, and richness of phenolic compounds. Three genetic loci, Ggc (grey ground colour), Tgc (tan ground colour), and Scp (seed coat pattern), were previously identified using humans to classify lines in various *Lens culinaris* populations. There tends to be inconsistency among these ratings, however. To improve phenotyping accuracy and investigate if these genes can be found in wild germplasm, we employed a digital-imaging platform, BELT, on a lentil diversity panel (LDP) and three interspecific inbred populations (LR-68, LR-70, and LR-86), to obtain color metrics. Three color indicators: L* mean for lightness, a* mean for green to red, b* mean for blue to yellow, were used to describe seed coat color in a 3-dimensional space. GWAS and QTL analysis identified associations among all populations corresponding to the Ggc, Tgc, and Scp loci which were consistently seen on top of chromosome 2, bottom of chromosome 3, and top of chromosome 6, respectively. For seed patterning QTL, the associations with different seed coat patterns suggests that this locus harbors an allelic series resulting in multiple different seed color patterns.

Keywords: lentil, seed, color, phenomics

PHENOMICS-ENABLED DISSECTION OF DROUGHT RESPONSES IN A FABA BEAN RECOMBINANT INBRED LINE POPULATION

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Faba bean (*Vicia faba* L.) is a strategically important legume for crop diversification in the UK and a sustainable source of proteins and other nutrients for food and feed use. However, its wider adoption is limited by sensitivity to water-deficit stress, which can reduce yield and affect seed protein traits. Breeding for drought resilience remains constrained by limited knowledge of the physiological and genetic determinants of drought-stress response, and by reliance on labour-intensive manual phenotyping methods. Leveraging the Advanced Plant Growth Centre's (APGC) fully controlled-environment and high-throughput phenotyping facilities, a genetically diverse F₉ recombinant inbred line (RIL) population derived from a UK commercial cultivar and a stress-adapted Afghan landrace will be characterised. The experiment will use 26 genotypes, including genetically diverse RILs representing contrasting seed protein traits, parental lines, and commercial checks. Plants will be grown under well-watered and drought regimes using automated irrigation and weighing to ensure reproducible water-deficit treatments. Time-resolved phenotyping will integrate RGB imaging for digital biomass and growth rate; chlorophyll fluorescence for photosynthetic performance; and hyperspectral imaging for physiological and biochemical signatures. Aligning with the National Alternative Protein Innovation Centre (NAPIC) objectives, these traits will be linked to measurements of harvested tissues to assess protein-related nutritional traits and to test their relationships with phenotypic performance. We hypothesise that lines with greater genetic contribution from the stress-adapted parent will show better growth, water-use efficiency, and maintenance of photosynthetic function under water-deficit stress. Also, that image-derived traits will detect stress responses earlier than endpoint measurements. This framework will support phenomics-enabled pre-breeding for drought-resilient, protein-rich faba bean.

Keywords: faba bean, phenomics, drought resilience

CROPGOBLIN: OPTIMISING LEGUME-BASED CROP SEQUENCES FOR CLIMATE MITIGATION AND NITROGEN EFFICIENCY

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CropGOBLIN, a new landscape-scale modelling framework, has been developed to reduce greenhouse gas (GHG) emissions from arable crop sequences (rotations) through the strategic inclusion of grain legumes, principally field bean, assessed across English regions. Results inform evidence-based decarbonisation while safeguarding cereal production by reducing reliance on synthetic mineral nitrogen (N) fertiliser - a major driver of agricultural N₂O emissions, fossil energy dependency, and water pollution. A central innovation of the approach is the ability to analyse radically different future national crop system scenarios, calculating emissions using IPCC Tier 2 methodology in-line with national emissions accounting. CropGOBLIN also applies rainfall-responsive, region-specific emission factors for soil N₂O, improving estimates of fertiliser- and residue-derived emissions. Validation against 11 years of UK National Atmospheric Emissions Inventory data demonstrates strong concordance, confirming robustness. Two optimisation engines underpin scenario development. Engine 1 reconstructs baseline crop sequences using crop-area data and sequence surveys, overcoming the absence of recorded crop-sequence datasets. Engine 2 applies linear programming with a weighted hierarchy (wheat > oilseed rape > barley) to design pulse-optimised crop sequences that increase legume inclusion (towards 15–20%) while minimising disruption to 'cash crops'. Simulations indicate that pulse areas could rise from ~7-18%, substantially reducing synthetic N demand through biological N fixation and lowering associated N₂O emissions. This national-scale assessment is complemented by a two-crop life cycle assessment (LCA) using participatory farmer data, enabling comparison of legume-to-non-legume versus non-legume-to-non-legume sequences. This allows refinement of synthetic N fertiliser offsets and identification of potential for further GHG reduction.

Keywords: field beans, biological nitrogen fixation (BNF), life cycle assessment (LCA), nitrous oxide (N₂O), crop sequence optimisation

EVALUATION OF THE AZODYN-PEA CROP MODEL, A TOOL TO STUDY PEA ADAPTATION STRATEGIES TO CLIMATE CHANGE

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In France, both spring and winter pea types are cultivated, differing in phenology and sensitivity to abiotic stresses. However, yield instability under climate change limits pea adoption in cropping systems. It is therefore essential to develop robust crop models that can accurately predict pea performance and support pea adaptation strategies to climate change. Azodyn-Pea is a process-based crop model that simulates pea growth and development, including yield components and grain protein content, as a function of genotype, management, and pedoclimatic conditions. Despite its potential, the Azodyn-Pea model has never undergone a global evaluation, limiting its improvement and use for decision support for agronomists. We performed the first multi-approach evaluation of Azodyn-Pea using an independent dataset covering 4,402 agro-pedoclimatic situations across France for dynamic and final outputs (leaf area index, biomass, yield, protein and nitrogen content). To identify sources of prediction error, we combined observed-simulated comparisons, global sensitivity analysis, and machine learning approaches. Analyses considered pea type, two irrigation levels, ten years and five contrasting sites. We found that winter pea biomass was simulated more accurately (nRMSE = 46%) than for spring pea (nRMSE = 57%). While yield and protein content were less accurately predicted, dynamic variables were well reproduced. Sensitivity analysis showed that all input parameters affected biomass, with soil parameters being the most influential. This evaluation highlighted the need to improve yield-related process formalism and identified the most influential genotype and soil parameters. The improved model will allow us to study pea adaptation strategies to climate change in France.

Keywords: legume crop model, global evaluation, sensitivity analysis, prediction, abiotic stresses

INTEGRATING FIELD AND CONTROLLED ENVIRONMENT PHENOTYPING TO FAST-TRACK HEAT TOLERANCE IN CHICKPEA

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Chickpea (*Cicer arietinum* L.) is vulnerable to high temperatures during reproductive development. In Australia, the increasing frequency of heat events during this critical window threatens yield stability, grain quality and grower profitability. Accelerating genetic gain for heat tolerance requires robust definition of the stress environment, reliable measurement of the traits that matter under stress, and a clear pathway to deploy findings into breeding. Environmental characterisation of historical climate and yield data using crop model APSIM revealed that a syndrome of hot, dry, high-vapour-pressure-deficit conditions correlates more strongly with yield loss than temperature alone and this syndrome is intensifying with climate change. In 2025, multi-environment field trials used optimal and delayed sowing to impose differential reproductive-stage heat stress across 400 diverse genotypes, with all environments successfully experiencing a high level of heat stress. High-throughput multispectral drone imagery captured canopy dynamics throughout the season, providing a rich, scalable platform for characterising genotypic responses across environments. Complementary controlled-environment screening of 200 genotypes dissected a defined stage-specific heat response, with flower-to-pod conversion emerging as a reliable indicator of reproductive competence under defined heat stress. Together, these field and controlled-environment trait datasets underpin genomic analyses to identify deployable haplotypes and the development of novel donor germplasm for integration into chickpea breeding programs.

Keywords: heat tolerance, reproductive stress, high-throughput phenotyping, chickpea

A STEPWISE APPROACH TO IDENTIFY AND RANK YIELD-LIMITING FACTORS OF SOYBEAN IN TEMPERATE CLIMATES FROM ON-FARM DATA

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The integration of grain legumes (GL) into temperate agricultural systems remains limited due to high yield variability and insufficient knowledge of yield-limiting factors. This study aimed to build a generic methodology to identify and rank the main factors affecting soybean yield variability in France. Eco-climatic factors (EF) derived from weather data and a simple water balance model were added to data from 421 French farmers' plots collected at establishment, flowering and maturation stages from 2021–2024. First, from literature and Terres Inovia expertise, 19 presumed limiting EF were identified and characterized by ~80 candidate indicators differing in their calculation window, threshold and computation method. Second, partial least squares (PLS) regression and variable importance in projection (VIP) scores enabled pre-selection of one representative indicator per limiting factor and phenological period. This yielded a refined set of eco-climatic indicators enriched with 16 agronomic indicators. Third, the impact of factors on yield and their ranking were determined using PLS-VIP on the selected set. Fourth, impact thresholds for the predominant factors were estimated using threshold detection methods. Preliminary results highlight the predominant influence of EF during the reproductive phases, particularly water stress and high temperatures, followed by weed pressure at flowering onset. These results provide new insights into soybean production in temperate climates. The identified limiting factors, their ranking, and estimated thresholds will be combined with a simple phenological model to delineate soybean production feasibility areas under current and future climates. This methodology will subsequently be replicated for six other GL species.

Keywords: soybean, yield-limiting factors, eco-climatic factors, on-farm data, temperate climate

IDENTIFYING LIMITING FACTORS FOR GRAIN LEGUME CROPS YIELD IN FRANCE

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Grain legumes are still rarely grown in France. Trials were conducted during 3 years (2021 to 2023) in 3 locations in France (in the north, east and south) to learn more about 6 grain legumes: chickpea, lentil that had recently developed in France ; faba bean, lupin (cultivated on small areas) ; pea and soybean (to complete data on these already well-known crops). The aim of the experiment was to precise, for each crop, the development stages and the differences in sensitivity to abiotic stresses, elements necessary for developing a tool to help farmers choose among these crops and facilitate their integration into cropping systems. For pea, faba bean and lupin, winter types were studied on 2022 and 2023. They have suffered significant damage (birds, wild game, weeds) in the eastern and southern sites both years and could only harvested with high yields in the northern site. This result highlights the difficulty to grow this kind of crops. For spring crops, accidents during the cycle or harvest problems were encountered for soybean, lentil, chickpea and lupin. Over the 3 years and the 3 sites, the yield of each crop is closely related to rainfall from sowing to maturity, with a positive or negative effect depending on the crop. For soybean, post-emergence climatic conditions were also determinant, particularly in northern sites. These results gave information on (i) the feasibility and productivity of the 6 grain legume crops and (ii) the timing of development stages of each crop very useful in modeling.

Keywords: grain legumes, development stages, abiotic stresses, yield

UAV-BASED SPECTRAL INDICES AND RANDOM FOREST REGRESSION FOR PLOT-LEVEL SOYBEAN GRAIN YIELD PREDICTION IN TWO RIL POPULATIONS

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This study evaluated the potential of UAV-derived vegetation indices (VIs) to predict plot-level grain yield in two soybean RIL populations, AIB ('Aires' × 'Buga') and PB ('Pepita' × 'Buga'), grown in augmented field trials. Multispectral imagery was acquired at four growth stages, corresponding to 308, 636, 918 and 1554 growth degree units, and 17 VIs were extracted for each plot, complemented by in situ chlorophyll and plant height measurements. Random forest regression models were fitted on a full dataset including 74 predictors and on a reduced dataset containing 15 selected variables. Model performance was assessed by 10-fold cross-validation using the coefficient of determination (R^2), root mean squared error (RMSE) and mean absolute error (MAE). For the full model, predictive ability was moderate to high, with $R^2 = 0.63$, RMSE = 0.42 t ha⁻¹ and MAE = 0.33 t ha⁻¹, corresponding to a relative RMSE of 10.4% given a mean observed yield of 4.01 t ha⁻¹. In the reduced model, performance was only slightly lower ($R^2 = 0.61$, RMSE = 0.43 t ha⁻¹, MAE = 0.34 t ha⁻¹, relative RMSE = 10.8%), demonstrating that a compact set of UAV-derived variables can achieve similar accuracy for prediction of grain yield in soybean breeding trials. In the full model, importance was spread across many correlated vegetation indices, with late-season NDGR and TCARI plus mid-season CIG, NLI and CVI dominating, whereas in the reduced model importance was concentrated into a smaller set of canopy greenness and chlorophyll indices (M4_GLI, M2–M3_CVI, M3_TVI, M4_EVI).

Keywords: soybean, grain yield prediction, UAV spectral indices, random forest regression, high-throughput phenotyping, recombinant inbred lines

REGIONAL PROJECTION OF WINTER FROST RISK ON PEA CROP DUE TO WARMING IN A TEMPERATE CLIMATE

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In the recent climate warming, a subtle evolution of winter pea frost risk was observed: a paradoxical increase of frost stress events and a frost stress intensity decrease. Such results are questioning the future winter frost risk for peas. We assessed the winter frost damage evolution along 2006 to 2100 in Burgundy-Franche-Comté (French region - Western Europe) by using a combination of i) a dynamical downscaled climate data of two RCP trajectories (4.5 and 8.5) and ii) a winter frost stress model calibrated for pea (using varieties with different frost resistance levels and acclimation rates). Our results show that frost risk will not disappear with warming temperate climate and will change compared to the historical period (1980-2005). Up to a warming threshold of +1.1°C, the model predicts a slight decrease of mean number of frost events and a larger increase of the frost average intensity for the pea variety with a frost resistance level of -13°C. Whilst when this threshold is largely overpass (+3.2°C) a significant decrease of both frost risk events number and intensity mean is observed. This projected frost risk structure evolution changes compared to those observed along the 1960-2019s period (previous work). Finally, the projection shows a contrasted geography of the frost risk evolution. This geographic trend depends on the frost resistance level and acclimation rate of the pea variety. This work highlights the needs of breeding and crop strategy for winter pea adaptation to climate change to avoid the detrimental effects of frost.

Keywords: climate warming, crop model, winter pea, frost risk, adaptation

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AGROPROTEINKA



CROP TRUST

Securing the future of food is one of the defining challenges of our time. Crop diversity – the genetic variation within and among crops – is essential to addressing this challenge. It enables plant breeders and researchers to develop varieties with higher yields, improved nutrition, and resilience to heat, drought, and new pests and diseases. Conserving and using crop diversity is a foundation for climate adaptation, agricultural innovation, and global food security.

The Crop Trust is an international organization dedicated to supporting long-term conservation and use of crop diversity in genebanks. With partners worldwide, the Crop Trust strengthens the global system of genebanks that collectively safeguard more than 5.9 million seed samples. These collections are a vital resource for research, breeding, and sustainable agriculture.

Legumes are among the most important crops conserved in this system. Rich in protein and micronutrients, legumes contribute to food and nutritional security. They also support sustainable farming through nitrogen fixation and soil improvement. The Crop Trust supports legume collections held by CGIAR genebanks and national partners, including chickpea, lentil, cowpea, pigeon pea, grasspea, common bean, faba bean, and groundnut. These collections are widely used worldwide for breeding and research.

Through the International Treaty on Plant Genetic Resources for Food and Agriculture and its Multilateral System, crop diversity conserved in genebanks is actively exchanged and used. Over 800,000 seed samples are distributed annually to researchers and breeders, accelerating the development of improved crop varieties adapted to changing environments.

The Crop Trust supports the critical infrastructure for this conservation and use. An Endowment Fund provides stable, long-term financing for key collections and system-wide services for genebanks. These include digital platforms such as Genesys and GRIN-Global Community Edition, which strengthen data management, connectivity, and access to crop diversity information.

The Crop Trust also partners on the Svalbard Global Seed Vault, the world's ultimate safety backup for crop diversity. Deep in the Arctic permafrost, the icy shelves of the Seed Vault safeguard more than 1.3 million seed samples, including legume collections backed up through international collaboration and initiatives such as the Crop Trust's BOLD project.

Building and maintaining a resilient global genebank system requires scientific cooperation, investment, and knowledge exchange. The Crop Trust recognizes genebanks, researchers, breeders, and policymakers worldwide. Together, we can strengthen the conservation and use of crop diversity and ensure that the crop diversity underpinning resilient agriculture remains available for generations to come.

The Fernand Lambein Fund, established in 2023, continues the scientific legacy of Prof. Dr. Fernand Lambein (Ghent University, Belgium). Prof. Lambein was an esteemed phytochemist who dedicated his career to the study of *Lathyrus sativus* (grasspea). He recognized the legume's remarkable potential as a resilient, high-protein crop capable of withstanding extreme environmental conditions such as drought, salinity, and flooding. At the same time, he was aware of the risks associated with its prolonged use as a staple food and advocated for its consumption as part of a balanced diet. Convinced that science is inherently collaborative and must be shared, Prof. Lambein actively promoted awareness and engagement within the scientific community around this crop.



Prof. Dr. Fernand Lambein

Inspired by his vision and dedication, the Fernand Lambein Fund, founded by Lambein's daughters, aims to carry forward his mission by supporting research on grasspea and fostering a collaborative and inclusive scientific community. A central objective of the Fund is to build a strong, connected global grasspea community. To support this goal, we organize the International Lathyrus Day, the first conference dedicated exclusively to this legume. We also promote communication through the *What's New Around Lathyrus?* newsletter, a biannual publication on scientific advances, ongoing projects, and community activities.

Through the GROW-Grasspea initiative, the Fund further aims to strengthen digital infrastructure within the grasspea community by supporting updates to two existing platforms. GrasspeaNet (grasspea.net) connects laboratories worldwide and provides a



The Fernand Lambein Fund

platform for science communication, while Germinate is an open-access repository designed for storing, sharing, and visualising validated agronomic, phenotypic, genotypic, and breeding datasets.

Empowering early-career researchers is another key priority. Through travel grants, research support, and workshops, the Fund facilitates access to international platforms and encourages the next generation of scientists to engage in interdisciplinary research on grasspea.

By bridging research, collaboration, and outreach, the Fernand Lambein Fund seeks to unlock the full potential of grasspea as a climate-resilient crop and to contribute to sustainable, nutritious, and inclusive food systems worldwide.

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