ILS4

Fourth International Legume Society Conference

2023

19-22 September
Granada Conference
Center

Granada
Spain

LEGUME SOCIETY

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Book of abstracts

September 19th–22nd
2023

LEGUME
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WELCOME MESSAGE

Dear colleagues and friends,

It is our great pleasure to welcome you to Granada (Spain), to the 4th International Legume Society Conference, which will be held between the 19th and 22nd of September 2023. This International Conference, which follows our earlier conferences in Novi Sad (2013), Tróia (2016) and Poznań (2019), aims at stimulating knowledge exchange and interactions among researchers and stakeholders interested in promoting greater cultivation and use of grain and forage legumes, as a necessary path towards more sustainable food and feed systems and healthier diets.

The conference will cover a wide range of topics, organized in the following scientific sessions:

- Legume-based value chains: innovation and optimization.
- Legume-based cropping systems: performance, ecosystem services and profitability.
- Legumes for human and animal nutrition and health.
- Legume biodiversity and genetic resource exploitation.
- Genetics and omics-based legume crop improvement.
- Legume breeding: challenges, tools, strategies and achievements.
- Legume physiology, biochemistry and systems biology.
- Beneficial legume plant-microbe interactions.
- Understanding and enhancing legume crop tolerance to abiotic stresses.
- Understanding and enhancing legume crop tolerance to biotic stresses.

The conference will take place at the Granada Conference and Exhibition Centre, well equipped with the most advanced congress facilities, located in the heart of the city at walking distance to hotels and the monumental city centre. Granada is a World Heritage city declared by UNESCO. Besides the world-renowned Alhambra Palace, Granada offers to visitors the beauty of its historic buildings and countless palaces, Arab town houses and gardens. Besides, Granada is close to the National Park of Sierra Nevada and the sunny Tropical Coast.

Recently, Granada has been awarded City of Science and Innovation. The University of Granada, one of the oldest and most prestigious seats for learning in Spain, the Health Science Technological Park, and the presence of several research Institutes from CSIC, result in a fascinating scientific atmosphere to promote and generate knowledge through many different areas, including key aspects focused in sustainable agriculture, nutrition and human health.

We hope this conference will provide a great opportunity for getting back to physical meetings as we used to live in the pre-covid19 age, with a vision for innovation and science-driven socio-economic change that is increasingly needed for more sustainable human development. We hope that you will enjoy not only the scientific program but also the Andalusian hospitality and the social activities that we will organize, to offer you an unforgettable stay in the beautiful city of Granada.

Dr. Paolo Annicchiarico
President of the International Legume Society

Dr. Alfonso Clemente
Chairman of the Organizing Committee

KEY SPEAKERS

Key Speaker

Session 1. Legume-based value chains: innovation and optimization

Bálint Balázs

Senior research fellow and managing director of ESSRG Non-profit Ltd. Budapest. Hungary

TRANSFORMATIVE POLICIES FOR DYNAMIC LEGUME VALUE CHAINS

Despite the potential of legumes to improve the sustainability of arable cropping systems and enhance the quality of farmed animal and human diets, the production and consumption of legumes in Europe are low, whereas their demand for feed is high. This insight also called a legume paradox, was shared by almost 600 stakeholders from the TRUE project partnership and beyond. We recorded the resonance among diverse value chain actors about more sustainable food systems where legumes play a role. The presentation offers a sociological understanding of stakeholders' policy assessment and intervention points leveraging barriers that hinder the production and consumption of legumes in the EU.

Key Speaker

Session 2. Legume-based cropping system: performance, ecosystem services and profitability

Christine Watson

Professor of Agricultural Systems, SRUC, Aberdeen, United Kingdom.

CLOSING THE LEGUME GAP – FOR PEOPLE, PLANET AND PROFIT

The inclusion of legumes in cropping systems is one mechanism for achieving crop diversification, seen by many as a way of improving the productivity of farming systems while increasing the delivery of ecosystem services. Here we use examples from Europe to illustrate how the inclusion, and management, of grain and forage legumes in agriculture contribute to food and feed production, profitability and ecosystem service delivery.

Key Speaker

Session 3. Legumes for human and animal nutrition and health

Karen A. Cichy

Research Plant Geneticist. Sugarbeet and Bean Research Unit. USDA- Agriculture Research Service. East Lansing. Michigan. USA

IS CONVENIENCE AT ODDS WITH NUTRITION AND HEALTH? COMPARING WHOLE BEANS WITH BEAN FLOURS

Most people appreciate the nutritional and health benefits of legumes in general and common dry beans (Phaseolus vulgaris) specifically. Beans are a major feature of the Mediterranean diet. They are rich in dietary fiber, protein, and minerals. But just because people know beans are good for them, doesn't mean they choose to eat them. Bean consumption is dropping in many parts of the world and one of the major reasons is lack of convenience/difficulty of preparation. To improve the convenience, beans can be milled and used as a flour in various products such as pasta or breads. Whole beans and bean flours are compositionally similar to each other since dehulling is not common with this legume. The major differences between whole beans and flour are that 1) milling breaks the cotyledon cell wall whereas in whole beans the cell wall remains intact through the cooking process and 2) the amount of water used in the cooking process is greater with whole beans. The nutritional, health and convenience implications of whole beans vs bean flours will be discussed, and research findings will be presented.

Key speaker

Session 4. Biodiversity and genetic resource exploitation

Roberto Papa
Università delle Marche. Italy

THE COMPLEX EVOLUTIONARY HISTORY OF COMMON BEAN

Common bean (Phaseolus vulgaris L.) is the most important grain legume for human consumption and, along other food legumes, is crucial for all agriculture-related societal challenges, including climate change mitigation, agrobiodiversity conservation, enabling healthy diets for all. The specific features of the Phaseolus genus have made it a unique example for the study of crop evolution. Moreover, the well-documented history of multiple domestications in Phaseolus vulgaris L. (common bean) and its further adaptation to different environments have provided the opportunity to investigate evolutionary issues considering both the origin of the species and the primary and secondary domestication process including the spread of the crop outside of centres of domestication.

The specific features of the Phaseolus genus have made it a unique example for the study of crop evolution. Moreover, the well-documented history of multiple domestications in Phaseolus vulgaris L. (common bean) and its further adaptation to different environments have provided the opportunity to investigate evolutionary issues considering both the origin of the species and the primary and secondary domestications process that include the spread of the crop outside their centres of domestication.

Will be presented the most significant outcomes obtained in common bean by the projects BEAN_ADAPT, PARDOM, BRESOV and INCREASE including the results of the INCREASE citizen science experiment that are relevant in the understanding of the process of the adaptation of common bean in Europe.

Key speaker

Session 5. Genetics and omics-based crop improvement

Sukhjiwan Kaur

Senior Research Scientist- Genomic and Predictive Breeding. Agriculture Victoria. Melbourne. Australia

SOWING THE FUTURE: PREDICTIONS, SIMULATION & SPEED BREEDING FOR LEGUMES

With increasing human population, climate change and shift towards plant-based diets, there is an urgent need to increase the current rate of genetic gain in food legumes to meet future demands. A variety of innovative technologies have emerged in recent years that can be synergistically integrated to facilitate trait improvement and accelerated crop breeding. Genomic selection (GS) can predict the breeding values of individuals early in the breeding cycle to reduce the generation interval. To maximise overall genetic gain, simulation modelling can be used to optimise the deployment of GS in breeding. Speed breeding can further shorten the generation time to accelerate trait stacking and variety development. By combining these techniques, breeders can develop crops that are better adapted to changing environmental conditions and that have improved yields and disease resistance, ultimately leading to an improvement in global food security. This keynote will focus on case studies from the Australian pulse breeding and pre-breeding programs that clearly demonstrate the potential benefits of combining these approaches to accelerate breeding and improve crop production.

Key Speaker

Session 6. Crop breeding: challenges, tools, strategies and achievements

Isabel Roldán-Ruiz

Scientific Director. Plant Sciences Unit. Research Institute for Agriculture Fisheries and Food (ILVO). Belgium

BREEDING PERENNIAL LEGUMES: CHALLENGES AND OPPORTUNITIES

The Plant Sciences Unit of the Institute for Agriculture, Fisheries and Food Research (ILVO) has a long tradition in breeding the perennial legumes Trifolium repens and T. pratense (white and red clover respectively). While the main breeding goals, such as forage yield and seed production, did not change throughout the years, the relative focus on specific selection criteria to achieve these goals have evolved. Examples of this are the most recent attention for persistence in red clover or trampling (and grazing) tolerance in white clover. Even though effective and efficient implementation of genomics approaches is not evident given the specific characteristics of these crops, some breeding programs already rely on genomic selection. Also, high-throughput on-field phenotyping tools are currently being explored, with promising results. In this presentation we will discuss the implementation of innovative breeding technologies in white and red clover, considering the specific challenges associated with their perennial nature and genetic characteristics.

Key Speaker

Session 7. Physiology, biochemistry and systems biology

Jérôme Verdier

INRAE Researcher at the Research Institute in Horticulture and Seeds (IRHS) located in Angers. France

SEED AND SEEDLING PERFORMANCES UNDER HEAT STRESS

Seed development needs the coordination of multiple molecular mechanisms to promote correct tissue development, seed filling, and the acquisition of germination capacity, desiccation tolerance, longevity, and dormancy. Abiotic stresses including heat stress can negatively impact these processes and upon the increase of global mean temperatures, global food security is threatened. Here, we explored the impact of heat stress on seed physiology, morphology, gene expression and epigenetic changes during seed development and seed germination.

In order to improve/homogenize seed quality of seed produced under abiotic stress conditions, we first identified genes involved in seed performance plasticity following heat stress. Here, we will focus on MIEL1 gene and how this gene acts as a plasticity regulator of seed germination by its potential epigenetic regulation during seed maturation.

In parallel to improve seed and seedling performances, we will explain how by bioengineering the desiccation process acquired during seed maturation, we successfully improved seed germination and seedling establishment under extreme drought conditions at early germination.

Key Speaker

Session 8. Beneficial legume plant-microbe interactions

Dulce Nombre Rodríguez Navarro

Scientific Researcher. Sevilla. Spain

ROLE OF BENEFICICIAL MICROBES IN LEGUME MANAGEMENT

Soil microorganisms have become an essential partner in crop management in the current scenario of Agriculture Sustainability as they can play fundamental roles in plant nutrition and phisiology, plant protection and environmental resilience.

Legumes display a special characteristic as they can interact with specific diazotrophic soil microorganisms -generally known as rhizobia- establishing a tight symbiotic relationship that turn out into a self-reliance on nitrogen acquisition as a distintive feature over other important crops. Rhizobia are able to fix atmospheric N2, the so-called Biological Nitrogen Fixation process.

In general, legume breeding and field management have been achived unconcerned the appropiated microbial partner. However, there exist a bulk up knowledge on the rhizobia-legumes interaction at both scientific and practical use levels since this plant-microbe association was worthwhile at the end of the 19th century. The conference will present approaches to soil microorganisms (greatly rhizobia) management, industrial manufacturing, field results of diverse legume performance under rhizobia N-feeding in adition to the economic and environmental advantages.

Key Speaker

Session 9. Understanding and enhancing legume crop tolerance to abiotic stresses

Kadambot Siddique

Hackett Professor of Agriculture Chair and Director, The University of Western Australia, Perth, Australia

ENHANCING ABIOTIC STRESS TOLERANCE IN GRAIN LEGUMES: PHYSIOLOGICAL AND MOLECULAR APPROACHES

Abiotic stresses are predicted to rise in the future owing to several reasons associated with global climate changes. These abiotic stresses (e.g. drought, heat, chilling and waterlogging, salinity) can result in severe threat to crop production in most countries. Grain legumes are well known for their impact on agricultural sustainability as well as their nutritional and health benefits. Abiotic stresses impose challenges for legume crops and has deleterious effects on the morphology, physiology, and reproductive growth of plants. Abiotic stresses at the time of the reproductive stage is becoming a severe limitation for production of grain legumes as their cultivation expands to warmer environments and temperature variability increases due to climate change. The reproductive period is vital in the life cycle of all plants and is susceptible to abiotic stresses stress as various metabolic processes are adversely impacted during this phase, which reduces crop yield. Grain legumes exposed to abiotic stresses during reproduction show flower abortion, pollen and ovule infertility, impaired fertilization, and reduced seed filling, leading to smaller seeds and poor yields. Through various breeding techniques, abiotic stress tolerance in major legumes can be enhanced to improve performance in the field. Omics approaches unravel different mechanisms underlying abiotic stress tolerance, which is imperative to understand the processes of molecular responses toward abiotic stress.

Key Speaker

Session 10. Understanding and enhancing legume crop tolerance to biotic stresses

Sabine Banniza

Professor and Strategic Research Chair in Pulse Crop Pathology. Crop Development Centre. University of Saskatchewan. Canada

CAN'T YOU JUST GENE-EDIT THAT PLANT TO MAKE MY PULSE CROP DISEASE RESISTANT?

The emergence of gene editing technology has revolutionized options for manipulating traits in plants leading to the impression in some that it will rapidly solve all problems, including disease susceptibility. Successful manipulation of traits by gene editing requires a comprehensive understanding of gene function and their roles in the networks regulating traits. This requirement is limited or non-existent in most of the host-pathogen systems in pulse crops. Most host-pathogen systems are regulated by complex networks with many interaction points with the pathogen rather than by single "major" resistance genes functioning as master switches as described in the gene-for-gene hypothesis. An example for this is the interaction of the hemibiotrophic pathogen Colletotrichum lentis with lentil where it causes anthracnose on all above-ground tissues. This pathogen bombards the lentil plant with a wide range of effectors, including extracellular proteins known to sequester fungal chitin to evade detection by the host, biotrophy–necrotrophy switch regulator NUDIX, and a wide range of carbohydrate-active enzymes (CAZymes) involved in plant cell degradation. In partially resistant Lens ervoides lines, the expression of thousands of genes changes in response to the invasion by C. lentis and an intricated network of co-expressed genes of both, the pathogen and the host can be visualized. To complicate this further, it has become apparent that pathogens also manipulate and exploit resistance mechanisms in plants to promote their proliferation, thereby obfuscating what is a host defense response rather than pathogen-induced. Aspects of this complex interplay between host and pathogen is not limited to this host-pathogen system, but has also been found in other pathogens, e.g. Ascochyta lentis and is emerging for the root rot pathogens in pea and lentil. While gene-editing technology may not provide quick solutions for these complex systems, they may become invaluable in the study of gene function.

SESSION ABSTRACTS

Session 1 Legume-based value chains: innovation and optimization

606/85. DRY FRACTIONATION AS A TOOL TO SUSTAINABLY INNOVATE THE LEGUME VALUE CHAIN AND SUPPORT THE VALORIZATION OF MARGINAL AREAS

Authors:

Davide De Angelis[1], Vittoria Latrofa[1], Giacomo Squeo[1], Francesco Caponio[1], Antonella Pasqualone[1], Carmine Summo[1]

Work centre:

(1) University of Bari, Department of Science of Soil, Plant and Food Science, Italy

Summary:

Objectives

Factors that negatively affect the legume supply in European Union involve the low productivity gains and the high variability of economic returns (Ferreira et al., 2021), which makes farmers not motivated to cultivate legumes. The development of legume-based ingredients such as protein concentrates, may promote the legume market expansion in the European Union and it is an object of different EU research calls. In fact, EU does not sufficiently produce legumes to meet the increasing demand of plant-proteins (Ferreira et al., 2021). From this perspective, simple and sustainable methods of protein production must be implemented. Dry fractionation is the most sustainable technology for protein extraction (Lie-Piang et al., 2023), as it is based on physical processes, e.g., a cyclonic fractionation or an electrostatic separation of a finely milled flour into two fractions mainly containing i) large starch granules and/or non-protein components; and ii) protein bodies, without any use of water or chemicals (De Angelis et al., 2021). We suggest that dry fractionation might also be a strategy to valorize the neglected and underutilized grains, facing the problems related to the loss of the genetic resources and of the biodiversity. In fact, neglected and minor grains are well adapted to local environments, require low input for their growth and they tolerate stresses in a way that they can be grown in marginal lands. This is a relevant and modern objective of the Italian scientific community which aims at the development of marginal areas enhancing agroecological and socio-economic sustainability (https://agritech-centre.it/). This would also promote the protein diversification and increase the self-sufficiency of protein production. The aim of this contribute is to review the use of the dry fractionation to produce legume-based ingredients, highlighting its versatility from the perspective of promoting the valorization of minor and neglected legumes.

Concise description of the work (materials & methods)

The bibliographic research was carried out considering studied published in the last 10 years, using Scopus and Web of Science scientific databases, including keywords e.g., “dry fractionation”, “legumes”, “ingredients”, “protein”, and their combinations. A total of 89 articles, composed of 16 reviews and 73 research papers were collected.

Main Results

The number of research articles concerning the application of the dry fractionation has increased over the years, with more applications on legumes compared to cereals and other crops. Moreover, the majority of the studies are focused on pea and faba bean and pea (Figure 1). However, a total of 10 different legume crops have been studied, confirming the versatility of the dry fractionation process and its potential in supporting the valorization of the minor crops. Overall, the protein obtained with dry fractionation are characterized by peculiar functional properties, including a high protein solubility, high foaming ability and foam stability, high gelling ability. Such properties have been exploited in the formulation of different food, such as bakery products, pasta, as well as other innovative food products such as meat and dairy analogues.

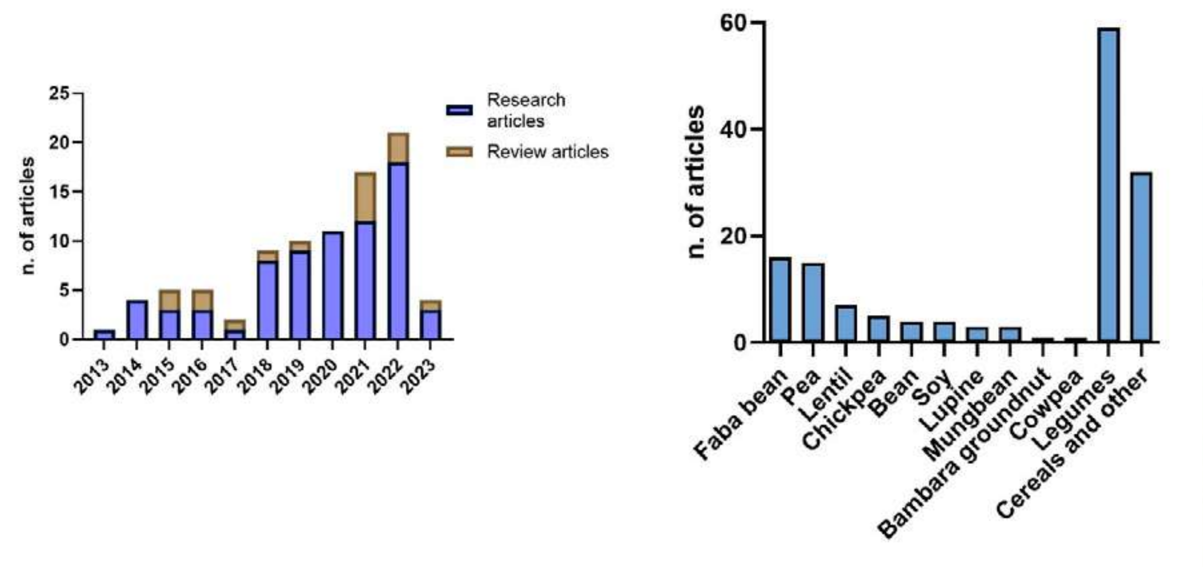

Figure 1. Number of published articles in the last 10 years concerning the dry fractionation (left) and species investigated (right).

Conclusions

In conclusion, our review highlight as the dry-fractionated protein needs to be improved, from the perspective of promoting the utilization of a wider range of plant materials. Moreover, the presence of antinutrient compounds and the intense sensory profile characterized by legume notes appear to be the most relevant factors to overcome.

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606/146. TRAIT PREFERENCES AND LENTILS VARIETAL ADOPTION IN CENTRAL ETHIOPIA: A MULTISTAKEHOLDER APPROACH

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Agricultural technologies, including modern crop varieties, are a critical measure for improving productivity, meeting food security needs, and bridging inequalities. This notwithstanding, adoption of some modern crop varieties in sub-Saharan Africa (SSA) tends to be low, with factors such as limited information, poor access to inputs, and risk averseness cited as reasons for low adoption. Few studies in SSA, and Ethiopia particularly, examine the influence of lentil trait preferences on adoption, and the ones that do only look at farmers' perspectives who are often treated as a homogenous group. This is despite the importance of lentils as a subsistence and growing market crop, and the fact that diverse factors may determine adoption among farmers. To address these knowledge gaps, this study used a mixed methods approach involving multiple stakeholders to understand gendered patterns in lentil varietal adoption and trait preferences. The findings revealed low adoption rates for improved varieties for women and men alike due to poor disease resistance, and insufficient attention from the breeding programs to preferred processing and consumer traits, as well as the differentiated needs of farmers. Paying attention to these trait needs serves to inform gender-intentional breeding and improve the income generation potential of lentil varieties for diverse farmer groups. As such, we recommend sex- disaggregated data collection from socially differentiated groups and market representatives in order to inform breeding priorities along with the development of multiple varieties that suite different needs.

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606/150. MAKING ROOM FOR LEGUMES: A CONSUMERS PERSPECTIVE ON LEGUMES INCORPORATION ON PROCESSED PRODUCTS

Authors:

Ana Ines Estevez Magnasco[1], Dominic Lemken[1]

Work centre:

(1) Bonn University. Bonn. Germany

Summary:

Objectives

Our society is going through several shifts, such as climate change, a growing world population, increased consumption of animal products and unhealthy diets in industrial nations such as Germany; it is imperative to encourage better nutritional behaviours. Alternatives to our current food choices in this environmentally and nutritionally challenged food system are needed, and legumes can be part of a solution. Unfortunately, the consumption of legumes is very low when compared to other food groups, such as meat, grains, tubers and cheese

Concise description of the work (materials & methods)

We wanted to see what variables influence the willingness of consumers to use processed products from legumes with a Transtheoretical Model approach and the image that selected legumes have among consumers. To evaluate this, we collected data from two surveys of German consumers. The preference for six types of legumes was assessed, with the intention of consumers to accept legumes in processed products as a dependent variable, and health, price, usage, taste, environmental impact, naturalness, protein content and potential use for a new product as the groups containing the independent variable.

Main Results

We discovered that when the question of pre-contemplation was evaluated for the Transtheoretical Model, 55% of the consumers didn't want to buy products that have lupine in a processed form, followed by soybeans, 48.6%, and chickpeas,42%. Moreover, on the contemplation question, I want to buy products that contain... in a processed form but don't pay attention, 32.9% replied that they were willing to accept large beans in processed products, and 32.1% would contemplate buying products with large peas in them. From the image analysis with the radar plots, we see that in terms of taste and consumption when being a child, lentils, green beans, and peas showed the highest likability scale. Taste has the greatest influence on a possible change in behaviour towards processed legume products; therefore, this result results of key interests for us.

Conclusions

Findings from our study shed some light on the area of acceptance and awareness of legumes among consumers in Germany. We have found that positive qualities are to be communicated more strongly, and that facts such as a high protein content or sustainability are largely misjudged by consumers today. The results on both questions are complex, where several assumptions are embedded, that must be considered in future research.However, they provide information for further research that enables a focus on decisive influencing variables.

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Session 2 Legume-based cropping systems: performance, ecosystem services and profitability

606/5. ROLE OF LEGUMES TO IMPROVE RESEILENCE OF SE AUSTRALIAN FARMING SYSTEMS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Due to the increasing global population estimated to reach 9 billion by 2050, food production needs to be increased up to 110% (1) to provide the needed energy and nutrients for the growing global population. But is this possible? Diversified and sustainable farming systems are one of the many solutions proposed to help increase food production while preserving biodiversity and limiting chemical inputs. However, farming systems' resilience and profitability are often threatened by severe climatic events such as drought and floods. Within the scope of the Future Drought Fund-funded project 'Redesigning Broadacre Farming of SE Australia', we aim to improve the drought resilience of low to medium rain feed farms across this region. We have investigated the feasibility to incorporate 'new or not so prominently grown legumes' in the region into the existing cereal-oilseed-legume rotation and using foliar silicon application as a proven drought/stress mitigation strategy for the inclusion of new legumes. Our results indicated silicon application positively affected above-ground biomass, number of secondary branches, seed weight, pods per plant, seeds per plant, and grain yield for all leguminous species tested, despite water-logged field conditions. Plots treated with silicon showed a 4-11% increase in foliar silicon concentration compared to control plots, but no silicon accumulated in the grain. Post-harvest analysis indicated that foliar silicon application did not alter the concentration of seed protein, macro-, and microminerals. In conclusion, our first-year field-based results indicated that high value-less prominent legumes of this region such as lupin, chickpea, and lentil could potentially be incorporated into established farming systems to enhance crop diversification and foliar silicon application had a positive effect on mitigating response to plant stress.

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606/17. DOES WHEAT-SOYBEAN IN RELAY CROPPING OUTCOMPETE SEQUENTIAL DOUBLE CROPPING? DEVELOPING INNOVATIVE DIVERSIFICATION STRATEGIES

Authors:

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Summary:

Objectives

Cereal-based sequential double cropping systems (DCS) such as barley-maize have become common in some irrigated Mediterranean areas. However, they imply risks such as potential N losses to the environment and do not contribute to EU protein self-sufficiency. Recent assessments of DCS with soybean as an alternative to maize, have shown promising results (Simon-Miquel et al. 2023). However, double-cropped soybean productivity gets compromised when planted at late dates such DCS. The production of two crops per year under relay cropping systems (RCS), where both crops co-exist for a limited period of time (Figure 1), is seen as an opportunity for mild temperate areas (Lamichhane et al. 2023) where limited opportunity exists to grow two grain crops per year. However, its advantages are unknown in European warmer climates. The aim of this work was to evaluate the performance of wheat-soybean RCS in an irrigated Mediterranean area.

Concise description of the work (materials & methods)

Three cropping systems (i) soybean sole crop (SC) planted on a roller-crimped rye mulch, (ii) wheat-soybean DCS, and (iii) wheat-soybean RCS, combined with three soybean cultivars (P18A02, Luna, ES Creator) were assessed in a split-plot design with 4 replications. The field experiment was carried under on-farm conditions in 2022 in NE Spain (semiarid Mediterranean climate, clay-loam soil). Hybrid winter wheat (cv. Hybiza) was planted on November 18th 2021 at 120 seeds m-2 in the DCS and RCS. Wheat in DCS also represented the sole wheat treatment and wheat in RCS was planted in paired rows. Soybean was no-till planted between wheat row pairs in RCS (March, 25th) (Figure 1), on a rye cover crop terminated with a roller-crimper in SC (May, 4th) and after wheat harvest in DCS (July, 11th). The following variables were measured: crop establishment, first pod height, grain yield (dry matter) and protein, and soybean N derived from atmosphere (Ndfa, only in RCS)). Land equivalent ratio (LER) and its partial components (LERw, wheat; LERs, soybean) were calculated.

Main Results

Average wheat grain yield was 5577 kg ha-1 without significant differences between DCS and RCS. Similarly, wheat grain protein concentration was not different between DCS and RCS (12.1 g 100 g-1, on average). Soybean establishment was affected by the presence of mulch according to the cropping system, with lower values when planted on rye mulch in the SC (47%) or wheat crop residues in DCS (57%) compared to RCS (82%). It was expected that competition for radiation in RCS would force soybean to grow vertically leading to greater pod height than SC. However, the presence of rye mulch in soybean SC enhanced the length of the epicotyls, leading to same first pod height than RCS (0.152 m, on average). Soybean grain yield was greater in the SC (4091 kg DM ha-1) compared to RCS (1641 kg DM ha-1) and DCS (2068 kg DM ha-1). Soybean grain protein concentration was 36.1%, without differences between treatments. Soybean Ndfa in RCS was 67% of above-ground biomass N (89.6 kg N ha-1). Total LER ranged between 1.38 and 1.64 in DCS and between 1.37 and 1.58 in RCS, without significant differences between systems. Wheat presented LERw values higher than 0.5 (from 0.96 to 1.05), indicating a performance similar to SC while soybean in RCS was more affected by interspecific competition with LERs ranging between 0.36 and 0.52.

Figure 1 Wheat-soybean relay cropping system on-farm field experiment in Sucs, Lleida (NE Spain). Clockwise: soybean planting under no-till between paired wheat rows, soybean establishment, microclimate control, wheat and soy coexistence right before wheat harvest.

Conclusions

Our preliminary results show a similar performance of wheat-soybean RCS and DCS in terms of grain yield and LER in irrigated Mediterranean areas. Although the results obtained are promising, the management complexity of RCS makes them riskier for the farmers, needing more benefits to outcompete DCS.

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606/44. PARTICIPATORY PROJECT DESIGN TO INTRODUCE A NEW CROP IN SWEDEN

Authors:

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Summary:

Objectives

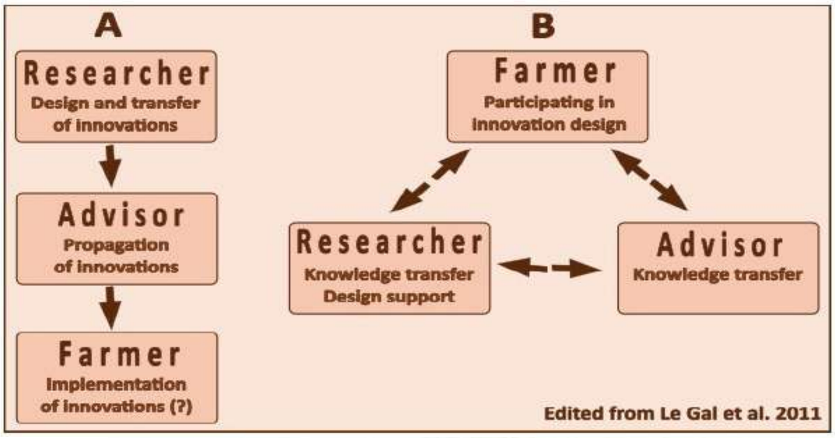
The cultivation of the narrow-leaf lupin (Lupinus angustifolius) and soybean (Glycine max) is very uncommon in Sweden, despite of cultivars suited to the climate of central Sweden (59°N) and successful cultivation experiences since 2006. These crops are tolerant to the root rot that infects peas and faba bean; their increased use in Swedish agriculture would allow for more efficient crop rotation in terms of legume interval. By introducing a novel participatory approach, we aim in the project PADILSO to increase the cultivation of lupin and soybeans in Sweden.

Concise description of the work (materials & methods)

Typically, communication of new knowledge gained from scientific research is an unidirectional process, and farmers are expected to be proactive in accessing and applying it, resulting in low adoption rates. We hypothesise that a multi-directional communication between researchers and practitioners facilitates the adoption of lupin and soybean cultivation in Sweden. Participation promotes the implementation of innovations by taking into account needs, limitations and farmer-knowledge and by evaluating the feasibility of a suggested innovation in the cropping system. Under the guidance of scientists, 30 farmers test to grow lupin and/or soybean for two years on their own land with self-designed cropping system. Farmers were offered seeds for an area of one hectare and a compensation payment in case of crop failure.

Main Results

By our new approach, knowledge is co-created. On the one hand, the guidance and financial compensation provided by the project offers security and a chance for farmers to test the crops on their fields using their own methods. On the other hand, scientists gain knowledge about crop performance under different management methods, soils, and climate zones. They also gain an understanding of farmers' knowledge, the specificity of the farming systems, and access to quality agricultural land, avoiding the heterogenic soils of field stations. At the end of the project, the results will be summarised in a manual to facilitate cultivation of these crops for other farmers.



Edited from Le Gal et al. 2011

Figure. Two processes to communicate new knowledge. (A) The linear process, where research communicates from top-down, which used to results in low adoption rates. (B) The participatory and interactive process, where the multi-directional communication facilitates the adoption of innovations. Participation promotes the implementation of innovations by taking into account needs, limitations and farmer-knowledge.

Conclusions

During the first project year, interest in the growth of these protein crops has become widespread across southern Sweden owing to the efforts of this project. So far, twelve field excursions have been held, all well attended and eagerly monitored by the press – coverage that may be crucial in the assimilation of these new legumes into Swedish agriculture. In the second year of the project, several participants bought their own seed to expand the test area, a sign that they have already adopted the new crop.

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606/56. INTERCROPPING WITH FABA BEAN IMPROVES MINERAL NUTRITION, GROWTH, AND YIELD OF WHEAT UNDER THE COMBINED STRESS OF WATER DEFICIT AND P LIMITATION

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Intercropping offers many benefits contributing to agriculture sustainability. These benefits are associated with complementarity or facilitation phenomena between the intercropped species (Chamkhi et al., 2022). We aim to assess the impact of intercropping with faba bean on wheat growth, yield, and grain quality under stress combining water deficit and P shortage in field. The experiment was conducted during two growing seasons (2020 and 2021) in farmer fields in Haouz area of Morocco. Faba bean (cv. Aguadulce) and wheat (cv. Karim) were grown as sole-crops or inter-cropped in low available P soil. After plant emergence, two irrigation regimes were applied, 80 % Field capacity (FC) for control plots and 40 % FC for stressed plots. The positive control received 80 kg/ha Monoammonium di-phosphate, while stressed plots were managed without mineral amendments. At the flowering stage, plant growth was assessed and at maturity, yield and quality parameters were determined. The results showed that intercropping improved wheat shoot dry weight (SDW) and root dry weight (RDW) reaching values of 6.63 g plant-1 and 1.06 g plant-1 instead of respective biomasses of 2.56 g plant-1 and 0.52 g plant-1 for sole-cropped plants, under the combined stress of water deficit and phosphate limitation. In addition, wheat total nitrogen, phosphate, and potassium contents were increased by 77.14%, 66.67%, and 113.33% respectively compared to sole-cropped plants under the combined stress. Moreover, grain yield, spike length, grain protein, and starch content were significantly improved under the same condition, by 23.39%, 26.12%, 8.29%, and 3.29% respectively. Thus, intercropping with faba bean could be a solution to enhance wheat production under stressful conditions, particularly in low-input agrosystems.

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606/130. GRAIN LEGUMES ENSURE REDUCTION IN EMISSIONS OF ARABLE SECTOR TO MITIGATE CLIMATE

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Summary:

Objectives

Nitrous oxide (N_2O) is the major greenhouse gas (GHG) linked to crop productions. Produced by natural soil processes, it can be greatly increased by the application of nitrogen fertilisers whose production is energy-intensive and which contributes to GHG emissions both upstream and on farm. Thanks to their symbiotic ability to fix air nitrogen, growing more harvested grain legumes (GL) is one of the promising solutions for arable sector for combining the contribution to climate mitigation and the enhancement of local plant protein production (Schneider & Huyghe 2015): absence of N application during the year of GL growth as well as reduction of the nitrogen dose requested for the crops in the following years. The absence of N2O emissions has been confirmed in field measurements in French conditions under pea crop contrary to fertilized crops (Jeuffroy 2013). In 2022, Terres Inovia has started studies to quantify mitigation levers, including system diversification with grain legumes, with its local partners of "Grand Est" region in France.

Concise description of the work (materials & methods)

Six main territories have been identified according to soil and climate conditions: Barrois, Brie, Champagne crayeuse, Champagne humide, Plateaux lorrains and Alsace. Eight case studies of cropping systems were drawn up to be representative of the recent average reality (data on operating context from standard field farms data base, technical and economic performances from regional statistics and interviews). For each initial crop system, a mitigation project was defined by inserting pea, faba bean or soya (+ 15 to 20% farm area). The emissions reductions (ER) was assessed according to LBC-GC method using the CarbonExtract® tool. Discounts were applied to ER so as to obtain carbon credits that the farmer could sell on the voluntary market.

Main Results

Figure shows the results for all the cases studied with the grain legume lever alone or combined with the "optimised" cover crop lever. The project in the Barrois-type farm produces net reductions of 69.7teqCO_2/year solely due to the inclusion of peas in the initial rape-wheat-wheat-barley rotation (R2), i.e. 0.39teqCO_2/ha/year. This represents -15% of the GHG emissions avoided. The additional insertion of cover crops in two intercrop periods improves carbon storage, increasing therefore the ER up to 0.54teqCO_2/ha/year (rebates included). To avoid the dilution of grain legume effect when the evolution of the system requires the addition of another wheat, the insertion of pea before oilseed rape would be also an interesting option. The quantifications carried out in the Grand Est region are backed up by other studies: 0.6 or 0.7teqCO2/ ha/year for peas or soya in the Grand Ouest region respectively (AgroSolutions) or for soya in Occitanie (Arvalis). Therefore, this represents a significant mitigation lever in the context of arable farming since the average of a set of real farm potential projects studied in Grand Est region (CarbonThink project) indicates 0.8teqCO2/ha/year. In addition, sensitivity analyses showed that ER can be increased by a further 10 to 20%, either when a better yield is obtained from the legume, or when better use is made of each of the effects on the following wheat crop (greater reduction in the nitrogen dose to wheat and/or greater gain in its yield).

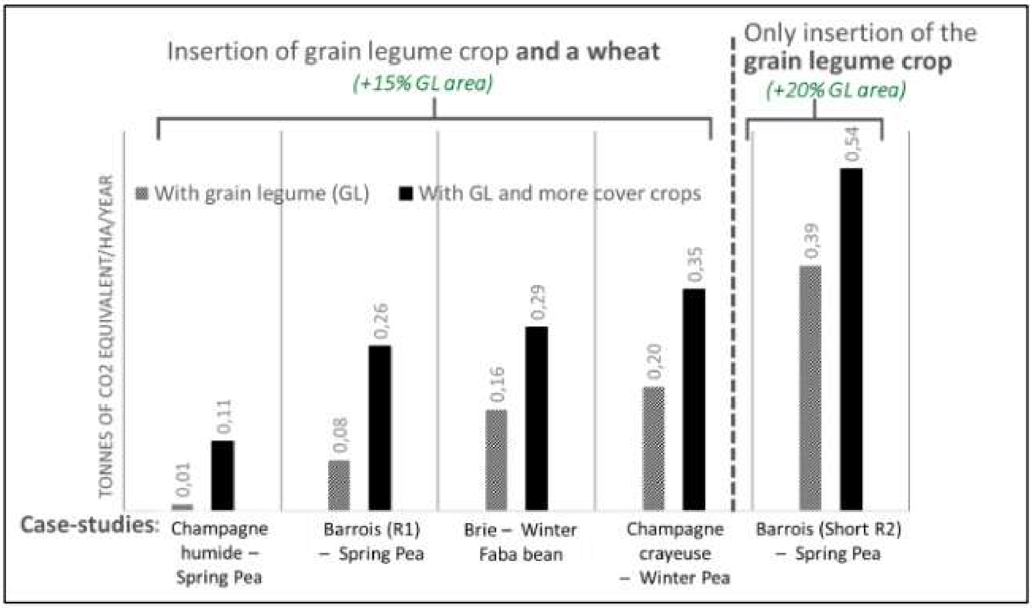

Figure. Net emissions reductions (ER) achieved by combining the "insertion of grain legume" and "optimisation of intercropping cover crops" levers using the LBC-GC method in several representative cases in the Grand Est region. Source: Terres Inovia 2022.

Conclusions

Inserting grain legumes in arable crops leads to a significant guaranteed contribution for climate change mitigation and this lever can be combined with cover crop increase. The implementation requires to enhance both the technical expertise in grain legume cropping facing climate hazards and in the system conception for benefiting of all legume ecosystem services at their highest levels.

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606/136. PRODUCTIVITY OF INTERCROPPING GRAIN LEGUME WITH WINTER CEREAL IN THE WESTERN MEDITERRANEAN

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Summary:

Objectives

Intercropping is a common practice in countries where inputs and mechanization are limited, and is increasingly adopted in the organic Western agriculture. Multiple meta-analyses demon-strated that this technique leads to land savings, of around 22% when compared to sole cropping of the same species (e.g. Yu et al., 2015). This can be attributed to various mechanisms such as complementary access to resources, cooperation in pest and pollinator regulation, and increas-ing resilience to pests, diseases and weeds (Brooker et al., 2015). However, intercropping gen-erate loss in grain compared with the most productive sole crop (Li et al., 2022). The present study, carried out in Spain, aims to assess the productivity of intercropping grain legumes with winter cereals in conventional agriculture in the northern Mediterranean, in order to determine whether it is an effective technique for food supply in this environment.

Concise description of the work (materials & methods)

An on-farm field experiment was established in the Ebro valley (Sucs, Lleida, Spain; 41°42'00.8"N 0°26'52.6"E) in a surface irrigated area, in collaboration with farmers, for 3 winter cropping seasons (2020-2021, 2021-2022, 2022-2023) in the framework of the EU Biodiversify project. Durum wheat/winter pea (Triticum durum Desf./Pisum sativum L.) and durum wheat-faba bean (Vicia Faba L.) intercrops were tested on a replacement design, and were compared together with their respective sole crops under two synthetic nitrogen (N) fertiliser scenarios (0N, control; 75N, 75 kg N ha-1 as top-dressing) in a randomized block design with 3 replications. Grain yields and nitrogen content were measured and the proportion of N derived from biological fixation (Ndfa) in the grain was determined using 15N natural abundance method.

Main Results

In the majority of scenarios, land savings were practically non-existent, with 7 out of 8 having Land Equivalent Ratio lower than 1, mainly due to lower legume productivity. Intercropping did not reach the grain productivity of the most productive sole species (durum wheat), with Transgressive Overyielding Index (TOI) below 1 in 7 out of 8 scenarios (Fig. 1). A complementarity in the use of N resource was observed with intercropping, presenting significantly higher proportion of Ndfa in pulses' grains ($p<0.05$). Intercropped fababean presented Ndfa in grain of 79%, whilst in sole cropping it was 56%. However, this increase did not result in higher N content in the grain of intercropped legumes compared to sole crop.

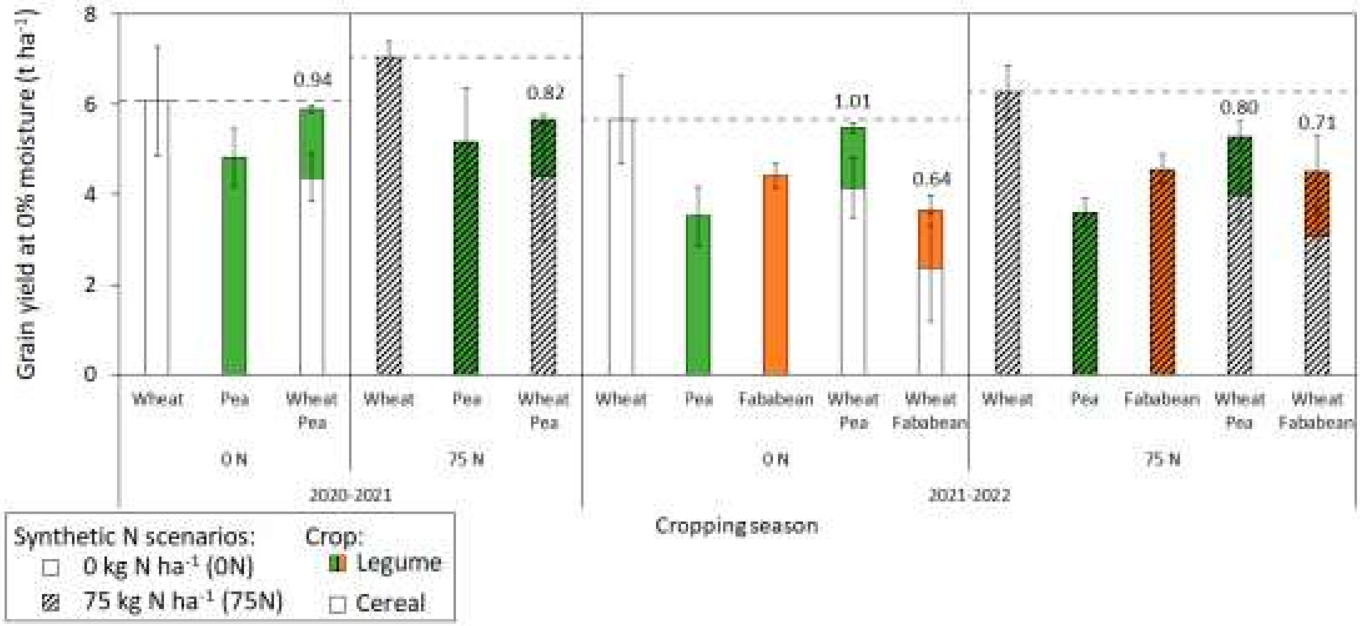

Fig.1. Grain yield of the species assessed as sole crop and in intercropping during two cropping seasons, in both synthetic N scenarios. Dotted lines represent the grain yield of the most productive crop in the mixture. Overyielding occurs when the total yield of a mixture surpasses this line, indicating a Transgressive Overyielding Index (TOI) > 1. For each scenario, TOI values are shown above the corresponding column. Error bars represent the standard deviation.

Conclusions

Intercropping did not result in land savings and was less productive than sole cropping, although it did promote complementarity between species. This underlines the need to evaluated this technique using several indicators in order to fully grasp its performance.

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606/165. POTENTIAL OF GRAIN LEGUME PRODUCTION TO SUBSTITUTE ANIMAL-BASED PROTEINS IN EUROPE

Authors:

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Summary:

Objectives

The considerable environmental consequences demand a radical restructuring of global food systems. A dietary transition from animal- to plant-based proteins would enhance healthy diets and reduce environmental impact. Adequate domestic

European production of grain legume crops is amongst the first steps in this protein transition. Faba bean (Vicia faba L.), pea (Pisum sativum L.) and soybean (Glycine max (L.) Merr) are currently the three most widely grown grain legumes in Europe. The objective of this study is to investigate the extent to which European grain legume production can be increased by 1) narrowing the yield gap, i.e., the difference between farmers' yields and yield potentials (Scenario 1), or by 2) expanding the areas of grain legumes (Scenario 2). We also estimate how much grain legumes can substitute meat-based proteins and its effect on the land footprint and GHG emissions, by using data from Poore and Nemecek (2018).

Concise description of the work (materials & methods)

For Scenario 1, potential yields (Ypot) for grain legume crops in Europe were estimated for the main producing countries following the bottom-up approach of the Global Yield Gap Atlas (GYGA, www.yieldgap.org) which includes using crop growth simulation models. We calculated yield increases to 80% of the Ypot. For the remaining crop x country combinations, a regression model was used to estimate grain legume Ypot based on Ypot of barley (for faba bean and pea) and maize (for soybean). In Scenario 2, we assumed that 1/12th or 1/6th of the total cropland area is covered by the three grain legumes with actual yields.

Main Results

We found that to achieve Scenario 1, actual yields have to increase by a factor 1.39, 1.44 and 1.69, for faba bean, pea and soybean respectively (Figure 1b). Current grain legume area should increase 2.7 or 5.5 fold in Scenario 2 to fulfil our assumptions (Figure 1c,d). Current self-sufficiency of grain legumes for food can be achieved by 80% yield gap closure or by 1/12th grain legumes cropland area. Self-sufficiency for food and feed is well achieved by 1/6th grain legume of the cropland area. If these extra plant-based proteins were used directly for food rather than for feed, it could substitute 13% of the meat consumption in Scenario 1 and theoretically all meat consumption in Scenario 2 (but we assumed a maximum of 25% meat substitution) resulting in substantial land and GHG emission savings. Arguments in favour of increasing grain legume production in Europe include crop system diversification, decreased dependency on external inputs, and reduction of legume imports. Legume yields and areas in Europe thus need to increase. Areas of especially pea and faba bean are limited, because the crops are perceived as unprofitable. Increasing yield of these crops requires more investment in breeding. In addition, water limitation plays an important role in yield variation, and irrigation would increase yields significantly, especially for soybean.

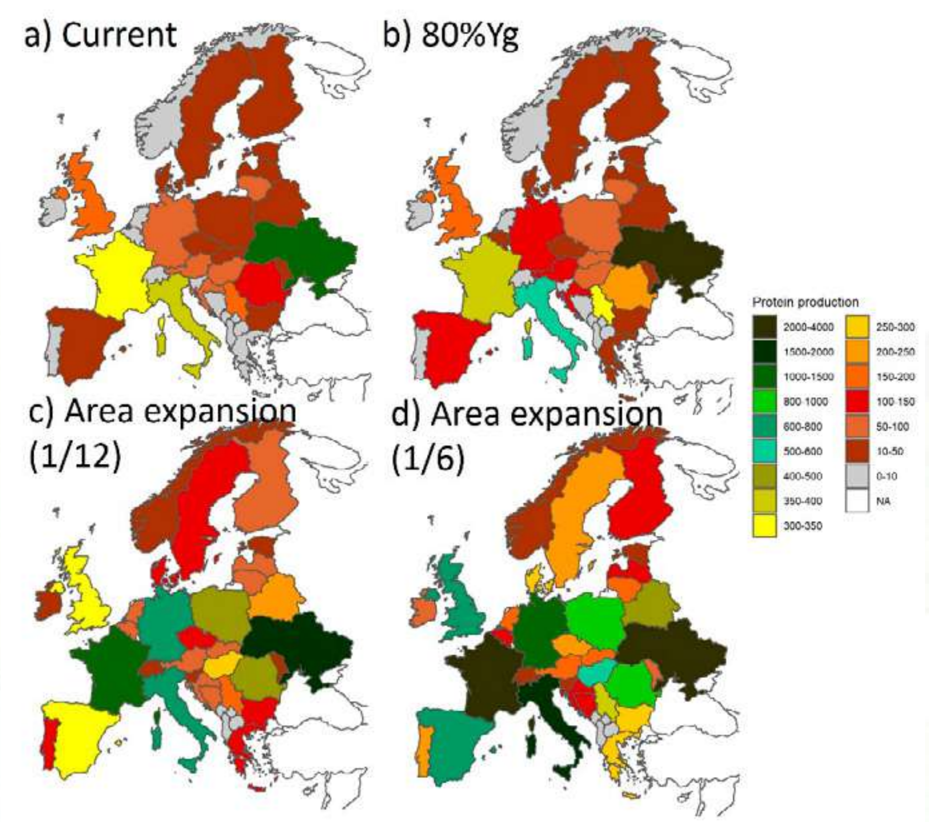

Figure 1 European digestible protein production (in 109 g) from faba bean, pea and soybean with current area and yields (a) in a scenario of: 80% yield gap closure (Yg) (b), area expansion of the legumes by 1/6th (c) and area expansion by 1/12th

Conclusions

Both higher yields and in particular area expansion of grain legumes are needed for Europe to become (more) self-sufficient in plant proteins for human consumption and certainly when including feed demands. Although with current price ratios it often seems not appealing to European farmers to increase production of grain legumes, other factors, including economic, benefits of grain legumes must be considered from a sustainable food system perspective.

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606/198. BETTER TOGETHER: INTERCROPPING FIELD PEA (Pisum Sativa) AND FABA BEANS (Vicia Faba) FOR INCREASED PRODUCTIVITY AND RESILIENCE IN A NORTH WESTERN EUROPEAN CLIMATE

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Summary:

Objectives

Global food security requires increased crop productivity to meet growing demand. The combined effects of biotic/abiotic stresses, climate instability and targeted reductions in pesticides input, make that requirement a challenge. Crop diversification along with the development of new agricultural practices promotes resilience of production systems to shocks and stresses, and are key actions to increase food production while limiting negative impacts on the environment. Introducing grain legumes is a particularly effective way of diversifying the common European cereal-based rotations, to break the build-up of diseases, pests and weeds with the added benefit of biological nitrogen fixation. Field pea is an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals. The high nutrient density of pea makes it a valuable food and feed commodity, constituting the third most economically important grain legume after soybean and beans. In Ireland, and other North European countries, production is minimal, in spite of the high yield potential of the crop in those countries. The main issue to overcome is the low crop standability, even when semi-leafless varieties are used. The potential of intercropping to assist a crop prone to lodging has long been recognized, specifically in barley-pea and oat-pea mixes. An alternative approach, that has been much less explored, is the intercropping of two legumes (Stelling, 1997). The aim of this study was to evaluate the efficacy of intercropping faba bean with pea, to increase pea standability, therefore extending the harvest window for this crop and decrease the risk of crop losses.

Concise description of the work (materials & methods)

Four field trials were done over three years covering both winter and spring sown varieties. Trials were set in randomized block designs with 3-4 replicates. Lodging was scored at maturity before harvest. Yield was recorded and a sample collected from each plot to determine TGW, moisture, protein and species ratio.

Main Results

Our research shows that the intercropping of pea with faba bean significantly increased pea standability. The mechanical support provided by faba bean to the field pea in mixed plots effectively prevented the pea crop from lodging in all experiments, compared to plots where pea was the sole crop (Figure A). Furthermore, the presence of both crops increased productivity per m2, regardless the presence of lodging (Figure B).

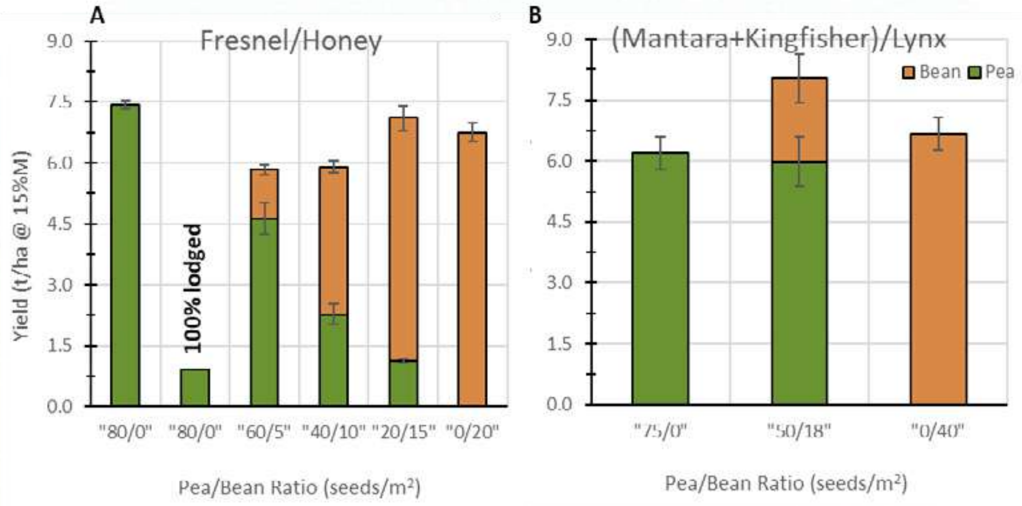

Figure. Mean yield (t/ha) ± SE of field peas (green column) and faba bean (orange column) in intercropping trials sown in winter (A) and spring (B). Trials were layout in a randomized block design with 3 replicates (winter) and 4 replicates (spring), plot size = 30 m2. (A) Winter sown (26.11.2020) intercropping trial with field pea variety Fresnel in sole plots or in mixed plots with faba bean variety Irena, at 3 different proportions and total seed rate, as listed. Sole field pea plots were harvested on the 26/07/2021 (no lodging observed) when the pea crop was ripen, and again on the 31/08/2020 (pea crop in sole plots flat on the ground) when the bean crop was ripen. Mixed and sole faba bean plots were harvested at the later date. (B) Spring sown (30/03/2021) intercropping trial with a mixed field pea combination (Mantara+Kingfisher) in sole pea plots and in intercropped plots with faba bean, variety Lynx. All plots harvested on the 01/09/2021. No lodging observed in this trial.

Conclusions

Further trials are necessary to tailor intercropping agronomy for the available varieties of pea and bean, which was a challenge through the course of this study. In addition, the market for a mixed crop needs to be established so that post-harvest separation and processing can be ensured to deliver an added value for the primary producer. This work is being continued as part of VALPRO Path, an EU funded research project tasked with increasing the added value potential of plant protein crops.

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606/241. BELOWGROUND INTERACTIONS – THE KEY TO FUTURE RESILIENT AND SUSTAINABLE LEGUME BASED CROPPING SYSTEMS?

Authors:

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Work centre:

(1) University of Copenhagen. Copenhagen. Denmark

Summary:

Objectives

Including more legumes in cropping systems is a climate-smart solution to increase sustainability, soil fertility, and cropping systems diversification, while reducing nitrogen inputs. Nevertheless, enhancing legume production in temperate regions for both food and feed presents various challenges that must be acknowledged and necessitates further research in order to achieve successful implementation (Wacker & Dresbøll, 2023). The main benefit of incorporating legumes into cropping systems is biological nitrogen fixation reducing inorganic fertilizer input and increasing the potential carbon sequestration. Furthermore, in cereal dominated cropping systems the diversification by legumes will lead to lower disease and weed pressure. However, several challenges exists, including 1) biotic and abiotic stresses limiting yields and yield stability, 2) secondary compounds hampering use for consumption and potentially constituting an environmental risk from exudation and 3) inputs and losses of nitrogen that can be uncertain and environment dependent. Belowground dynamics play a key role in improving sustainable legume production, such as tolerance to abiotic stress, BNF, nutrient use efficiency, and water use efficiency. At University of Copenhagen, we aim to investigate belowground traits of grain and forage legumes, as part of sustainable solutions for crop production that is adapted to a changing climate.

Concise description of the work (materials & methods)

Specifically, we investigate root growth development and function in grain legumes such as faba bean and peas, as well as forage legumes such as white clover, red clover and lucerne. The experiments are in most instances conducted until plants reach physiological maturity in soil in an environment, which does not restrict deep root growth to ensure agronomic relevance. Thus, experiments are conducted in rhizotubes or in the field, roots are analysed by imaging and AI based software, water content monitored by TDR sensors and root function determined by tracer uptake.

Main Results

The results from ongoing experiments show genotypic variation in root growth development, rooting depth, spatial resource use differentiation and resource use efficiency. Further, we show plastic responses of root and shoot traits to environmental conditions such as water deficit. In addition, results show interactions between different traits, such as drought stress and BNF based on 15N and 13C stable isotope discrimination.

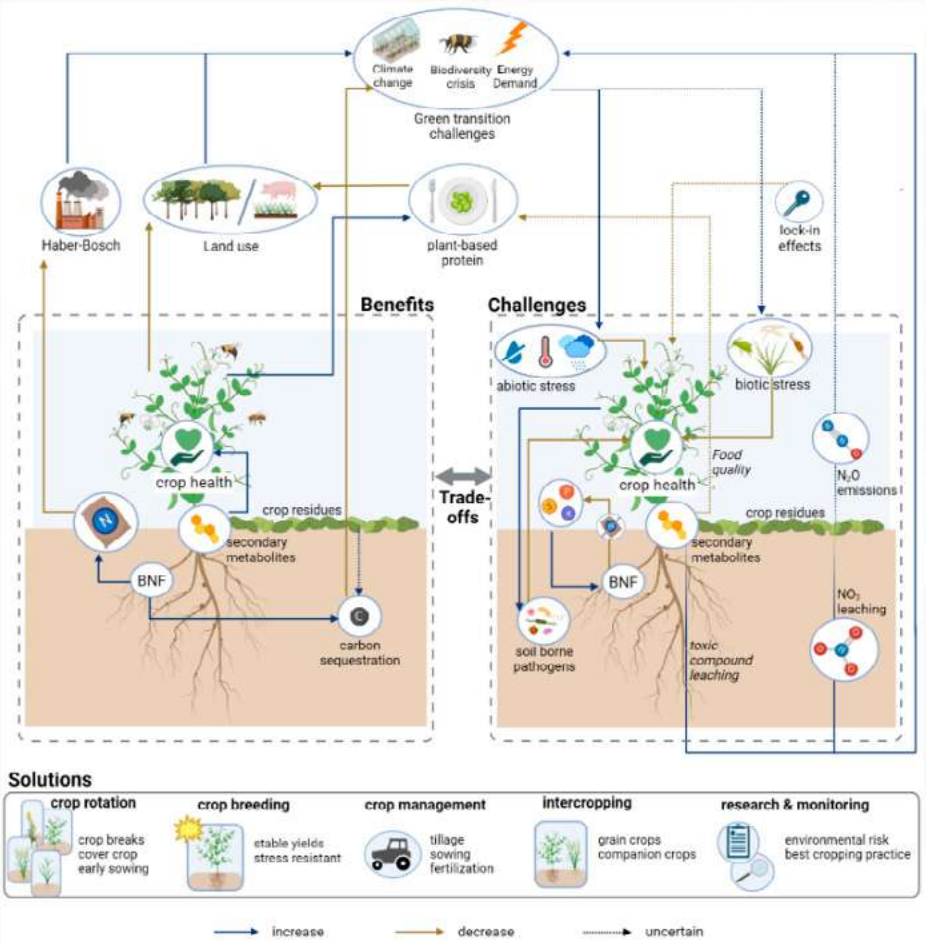

Figure. Benefits (left) and challenges (right) of grain legume production in temperate climates are depicted in two mirrored soil-plant systems and in connection to challenges of the agricultural green transition. Blue arrows (->) indicate an increasing effect, yellow arrows (->) indicate a decreasing effect. Arrows in dotted lines indicate uncertainty of the effect or effect size. Opposite effects in the separate sides indicate trade-offs. The broken line around the soil plant system depicts field system boundaries. Arrows passing the field system boundary show effects beyond the field system. Above the soil-plant systems are effects of higher system levels connected with increased grain legume production, as well as economic-political aspects e.g. lock-in effects. Below the soil-plant systems are potential solutions listed that can assist in mitigating some of the adverse effects. Figure created with biorender.com and published in Trends in Plant Science (Wacker and Dresbøll, 2023).

Conclusions

Thus, our work shows that there are complex interactions at play, and that we need to include the perspective of belowground dynamics combined with aboveground dynamics to understand benefits and challenges of legumes in future resilient and sustainable cropping systems.

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606/250. COMPARING THE CROP SEQUENCES WITH LEGUMES AT RESEARCH AND COMMERCIAL SCALE

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Summary:

Objectives

Diversifying a crop sequence to include a pulse legume can not only be profitable but increases the resilience and sustainability of modern farming systems. Benefits can include increased soil nitrogen (N) availability and improved rotational and environmental outcomes. There are many flexible end-use options for pulses including grain, forage (hay/silage/grazing), or brown manuring (BM) with different market opportunities compared to cereals and canola. Sowing large-seeded pulse legumes into retained cereal residues in modern no-till diverse crop sequences has allowed farmers to retain all their stubble, reduce issues of soil N-tie up when non-legumes are sown into cereal, and assists in converting high carbon cereal stubble into stable soil organic matter.

Concise description of the work (materials & methods)

A series of replicated field experiments over a 12 years period were examined.

Main Results

The results confirmed that including a pulse legume (grain for either human or animal consumption, or as a source of BM) into a 3- or 4-year cropping sequence, can be as profitable as the Baseline sequence of canola-wheat-wheat currently favoured by growers (Table 1). The average annual gross margin (GM) of Baseline sequences with low N and high N inputs, were much lower than sequences which included a pulse for human consumption or for animal feed, but exceeded those where a pulse was used for hay or BM. The systems incorporating a pulse were less risky (higher profit:cost ratio) and required significantly less inorganic N fertiliser ($53-$129/ha/year) compared to the Baseline sequences. In experiment 4, there was an average 64 kg N/ha increase in soil mineral N immediately prior to sowing the following crop after pulses, compared to after cereals.

In sequences where a pulse preceded canola prior to a cereal crop (i.e. "double-break"), there was a significant reduction (92%) from the initial herbicide-resistant average annual ryegrass (ARG) populations (1840 plants/m2) compared to a 262% increase in the Baseline(L) sequence. Herbicide costs were reduced by $30 /ha/year in diverse sequences.

Despite the compelling evidence of economic and environmental benefits of diverse systems, adoption on commercial farms in Australia remains low.

Experiment	Years	Crop sequence by Nitrogen Strategy	Average GM ($/ha/year)	Profit:Cost ratio	Cost Nitrogen Fertiliser ($/ha/yr)	Annual ryegrass seedbank at the end experiments (seeds/m2)
1	2011-13	Canola(H)-Wheat(H)-Wheat	$749	1.3	$166	-
		Canola(L)-Wheat(L)-Wheat	$811	2.0	$131	-
		Lentil-Canola(L)-Wheat	$708	1.9	$83	-
		Lupin-Canola(L)-Wheat	$674	1.6	$113	-
		FieldPeaBM-Canola(L)-Wheat	$511	1.7	$72	-
2	2012-15	Canola(H)-Wheat(H)-Wheat(H)	$883	1.5	$220	219
		Canola(L)-Wheat(L)-Wheat(H)	$803	2.1	$134	2222
		Lupin-Canola(L)-Wheat(L)	$793	1.9	$133	63
		LupinBM-Canola(L)-Wheat(L)	$552	1.5	$72	110
3	2014-18	Canola(H)-Wheat(H)-Wheat(H)	$454	0.8	$111	898
		Canola(L)-Wheat(L)-Wheat(L)	$328	0.7	$107	7406
		PulseHay-Canola(L)-Wheat(L)-Barley(L)	$478	1.0	$72	250
4	2018-22	Canola(H)-Wheat(H)-Wheat(H)	$1,234	1.7	$441	-
		Canola(L)-Wheat (L)-Wheat(L)	$1,192	1.9	$262	-
		Fababean-Canola(L)-Wheat(L)	$1,341	2.0	$174	-
		PulseHay/BM-Canola(L)-Wheat(L)	$1,026	1.6	$213	-
		Chickpea-Wheat(L)-Chickpea-Wheat(L)	$1,097	1.9	$55	-
Averages across experiment						
Baseline High N		Canola(H)-Wheat(H)-Wheat	$830	1.3	$235	559
Baseline Low N		Canola(L)-Wheat(L)-Wheat	$784	1.7	$159	4814
High value legume		Lentil/Chickpea-Canola(L)-Wheat(L)	$903	1.9	$69	
Low value legume		Lupin/Fababean-Canola(L)-Wheat(L)	$936	1.8	$140	141
BM/Hay		PulseHay/BM-Canola(L)-Wheat(L)	$672	1.4	$119	

Table 1: The average gross margin (GM), profit:cost ratio, nitrogen fertilizer expense and final annual ryegrass seedbank populations in a Baseline sequence at low(L) and high(H) nitrogen strategies, and from crop sequences including a pulse legume at low nitrogen strategies.

Conclusions

We explored reasons for this by scaling up the results (from experiment 3) to a commercial scale farm (3000 ha or 5000 ha). Only when a business was already managed at a high level of efficiency that changing management strategies to include legumes was effective and profitable for growers by reducing the (i) amount of synthetic N required, (ii) ARG weed seedbank, and (iii) economic fluctuations at the whole-farm level. However, when assessing the potential for inclusion of pulses into an existing Baseline sequence, there are several factors outside of production and financial performance that constrain the adoption of legumes commercially. These include legume grain price volatility, export market and volume issues, lack of accumulators for bulk marketing, skills/aptitude/attention to detail, capital resources of the farm, land class suitability, and capacity to manage increased disease and crop failure risks. Our work suggests that the outcomes of experimental research on the value of diverse crops into cereal-based systems should be closely integrated with a clear understanding of farm- and industry level constraints and the extent to which the existing system has been optimised.

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606/16. CROP DIVERSIFICATION IN MEDITERRANEAN NO-TILL RAINFED AREAS – A STRATEGY TOWARDS N EFFICIENT CROPPING SYSTEMS?

Authors:

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Work centre:

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Summary:

Objectives

Under Mediterranean conditions, water availability is the main limiting factor for crop productivity. No-till cereal-based systems have been adopted as an adaptation strategy to these dry conditions. However, in the long term, such systems can increase the risk of some biotic and abiotic stresses. In addition, the increases in production costs (namely, N fertilizer) and European policies require farmers to rethink their cropping systems. Crop diversification can be a lever to alleviate some of these problems and maintain sustainability in the long run (Kirkegaard et al., 2014). We aimed to evaluate diversification and N fertilization as strategies to improve N use efficiency (NUE) at the cropping system level by assessing its levers: productivity and N sources.

Concise description of the work (materials & methods)

Four cropping systems combined with four levels of top-dressing N fertilization (0, 40, 80 and 120 kg N ha-1) were assessed in a three-year on-farm field experiment in NE Spain. The farm implemented no-till techniques 20 years ago. Mean annual precipitation and evapotranspiration are 433 and 1000 mm, respectively. Cropping systems consisted of continuous winter wheat (WCS) as the reference, and three three-year rotations with pea (PCS), faba bean (FCS), or a multi-service cover crop (MSCS) based on spontaneous vegetation chopped at flowering. We used a multi-level assessment, with crop, pre-crop and cropping system levels (Simon-Miquel et al., 2023). Crop level included legume performance and contribution by biological N fixation (using 15N natural abundance method, (Unkovich et al., 2008)). At the pre-crop level, we evaluated the effects of alternative pre-crops on wheat performance. Cropping systems level included energy and protein productivity, N sources and N use efficiency for energy (NUEe) and protein (NUEp) production.

Main Results

Faba bean yield was higher in 2020 (4251 kg ha-1) than in 2021 and 2022 (1472 kg ha-1 on average). Except in 2022, biologically fixed N was higher than grain N (31 kg N ha-1, on average). Pea yield was lower, 510 kg ha-1, and biologically fixed N was higher than grain N by 17 kg ha-1. The lower pea yields were caused by a crop failure in 2020 caused by severe lodging, and the dry conditions in 2021 and 2022. After alternative pre-crops, significantly higher wheat yields (4986 kg ha-1, on average) than continuous wheat (4121 kg ha-1) were observed. Energy yields at the cropping system level were 81 and 53 GJ ha-1 yr-1 in the WCS and the other cropping systems, respectively (Figure 1A), which led to the highest NUEe. Protein yield increased across cropping systems and N fertilizer rates (Figure 1B). At low N fertilizer rates, cropping systems including legumes (FCS and PCS) showed higher protein yields compared to WCS and MSCS (Figure 1B). WCS had the highest response to N fertilization. At a fertilizer rate of 40 kg N ha-1, WCS outyielded PCS, while FCS had higher protein yields regardless of the N fertilizer rate. Protein yield increased proportionally to N supply (either biologically fixed or fertilizer), leading to a stable NUEp (1.69 kg protein kg N supply-1).

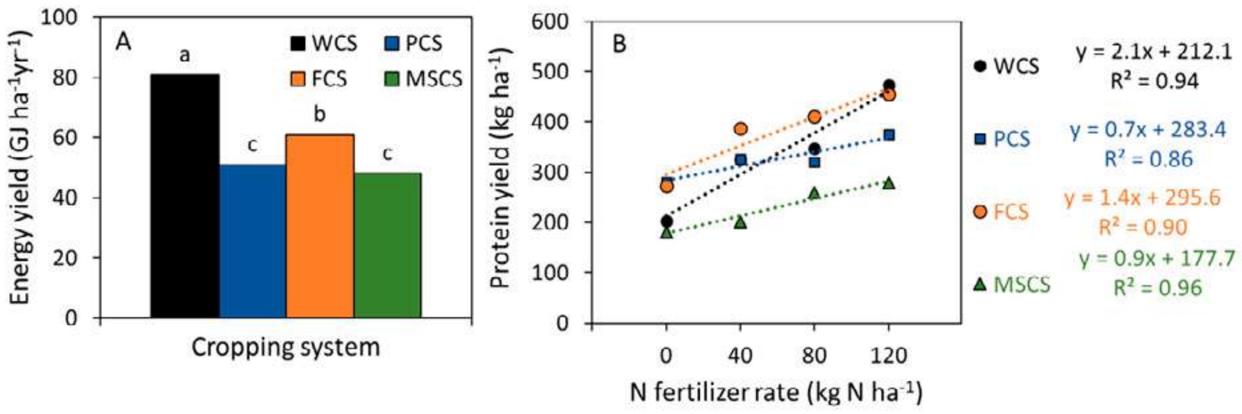

Figure 1 Annualised energy yield depending on the cropping system (wheat (WCS), pea (PCS), Faba bean (FCS) and multiservice cover crop (MSCS) cropping systems (A) and Linear regression between annualised protein yield of the four mentioned cropping systems against increasing levels of N fertilization (B). Levels not connected by the same letter are significantly different.

Conclusions

Using a multi-level approach allowed us to better characterize the performance of alternative cropping systems. We observed a positive effect of grain legumes and multi-service cover crop on the succeeding wheat and net input of fixed N in the legume cropping systems (N fixed was larger than N removed by grain). At the cropping system level, crop diversification led to a decrease in energy productivity. However, protein production showed promising results when cropping systems were diversified with legumes, especially faba bean, regardless of the N fertilizer rate.

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606/25. SOIL MICROBIAL DIVERSITY MEDIATED BY LEGUME CROP MEASURED BY BIOLOG® ECOPLATES™ AND API-ZYM

Authors:

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Summary:

Objectives

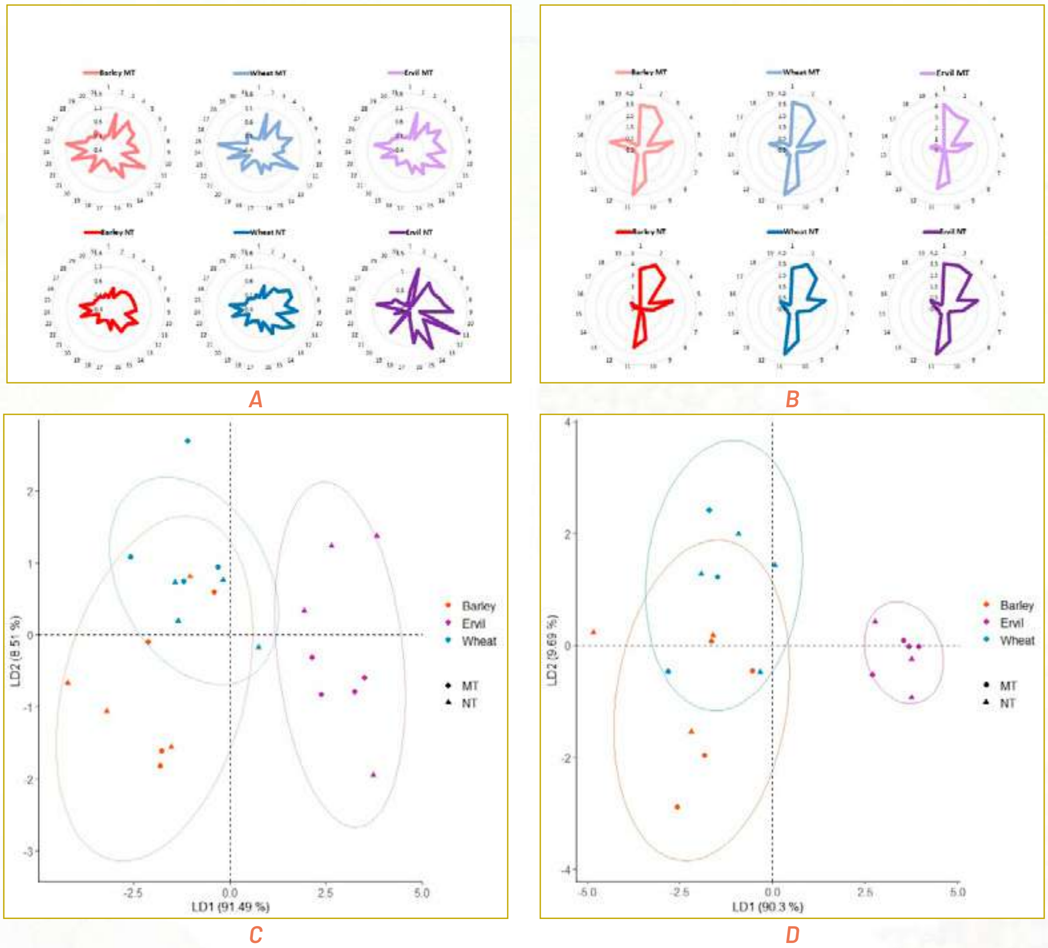
The potential benefits of increasing crop diversification in the EU can be summarized in ecosystem services. Crop diversification in Mediterranean rainfed rotations are based mainly in legume crops. One of the services provided by crop diversification is increase soil microbial functional diversity. In this work we proposed to analyse it by Biolog® EcoPlates™ (Biolog Inc., Hayward, CA, USA) and API-ZYM strips (#25200, Biomerieux), which have been reported as methods to study the response of microbial communities in a changing environment (Martinez et al 2016, Flynn et al 2017)

Concise description of the work (materials & methods)

We used a rainfed long term essay stablished in 2004, which involved minimum tillage vs. no-tillage practices in a randomized block design with four replications. The stablished crop rotation is: wheat/barley/legume crop. The legume crop was ervil (Vicia ervilia (L.) Willd.) in 2022. Superficial soil (0-15 cm depth) of the 24 plots were sampled in May 2022 (at grain filling in cereals and ervil). Soil samples were processed at laboratory as described by Flynn et al (2017), and modified by Sofo and Ricciuti (2019) for BIOLOG® ECOPLATES™. Each well of EcoPlate array (Biolog, Hayward, CA) contains a proprietary minimal media as well as one of 31 carbon sources and water, per triplicate. A tetrazolium dye reacts forming a distinct purple colour, which intensity is proportional to the level of metabolic activity (measuring absorption at 592 nm using a plate reader). In the case of the API ZYM assay, samples were processed as described by Martinez et al (2016) with a longer period of incubation. The API ZYM assay is a semi-quantitative commercial chromogenic enzymatic Kit, and includes 19 enzymatic reactions involved in the breakdown of different biomolecules. The colour reactions were read in a numerical scale from 0 to 5 according to the colour chart provided by the manufacturer by, at least, five independent observers.

Main Results

Only four out of 31 substrates of Biolog ecoplates resulted statistically significant for tillage factor: D-xylose [13], D-glucosaminic acid [17], glycyl-L-glutamic acid [29] and putrescine [31]; none for crop. None of functional diversity indices calculated (richness: S, Shannon's diversity and evenness indices: H' and E, respectively) resulted statistically significant. Nonetheless, in figure 1 (A) small differences could be observed in some substrates, especially in the case of ervil in no tillage, in which more activity was observed for more substrates. Linear discriminant analysis was able to distinguish crops, specially ervil against cereals (Figure 1 C). Results obtained by API-ZYM strips were similar. Only two out of 19 enzymatic reactions resulted statistically significant for tillage factor and none for crop: Lipase C14 [4] and beta-Glucosidasa [16]. None of biodiversity indices resulted statistically significant. Minimum differences could also be observed in figure 1 (B). Finally, linear discriminant analysis distinguished ervil from wheat and barley (Figure 1 D), although variability among crop samples was higher than for Biolog results. Either due to the number of substrates used, per triplicate in each plate, either due to the sensitivity of the chemical reactions evaluated, or due to the quantitative value obtained from measuring the test result with a plate reader, Biolog seems to have a higher resolution to assess differences among different samples.



A *B*

C *D*

Results obtained by soil samples analysed by Biolog Ecoplates (A) and API-ZYM strips (B) depending on crop (barley, ervil and wheat) and soil tillage management (MT, minimum tillage; NT, no-tillage). Linear discriminant analysis of Biolog (C) and API ZYM (D) results.

Conclusions

Nevertheless, API-ZYM is a simpler and cheaper method. Both tests are useful and cheaper tools for assessing soil microbial functional diversity among crops and also between soil tillage management, according to linear discriminant analysis, although have less resolution than other methods used as metagenomics.

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606/49. EFFECT OF INTERCROPPING CHICKPEA (CICER ARIETINUM) WITH A COMPANION PLANT ON WEED CONTROL DURING EARLY GROWTH

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Work centre:

(1) School of Agricultures - ESA. Angers. Francia

Summary:

Objectives, Description, Main Results & Conclusions

Grain legumes are key species for the agroecological and dietary transitions due to the numerous services they can provide. However, they are seldom introduced in cropping systems because of the technical barriers associated with their cultivation. For instance, chickpea is highly sensitive to weeds during its early growth leading to unstable yields at harvest (Yadav et al., 2007). Besides, very few references are available under European pedoclimatic conditions since most of chickpea global production is attributed to Asia. Many studies have shown that intercropping was a relevant lever to optimize resource use, improve weed control and stabilize yields (Corre-Hellou et al., 2011). This study investigates whether intercropping chickpea with a companion plant allows to facilitate its production by improving its competitive ability against weeds compared to the sole crop. In a field experiment carried out in western France (2023, 2024) we studied five chickpea-based intercropping systems using five species of companion plants varying in their morphological (growth habit, root system), physiological (nitrogen nutrition) and phenological traits (earliness): spring oat (Avena sativa), Egyptian clover (Trifolium alexandrinum), white mustard (Sinapis alba), faba bean (Vicia faba) and Sudan grass (Sorghum x drummondii). The intercrops were sown in an additive design (100:50) in a randomized complete block design with 8 replicates. Mechanical regulation of the companion plant and weeds in chickpea's inter-row was studied as a means to manage interspecific competition leading to two different treatments : mowing at chickpea flowering or no mowing. Ground cover and accumulation of dry matter (chickpea, companion plant, weeds) were measured. Differences in ground cover and weed suppression ability were observed between intercrops. Apart from producing references about chickpea-based intercropping systems, this study provides further knowledge on mechanical regulation as a lever to manage trade-offs between services in intercrops.

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606/52. INTERCROPPING OF FABA BEANS AND PEAS IN ORGANIC FARMING SYSTEMS

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Summary:

Objectives:

The main objectives are to see how different phenotypes/growth habits responds to various seeding ratios (pea : faba bean) in terms of competition, standability, and seed- and biomass yield. We also want to see if this intercropping combination can facilitate cultivation of a wider range of pea varieties (i .e landraces and old cultivars) that might have added value related to ecosystem services as well as culinary and cultural attributes.

Method/Description:

During three years (2021-2023) we tested various combinations of pea and faba bean varieties (10 pea and 3 faba types, sole crop controls for a subset of them) with very distinguished growth habits. The plot sizes were 3x2 m and we used complete randomized block design with three replications (26x3 plots). Sub-samples of 1 m2 were taken in each plot for weeds, seed, and biomass yields. Plant legth/population stand height were measured every 15 days and early ground cover were measured with the Canopeo-app developed at Oklahoma State University. Days from sowing were measured for 50% flowering as well as pod fill and maturity.

Main Results:

The harvest from the third year is still being processed. Data from the first two years indicates that this intercropping combination can facilitate old cultivars to have similar LER values as modern pea cultivars under organic (low input) conditions. We have also seen how different cultivars differ in their weed competition as well as competition on the companion crop.

Conclusion:

This intercropping combination might be an alternative to cereal-pea intercropping when farmers wants to maximize the benefits of legume cultivation (i e. N-fixation and high protein content) for the legume spot in his/her crop rotation. Especially for biomass production we have seen strong indications that pea and faba bean intrecropping can enhance the yields by facilitating cultivation of more vigorous pea cultivars.

606/64. BARLEY – PEA INTERCROPPING: EFFECT OF SOWING DENSITY ON HAY YIELD

Authors:

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Summary:

Objectives

Legume – cereal mixed cropping is an interesting way to achieve sustainable productions by its agronomic advantages like to increasing biodiversity, improving resource use efficiency (light, nutrients, soil…), and enhancing crop resilience. To accomplish this goal, sowing density should be optimized to improve resource use efficiency and crop yield simultaneously (Li et al., 2013).

The objective of this work was to evaluate the impact of different sowing densities on the hay yield of a barley landrace – commercial fodder dry pea intercropping.

Concise description of the work (materials & methods)

To achieve this goal, during 2020-2021, field trials were carried out under organic farming at three different sites located in north central Spain - Castrillo de la Guareña, Viñalta and Zamadueñas - representing low, medium and high potential yielding, respectively.

Treatments consisted of combinations of four seed rates (0, 33, 66 y 100 % sowing density of barley and pea as mono-crops) and an additional 50 – 50 combination, so 16 treatments were assayed in a four-replication randomized complete block experimental design (12 m2 plot- size). At each site, two samples of 0,25 m2 per plot were mowed at milk grain stage. Aboveground biomass was oven-dried (72°C, 72h), weighed and evaluated as hay yield.

An analysis of variance (ANOVA) was carried out considering treatment, location and their interaction as factors. All factors included in the analysis were significant ($p\text{-value} \leq 0.05$). Due to the influence of the location, representing 69% of Sum of Squares in the model compared to treatment (13%) and interaction (5%), a surface response analysis was performed for each location. The optimal combination of seed rates to obtain the highest hay yield was estimated through a surface response analysis (Inouye, 2001) choosing the highest adjusted root squared model between linear and quadratic regression simulations including predictors interaction.

Main Results

A second-order polynomial regression model without interaction and without pea quadratic term was the significant model ($p\text{-value} \leq 0.05$) with the highest R-squared in all sites (adjusted R2-values were 0.39, 0.42 and 0.52 for Castrillo, Viñalta and Zamadueñas, respectively). Barley seed rate was distributed in a quadratic way, while pea was distributed in a linear way.

Pea seed rate was significant only in Castrillo de la Guareña, the low potential yielding site: yield increased 9.2 kg ha-1 per unit of pea seed rate. According to the model, maximum yield would be obtained by 85.2% barley – 100% pea (Fig 1).

In the medium and high potential yielding sites, pea linear term was no significant in the model, so hay yield seems to depend on barley exclusively. Biomass increased in a quadratic way per each barley sowing density unit until 68.6% and 75.6%, the optimum seed rate in Viñalta and Zamadueñas, respectively.

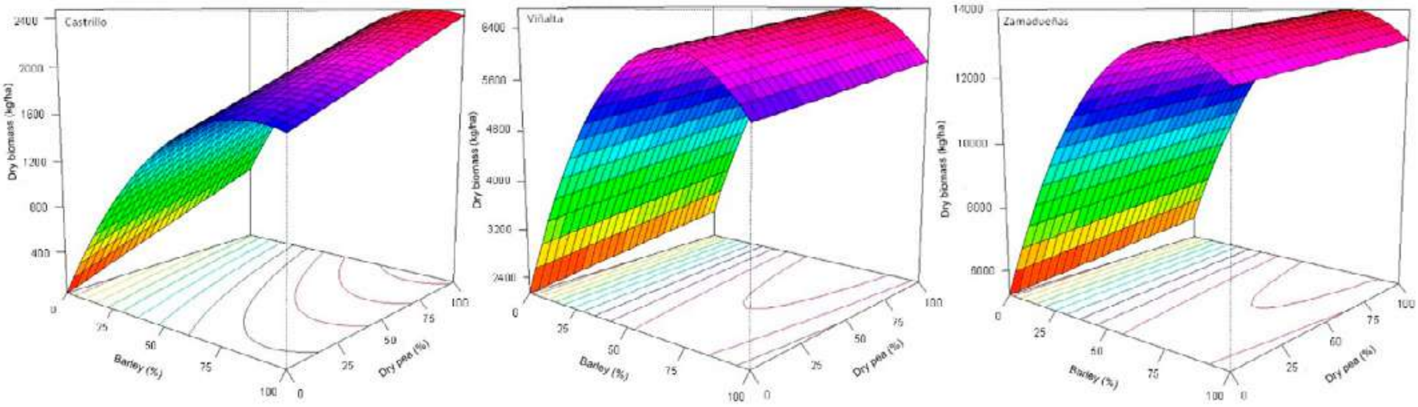

Fig 1. Response surface analysis for the mixed crop hay yield (kg ha-1) as a function of barley and pea seed rates.

Conclusions

The performance of mixed cultivation depended extremely on the locality.

In this study, regarding hay yield, there was no advantage of intercropping versus typical barley monocrop in the medium and high potential yielding sites.

The sowing density had a great impact on hay yield, which depended mainly on the dominant crop (barley), while dry pea had significant influence only in the low potential yielding site.

Therefore, cereal-legume intercropping was confirmed as an interesting strategy to increase hay yield in low input production systems.

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606/71. INTRODUCING SOYBEAN IN CROP ROTATIONS FOR CLIMATE CHANGE MITIGATION IN NORTH-EAST EUROPE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

In the current European cropping systems only 1.5% of arable land is dedicated to cultivating legumes. It leads to a deficit of 70% of high-protein crop commodities for animal feed in Europe. This way current food chains are unsustainable from consumers, producers, climate and the environment points of view. Our research aims to investigate if diversification of crop rotation by soybean can advance the implementation of Green Deal goals, such as climate change mitigation and adaptation and what role in it has plant-specific symbiotic and endophytic bacteria. Our study presents the data for 9 last years of soybean cultivation in Lithuania.

Our results showed that soybean might be a good tool to ensure food security in the face of climate change (Toleikiene et al., 2021). With increased co-occurrence of drought and uneven moisture during the growing season, soybean competes well in productivity with traditional legume plants - field pea and fababean. Isotope based quantification of nitrogen fixation in symbiosis with different microorganisms gave important tender to climate change mitigation. Using B. japonicum ensured effective N fixation from the atmosphere for soybean up to 175 kg N ha-1. Several bio stimulants were used to stimulate higher efficacy of different cultivars of Bradyrhizobium japonicum, grouped with different soybean varieties. The effect of herbicides and fertilisation on nitrogen fixation was investigated. Also, soybean was shown to have equal or higher rotational residual effect on subsequent cereals than field pea. Our study gives the list of advantages and disadvantages of cultivating soybean plants in North-East Europe and also presents the symbiotic bacteria cultivars for effective nitrogen fixation.

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606/83. CEREAL-MAIZE VS. LEGUME-MAIZE DOUBLE-CROPPING: IMPACT ON CROP PRODUCTIVITY AND NITROGEN DYNAMICS UNDER IRRIGATED MEDITERRANEAN CONDITIONS

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Summary:

Objectives

In irrigated maize systems, the inclusion of another crop during the fall season can increase economic profitability and provide environmental benefits (Liu et al., 2022). Besides, the introduction of legumes can reduce the dependence of the rotation on nitrogen (N) inputs. Therefore, double-annual cropping is an interesting diversification strategy to increase these high-intensive management agrosystems sustainability (Franco-Luesma et al., 2022). The objective of this work was the evaluation of the impact of crop diversification system and different N rates on the crop productivity and N dynamics in a flooded irrigated maize under Mediterranean conditions.

Concise description of the work (materials & methods)

To achieve this objective, two double-cropping systems (barley-maize, BM; pea-maize, PM) and 3 N rates (unfertilized, 0N; medium: MN; high rate, HN) were evaluated in a field experiment located in Zaragoza (NE Spain) during two years (2019; 2020). In BM, the N rates assessed were 0, 125 and 250 kg N ha-1, and 0, 200 and 400 kg N ha-1 for the barley and maize phases, respectively. Meanwhile, in PM, pea did not receive N fertilization and the maize N fertilization was reduced in 50 kg N ha-1 compared to BM system. Crop productivity was evaluated after harvest. N use efficiency (NUE) was calculated for each phase and system. Soil mineral N (Nmin, 0-90cm) was determined prior to sowing and after harvest.

Main Results

Both years, barley achieved a greater yield than pea. However, the maize following pea obtained a higher grain yield than the one following barley in 2019, and no differences were found in 2020 (Fig.1A). Both MN and HN led to higher yields in 2019, whereas the three N rates were differentiated in 2020 (Fig.1B). The legume had a residual effect on the following maize N uptake, as values were greater in PM than in BM in the unfertilized plots (Fig.1C).

N use efficiency indicators showed a better N use in 2019 than in 2020. The PM had a higher NUE than BM, and MN greater than AN; being differences more marked in 2019.

Prior to maize sowing in 2019, the HN rate in barley led to higher soil Nmin than other rates, and than pea, mainly in upper layers, whereas no differences were observed in 2020. Both years, after maize harvest, the cumulated Nmin in the profile was higher in BM than in PM (62±8 vs. 16±3 kg N ha-1).

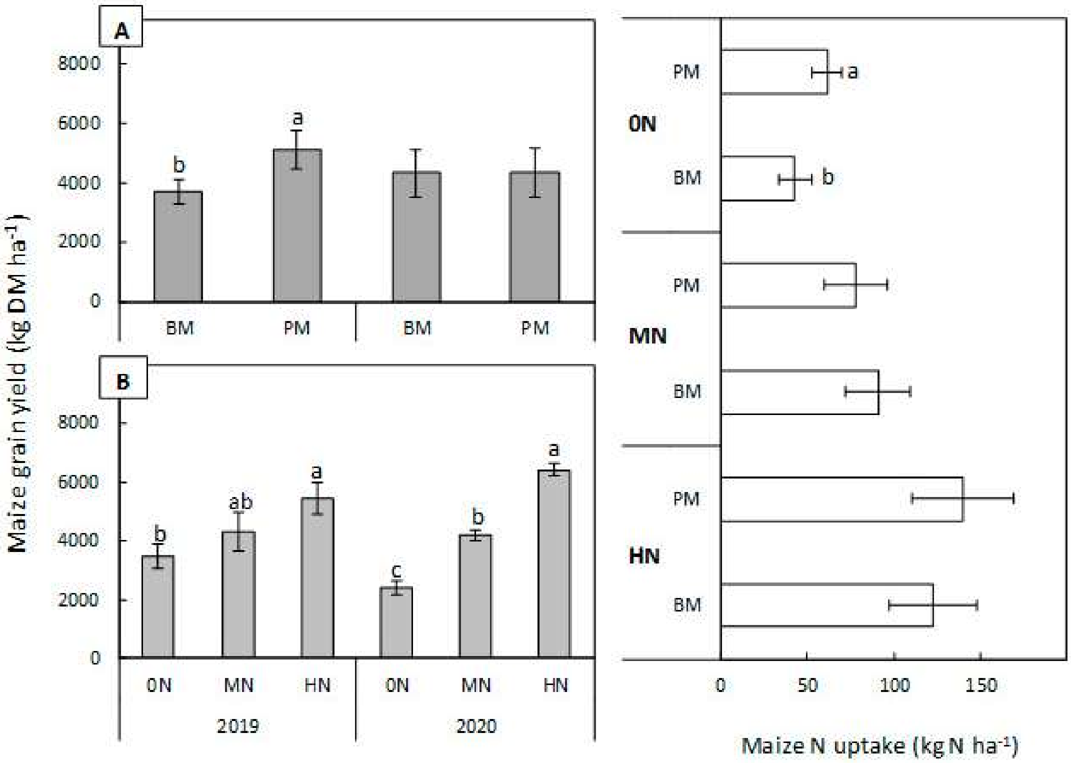

Fig.1. Maize grain yield presented for the treatments resulting from: (A) the cropping system (BM, PM) through the year (2019, 2020); and (B) the N rate (0N, MN, HN) through the year. (C) Maize N uptake presented for the treatments resulting from the rotation through N rate. Letters indicate differences between treatments (P≤0.05).

Conclusions

In this study, both cropping diversification and N rates affected productivity and N dynamics. An increase in the N rate led to higher crop productivity but differences between medium and high N rates were observed the second year. The medium N rate led to a better N use. The inclusion of a legume allowed the N rate reduction in the following crop and increased the maize yield one of the years. These results together with the pea influence on the following maize N uptake pointed out the importance of considering the N release from legumes. Besides, this did not lead to a higher Nmin after pea, and values were lower than BM after maize harvest, therefore, the pea-maize cropping system showed a greater potential to reduce the nitrate leaching risk.

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606/88. YIELD RESPONSE OF TWO CHICKPEA (Cicer arietinum L.) CULTIVARS TO RHIZOBIUM INOCULATION AND P-FERTILIZER APPLICATION

Authors:

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Summary:

Objectives

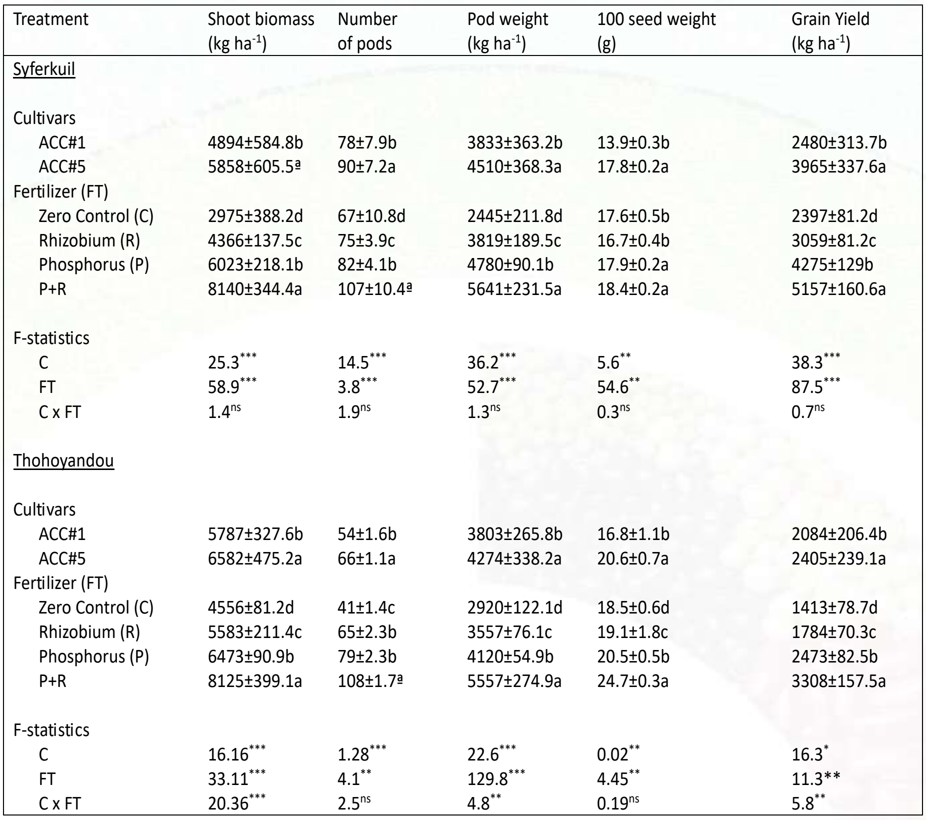
Low soil fertility is one of the major constraints to the productivity of chickpea and other legumes. Application of fertilizer (inorganic, organic, biofertilizer) may enhance growth, yield and overall productivity of legumes especially in marginal soils. We have shown that Rhizobium inoculation and P fertiliser, either individually or in combination, increased accumulation of rhizosphere nutrients (Ogola et al., 2023). However, there is a dearth of information in literature on the effect of phosphorus and rhizobium inoculation on yield and yield components of chickpea in contrasting soil types. Therefore, this study assessed the effects of P fertilizer application and rhizobium inoculation, either individually or in combination, on grain yield and yield components of 2 Desi chickpea cultivars (ACC#1 and ACC#5) at two sites, characterized by contrasting soil types, in NE South Africa.

Concise description of the work (materials & methods)

A field experiment was established at Syferkuil (loamy sand soil) and Thohoyandou (clay soil) in 2018. The treatments, each with three replicates in a randomized complete block design, consisted of a factorial combination of four fertilizer levels: zero control, 90 kg P ha-1, rhizobium inoculation, 90 kg P ha-1 plus rhizobium inoculation and two desi chickpea genotypes (ACC#1 and ACC#5).

Main Results

Grain yield was consistently greater in ACC#5 compared to ACC#1 (2405 vs 2084 kg ha-1 & 3965 vs 2405 kg ha-1) at both sites (Table 1). A similar trend was observed with the yield components (Table 1). Also, rhizobium inoculation (3059 & 1784 kg ha-1), P application (4275 & 2473 kg ha-1), and P application plus rhizobium inoculation (5157 & 3308 kg ha-1) recorded greater grain yield compared to the zero control (2480 & 1413 kg ha-1), respectively at both sites (Table 1) but a combination of P and rhizobium inoculation exhibited greater increases suggesting an additive action of rhizobium inoculation and fertilizer P (Wolde-meskel et al., 2018). The response of yield components followed a similar trend to grain yield (Table 1). The better yield performance by ACC#5 and a combination of P and rhizobium inoculation could be associated with greater accumulation of rhizosphere nutrients that was observed in those treatments (Ogola et al., 2023).



Treatment	Shoot biomass (kg ha^{-1})	Number of pods	Pod weight (kg ha^{-1})	100 seed weight (g)	Grain Yield (kg ha^{-1})
Syferkuil					
Cultivars					
ACC#1	4894±584.8b	78±7.9b	3833±363.2b	13.9±0.3b	2480±313.7b
ACC#5	5858±605.5ª	90±7.2a	4510±368.3a	17.8±0.2a	3965±337.6a
Fertilizer (FT)					
Zero Control (C)	2975±388.2d	67±10.8d	2445±211.8d	17.6±0.5b	2397±81.2d
Rhizobium (R)	4366±137.5c	75±3.9c	3819±189.5c	16.7±0.4b	3059±81.2c
Phosphorus (P)	6023±218.1b	82±4.1b	4780±90.1b	17.9±0.2a	4275±129b
P+R	8140±344.4a	107±10.4ª	5641±231.5a	18.4±0.2a	5157±160.6a
F-statistics					
C	25.3***	14.5***	36.2***	5.6**	38.3***
FT	58.9***	3.8***	52.7***	54.6**	87.5***
C x FT	1.4^{ns}	1.9^{ns}	1.3^{ns}	0.3^{ns}	0.7^{ns}
Thohoyandou					
Cultivars					
ACC#1	5787±327.6b	54±1.6b	3803±265.8b	16.8±1.1b	2084±206.4b
ACC#5	6582±475.2a	66±1.1a	4274±338.2a	20.6±0.7a	2405±239.1a
Fertilizer (FT)					
Zero Control (C)	4556±81.2d	41±1.4c	2920±122.1d	18.5±0.6d	1413±78.7d
Rhizobium (R)	5583±211.4c	65±2.3b	3557±76.1c	19.1±1.8c	1784±70.3c
Phosphorus (P)	6473±90.9b	79±2.3b	4120±54.9b	20.5±0.5b	2473±82.5b
P+R	8125±399.1a	108±1.7ª	5557±274.9a	24.7±0.3a	3308±157.5a
F-statistics					
C	16.16***	1.28***	22.6***	0.02**	16.3*
FT	33.11***	4.1**	129.8***	4.45**	11.3**
C x FT	20.36***	2.5^{ns}	4.8**	0.19^{ns}	5.8**

Table 1. Effect of Cultivar, Rhizobium and P application on gran yield and yield components of chickpea grown at Syferkuil (loamy sand soil) and Thohoyandou (clay soil) in 2018.
*Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and ns = not significant*

Conclusions

These preliminary results indicate that chickpea productivity in this region may be enhanced by a combined application of fertilizer P and rhizobium inoculation irrespective of soil type but further studies, incorporating more chickpea genotypes, are recommended before definite conclusions can be drawn

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606/99. MIXED INTERCROPPING BETWEEN FOUR LENTIL AND THREE HULL-LESS BARLEY VARIETIES: PERFORMANCE UNDER SWISS CLIMATIC CONDITIONS

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Summary:

Objectives, Description, Main Results & Conclusions

The adoption of alternative systems promoting underrepresented arable crops has the potential to increase agrobiodiversity while maintaining high crop yields and quality. Mixed intercropping (MIC) of niche legumes with cereals is a

promising approach to achieve these goals. Within the European project CROPDIVA, a two-year field experiment was conducted at two locations in central Switzerland to assess the agronomic performance of three spring hull-less barley varieties (Oak Ruby, AF Cesar, Goljiat) and four spring lentil varieties (Anicia, Beluga, Grüne Berry, Château) under a low-input system. Using an identical overall plant density, different cropping systems (pure versus mixed) and fertilisation levels were compared. It was assessed whether MIC would improve land use efficiency, reduce the pressure of weeds, diseases and pests, and reduce lodging of lentils, while achieving comparable yields to those crops in pure stands. Phenotypic evaluations included BBCH recording, number of tillers, weed volume, incidence of diseases and pests lodging and synchronisation of maturity. Post-harvest evaluations focused on yield and quality (hectolitre weight, protein content, separation ability). Additionally, seed health tests for all hull-less barley varieties were conducted to assess possible effects of cropping systems and locations. Preliminary results indicate that, regardless of the location, the lentil varieties Anicia and Grüne Berry achieved higher yields compared with pure stands, while Beluga showed no significant differences between the cropping systems. All mixtures, except Grüne Berry, showed a greater land use efficiency, proven by land equivalent ratios values above 1. Furthermore, the hull-less barley varieties AF Cesar and Oak Ruby were found to be more suitable companion plants for lentils compared with Goljiat, which overgrew all lentil varieties. The results of currently running analyses, including the seed health and various quality parameters, will be presented and discussed. This research is part of the CROPDIVA project (Horizon 2020, grant agreement NO 101000847)

606/137. INTERCROPPING OF VETCH (VICIA SATIVA L.) AND CEREALS FOR GRAIN AND PROTEIN PRODUCTION

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Summary:

Objectives

In organic farming systems, grain legumes offer, next to agronomic advantages (Böhm et al. 2020), a protein-rich and local produced feed stuff. Faba bean and pea are often used grain legumes but have certain demands on their environment. Common vetch (Vicia sativa L.) which can be cultivated even in dry climates and on soils of low pH could be an alternative for the nutrition of monogastrics. The interdisciplinary project 'WickEiweiss', funded by the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture, examined the production and use of vetch for feeding. The objectives for the part of crop production were to find mixtures of high grain yields, high protein outputs as well as a good weed suppression.

Concise description of the work (materials & methods)

A field trial was conducted from 2017 to 2019 in a randomised block design with four replications and a plot size of 37.5 m^2 on a loamy soil at the Thünen Institute of Organic Farming. Five vetch cultivars of different growth types [Slovena, Jaga, Ina, Toplesa, Berninova] were tested sole cropped [120 germinable seeds per square meter (germinable seeds m^{-2})] and intercropped in alternating rows with spring wheat [2017-2019], spring rye [2017] and spring triticale [2018, 2019]. Each cereal was also grown sole cropped with 400 germinable seeds m^{-2}. The mixtures were laid out as a replacement design in the vetch:cereal-seed ratios of 25%:75%, 50%:50% and 75%:75% depending on the sole cropped sowing density. At stem elongation, flowering and ripening stage of vetch 0.5 resp. 1.0 m^2 was harvested for weed biomass determination. At ripening, the crops were harvested by a plot combine harvester and subsequently the grains were separated. Afterwards, grains of vetch and cereals as well as the weed biomass were grinded and analysed for nitrogen which was multiplicated with 6.25 to get the protein content. All statistical evaluations were done with SAS (SAS Institute Inc., Cary, NC, USA). Regression curves were estimated for the total and partial grain and protein yields with the procedure GLM. The evaluation of the weed biomass development over time were analysed with a time series and a post-hoc Tukey-test.

Main Results

The weather conditions in the three years were quite different in precipitation and temperature. The total and vetch grain yield were highly influenced by the weather, so in the dry and warm year 2018 the proportion of vetch in the mixture was low, but a total yield loss was prevented by the cereal. The high-growing vetch Slovena reached the highest total grain yields (3.4 – 3.7 Mg ha-1) in a share of 30-48 % vetch in the seed mixture with spring wheat. Also, the highest total protein yields were achieved in 2017 and 2019 by the vetch cultivar Slovena (1.1 Mg ha-1) in a mixture with spring wheat and a share of 40 % vetch in the seed mixture (Figure).

The weed biomass in the crop stand increases with an increasing share of vetch in the mixture but all mixtures had a better weed suppression than the corresponding sole cropped vetch. High-growing vetches and mixtures with a share of cereal over 50 % had a better weed suppression than low-growing vetches and mixtures of lower cereal proportions.

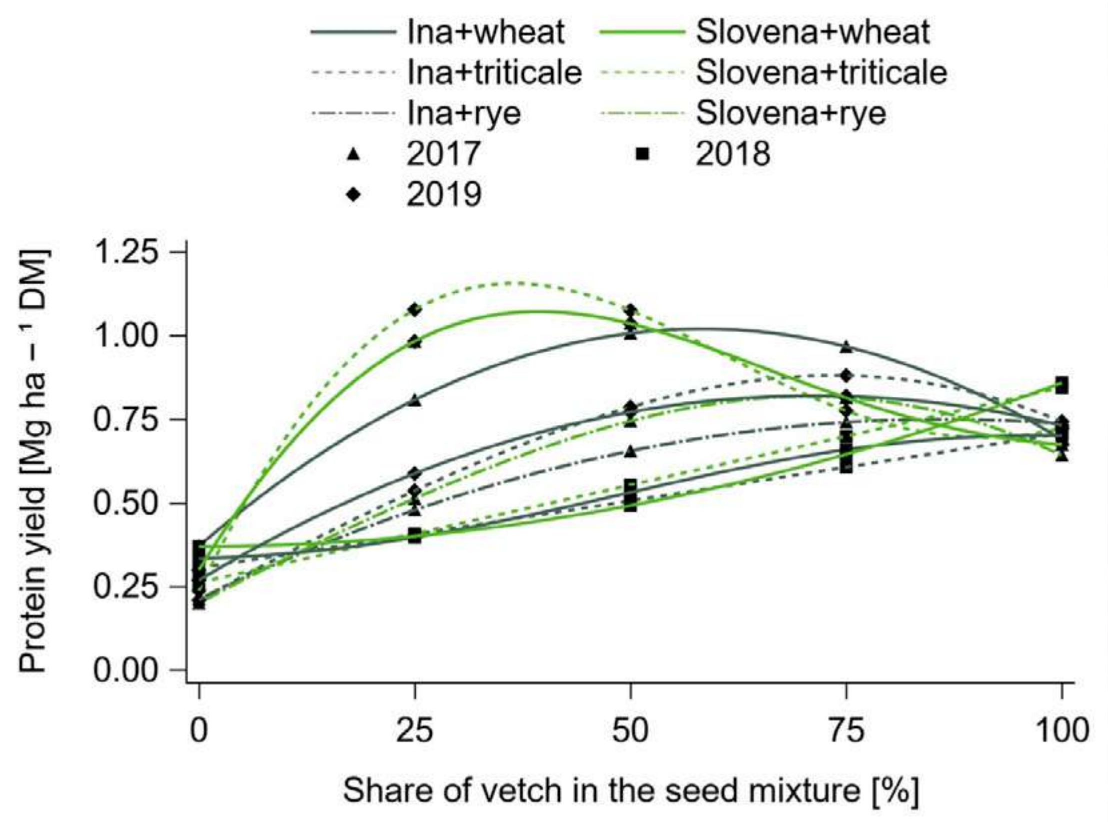

Figure. Regressioncurves of the total protein yield of a low-growing (‚ Ina') and a high-growing (‚ Slovena') vetch intercropped in different proportions of spring wheat, spring triticale and spring rye from 2017 to 2019. A share of 0 % vetch in the seed mixture is equal to the sole-cropped cereal whereas 100 % is the sole-cropped vetch.

Conclusions

Vetches can be well intercropped with spring wheat and triticale, diversify crop rotations and are a protein-rich, local produced feed stuff.

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606/164. THE EFFECT OF INTERCROPPING WITH HAIRY VETCH ON THE GRAIN YIELD AND QUALITY OF WINTER WHEAT

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Summary:

Objectives, Description, Main Results & Conclusions

Intercropping of cereals and legumes is considered as an opportunity to increase the grain yield and quality in organic farming. The field experiment was carried out at Estonian Crop Research Institute (present: The Centre of Estonian Rural Research and Knowledge, METK) on organic land during two growing seasons (2019/20 and 2020/ 21) to evaluate the effect of intercropping winter wheat with hairy vetch on the yield and quality (protein and gluten content) of winter wheat. Winter wheat was sown at

the seeding rate of 350 germinating seeds m -2 as monoculture and in a mixture with hairy vetch. In the mixtures, three different seeding rates of hairy vetch were used (5, 10, and 20 kg ha-1). The results showed that in the first growing season (2019/20) the yield of winter wheat was significantly lower in intercropping with hairy vetch compared to winter wheat monoculture. The yield decreased and lodging increased with the higher proportions of hairy vetch. At the same time, hairy vetch improved the grain quality of winter wheat, protein and gluten content being highest with higher seeding rates of hairy vetch. In the second growing season (2020/21) hairy vetch over-wintered poorly and did not affect the yield or quality of winter wheat. It can be concluded that in intercropping systems, varieties with good winter hardiness should be selected.

606/186. USE OF INTERCROPPING TO CONTROL WEEDS IN FABA BEAN

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Summary:

Objectives

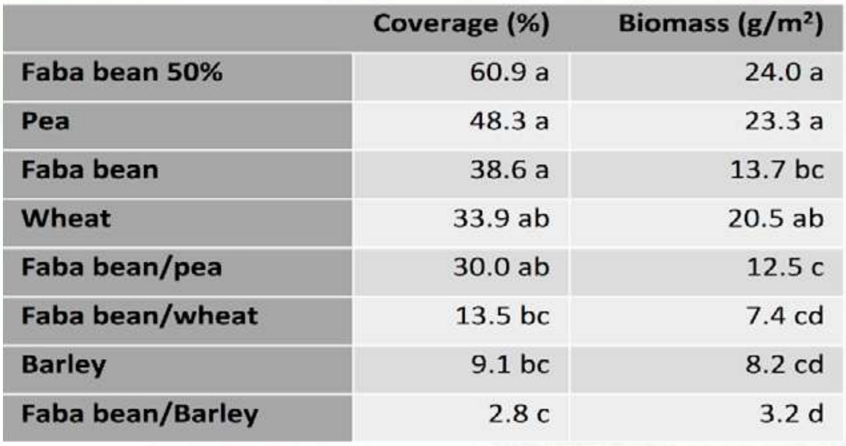
One of the main problems for the cultivation of faba bean is the control of weeds, which can reduce yields by as much as 50% (Karkanis et al., 2018). The only effective method of control is the use of herbicides, but this is an expensive option with a negative environmental impact. Intercropping appears as an alternative for weed control for different crops and environments (Gu et al., 2021). The main objective of our work was to establish if it is possible to control weeds in faba bean in the Guadalquivir Valley (South of Spain) by intercropping. In case this was so, a second objective was to investigate the mechanisms behind this control.

Concise description of the work (materials & methods)

Four field trials were conducted in Cordoba, mixing faba bean with either wheat, barley or pea. Two intercropping systems were tested: alternate with replacement at 50% and alternate with addition at 100%. Pure stands of the tested crops were also included, as well as faba bean at 50% density. Plot size was 2.8 x 3 m2. Weed coverage (as the percentage of plot surface covered by weeds) was assessed and weed biomass per plot was obtained. Additionally, an experiment under controlled conditions was carried out to investigate the possible role of allelopathy by barley in the suppression of weeds (Kremer & Ben-Hammouda, 2009). To this effect, two different experiments were performed: in one, barley plants were grown in pots and removed immediately prior to sowing four different weed species. In the other one, weeds were sown without removing barley plants. Controls were included in both cases. The number of weed plants and its biomass was determined 45 days later.

Main Results

Alternate intercropping with replacement did not provide control of weeds as compared to faba bean monocrop in any of the tested cases. With addition intercropping the combination of faba bean with barley proved to be effective in controlling weeds: 2.8% of coverage and 3.2 g/m2 of weed biomass in comparison with 38.6% and 13.7 g/m2 in faba bean monocrop (Table 1). The controlled-conditions experiment found that all four weed species were suppressed in those pots where barley was growing or had previously grown, with reductions as high as 75% for weed plant number and 50% for weed biomass. This points to a clear allelopathic effect by barley plants.



	Coverage (%)	Biomass (g/m²)
Faba bean 50%	60.9 a	24.0 a
Pea	48.3 a	23.3 a
Faba bean	38.6 a	13.7 bc
Wheat	33.9 ab	20.5 ab
Faba bean/pea	30.0 ab	12.5 c
Faba bean/wheat	13.5 bc	7.4 cd
Barley	9.1 bc	8.2 cd
Faba bean/Barley	2.8 c	3.2 d

Table 1. Average coverage (% of surface of plot) and biomass of weeds in the different treatments tested in the field experiments. Crop mixtures were made by addition alternate intercropping. Different letters in each column mean significant differences (Friedman's test, p<0.05).

Conclusions

Addition alternate intercropping of faba bean with barley is a promising method to control weeds in the Guadalquivir Valley. Allelopathy by barley appears as one of the main mechanisms explaining the weed reduction. Future work should focus on finetuning the best conditions for the mixture and identifying the best genotypes for this strategy.

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606/199. HOW TO ENHANCE LEGUME N_2 FIXATION AND N ACCUMULATION IN COMPLEX RAPESEED-SERVICE PLANT INTERCROPS?

Authors:

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Summary:

Objectives

Intercropping of frost sensitive service plants (SPs) with winter rapeseed (Brassica napus L.) became a common practice in Switzerland. Legume SPs intercropped with rapeseed are known to contribute to weed control, enhance soil microbial activity and contribute to nitrogen nutrition of rapeseed in spring (Dayoub et al. 2022; Drut et al. 2023). However, non-legume SPs are more competitive against weed than legumes. In order to combine these services, Swiss farmers designed complex mixtures of 4 to 10 SP species that included both legumes and non-legumes. Therefore, the goal of our study is to investigate the effect of SP mixture complexity on the potential services provided by three legume SPs with a specific focus on N_2 fixation.

Concise description of the work (materials & methods)

To answer this question, a greenhouse and a field experiment were carried out. The greenhouse experiment consisted of growing faba bean (Vicia faba L.) and grass pea (Lathyrus sativus L.) alone, or in intercropped with one to three other species in mesocosms. This experiment was fully described in Bousselin et al. (2021). In addition to this experiment, a field study was carried out from August to October 2019 in Swiss field conditions. In this experiment faba bean and Berseem clover (Trifolium alexandrinum L.) were intercropped with rapeseed alone or in mixtures of 2 to 3 SP species. In both experiments the soil was labeled using 15N and the proportion of legume N derived from N2 fixation (%Ndfa) was calculated thanks to the isotopic dilution method. The plant biomass and N contents were also measured.

Main Results

Our results demonstrate the high potential of fixation of both faba bean and Berseem clover either intercropped alone with rapeseed, or in more complex mixtures. The grass pea biomass was strongly affected by rapeseed competition which led to low N fixation and accumulation. The competition with rapeseed increased the %Ndfa of legumes both in field and greenhouse conditions. In intercropping with more than two SP species, the legume %Ndfa remained unimpacted and did not further increase (Figure).

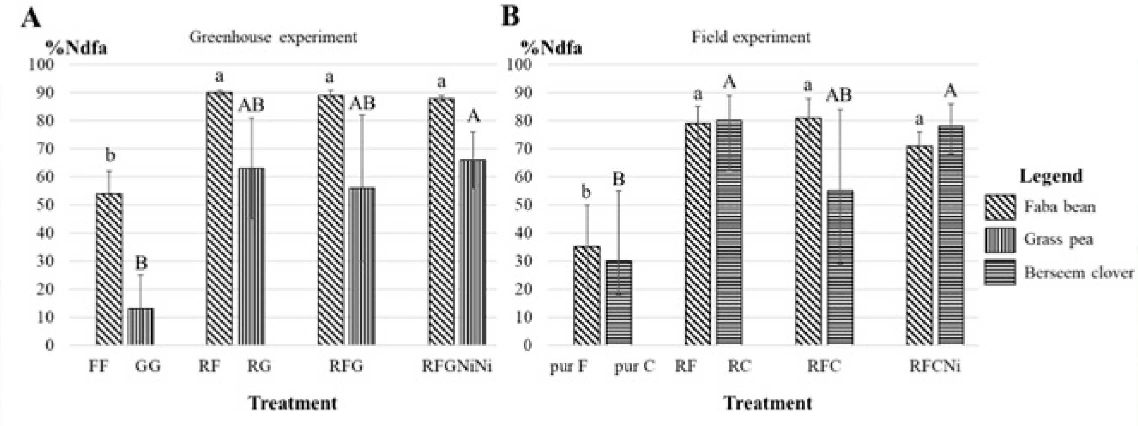

Figure. Legume N_2 fixation depending on intercrop species diversity.
Values are means of four replicates; bars represent standard deviations. Mean values with different letters were significantly different (P <0.05; Tukey HSD). Lower case letters are used to compare the %Ndfa of faba bean values across mixture complexity gradient; uppercase letters are used to compare the %Ndfa of grass pea and Berseem clover in the greenhouse and field experiment respectively. R: Rapeseed, F: Faba bean, G: Grass pea, C: Berseem clover and Ni: Niger (Guizotia abyssinica). In the greenhouse experiment the number of individual plants corresponds to the number of occurrences of the species code in the treatment name. In the field experiment rapeseed was sown at its recommended sowing density and the sowing density of each SP was equal to its recommended sowing density divided by the number of species in the mixture.

Conclusions

Consequently, although non-legumes are better competitors against weeds, they do not positively contribute to nitrogen accumulation in the SP mixtures compared to mixtures with only legumes. The number of species in the SP mixture did not allow to achieve high level of N fixation and accumulation together with high level of weed control in the meantime. However, it can contribute to adjust the trade-off between competition against weed and N related services.

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606/207. NITROGEN NUTRITION INDEX: A KEY INDICATOR OF THE AGRONOMIC DIAGNOSIS IN PEA

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Summary:

Objectives

In the context of the climate change and of pesticids reduction, diversification of crops can give solutions to improve the services given by the agro-ecosystems. Pea, as a legume crop, is autonomous for nitrogen, which can help to the agroecologic transitions. However, it was observed from the 5 last years in France that yields are low for this crop. Climate, drought stresses, in particular and high temperatures during flowering period, lead to an important limitation of seed number produced (Guilioni et al., 2003). They are often cited to explain these low perfomances (Benezit et al., 2017). Furthermore, Nitrogen Nutrition Index (NNI), calculated from the measures of dry matter and nitrogen content in the plant at the beginning of flowering, each year in the variety trials network for winter and spring pea, suggest that limiting factors are present at the beginning of the cycle, and may induce a default in nitrogen nutrition, which can reduce yield but also protein content in the seeds. The objective of the study presented here was to identify the limiting factors of NNI in a fields plots network in France in 2021 and 2022 (Cap Protéines project).

Concise description of the work (materials & methods)

NNI was calculated from the measures of dry matter and nitrogen content in the plant in 140 fields plots. Some information relative to the crop management (sowing date, soil type), climatic data, quantity of nodules or pests attacks (weeds, diseases, insects) were recorded.

Main Results

NNI values are more often lower than 1 for spring pea than for winter pea (Figure), which indicates that nitrogen nutrition is more often limited for this type of pea. Soil type could explain differences in NNI. Indeed, weak values were measured for the both types of pea in calcareous soils, especially in the Centre-West and the North-East of France. For spring pea, 2022, drier year than 2021, led to significant lower values of NNI. This underlines the need of good water conditions to promote nodulation. The sowing date has an impact on NNI such as some climatic variables. NNI is also related to the quantity of nodules, which were counted at beginning of the flowering. Fields plots with low nodules numbers at this stage achieved low NNI and low yields. This observation highlights the importance of successful installation of the crop and of a quick nodulation.

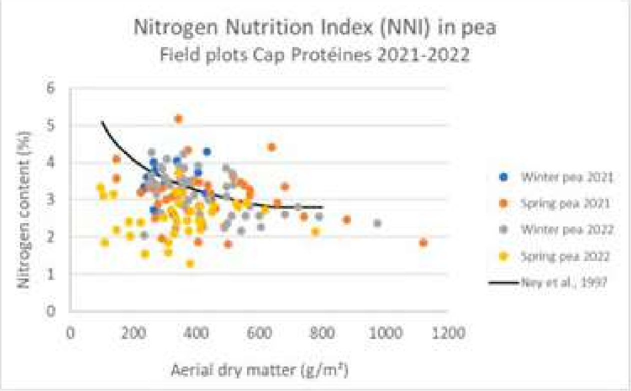

Figure. Nitrogen Nutrition Index measured in French fields in 2021 and 2022. Aerial dry matter and nitrogen content measured in pea plants were positioned relative to the dilution curve established for pea by Ney et al. 1997.

Conclusions

As shown in our results, at early stages, soil type, climate or sowing date could have effects on the NNI but not pests. Some results are in line with those obtained on soybean in a study in 2021-2022.

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606/213. EFFECT OF CUTTING STAGE ON BIOMASS YIELD, PROTEIN YIELD AND PROTEIN CONTENT OF DIFFERENT VETCH SPECIES AS WINTER CATCH CROP IN NORTHERN GERMANY

Authors:

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Summary:

Objectives

Legume winter catch crops increase biodiversity, root penetration over autumn and winter improves soil structure, protect against soil erosion and suppress weeds. When harvested the below ground biomass and crop residues remain in the soil and contribute to the optimization of the nitrogen utilization for the following crop. The biomass on the surface can be used as protein-rich roughage – a feedstuff with very low food competition. The aim of the experiment presented is to test the suitability of three different vetch species - hairy vetch (Vicia villosa (VV)), Hungarian vetch (Vicia pannonica (VP)), and common vetch (Vicia sativa (VS)) - as winter catch crops for the use as roughage depending on the cutting stage in Northern Germany.

Concise description of the work (materials & methods)

In autumn 2020, a field trial with randomized block design with four replications was carried out organically managed experimental station (53°46´N, 10°30´E, soil texture: sandy loam). On 15th of September 2020, five varieties of VV (cv. Savane, Dr. Baumanns Ostsaat, Hungvillosa, Latigo and Villana), two varieties of VP (cv. Beta and Detenicka) and two varieties of VS with improved winter hardiness (cv. Carbure and Rubis) were sown at a sowing density of 250 seeds m-2. To determine the potential as roughage, the whole vegetation cycle was considered. Thus, at four cutting stages (BBCH: 51 - 59, 65, 69 - 71, 71 - 79) a part of each plot was harvested with a cutter bar mower (Agria, cutting height 6 - 8 cm). The biomass yields of the vetch cultivars and the dry matter (DM) contents were determined (24h, 105°C). The samples were dried at 40°C and subsequently ground to pass a 1 mm sieve for the determination of the crude protein content (Kjeldahl, N*6.25). The generalized least squares gls function of the "nlm" package with a repeated measurement model for the target variables biomass yield, protein yield and crude protein content was used for statistical evaluation (program R: R x64 4.3.) (Pinheiro J et al. 2023; R Core Team 2023).

Main Results

All varieties could be harvested after winter. The crude protein content of the crops decreased within the growing period, while the forage yield increased. VP and VV achieved a significant increase in biomass from bud to flowering stage (p>0.05). Yields of 4.1 t DM ha-1 (VV) and 5.6 t DM ha-1 (VP) were obtained. At flowering stage protein contents of 222 g kg-1 DM (VV) and 190 g kg-1 DM (VP) were achieved. There is no significant difference in protein yield between species in the first and second cut. In contrast VS achieved a significant increase in biomass yield at pod formation stage. At pod formation VS reached a biomass yield of 5.1 t DM ha-1 (VS) with a protein content of 217 g 100 g-1 DM (VS). In the last two cuts VS showed significantly higher crude protein yields than VV.

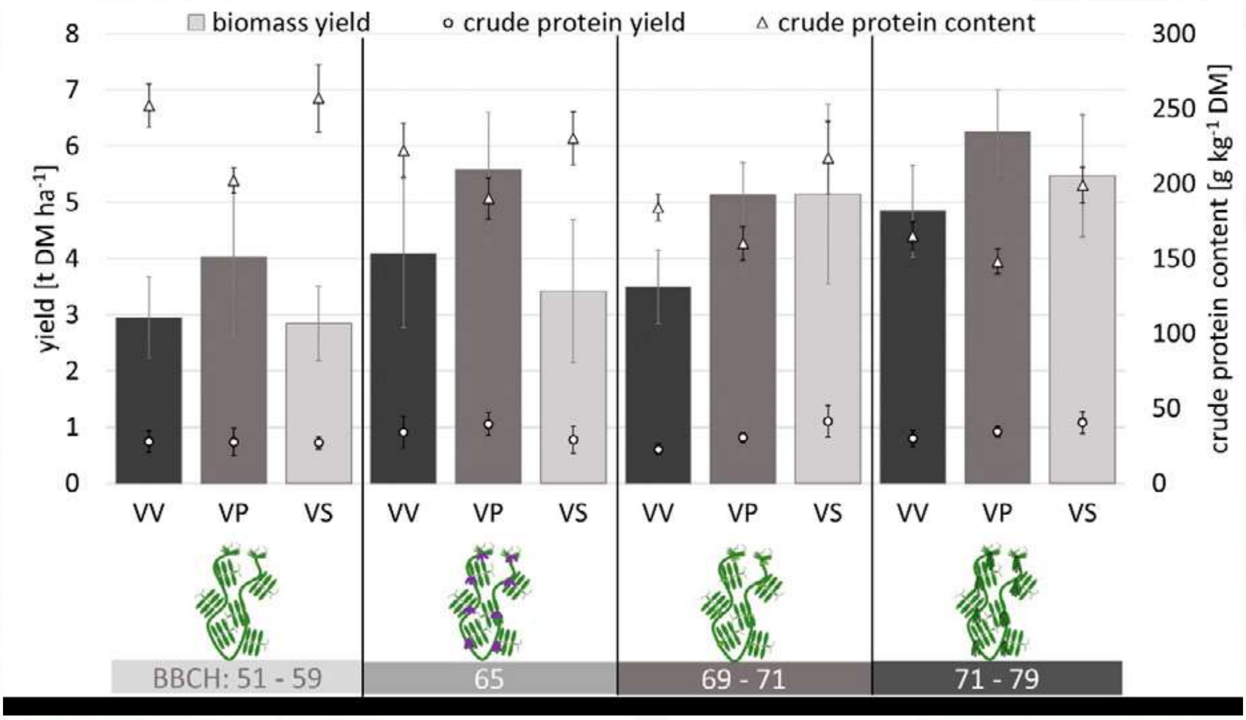

Figure. Biomass and protein yield as well as protein content of different Vicia species (hairy vetch (VV), Hungarian vetch (VP) and common vetch (VS)) at four different cutting stages.

Conclusions

The results indicate that all mentioned vetch species survive the winter and can be used as overwintering catch crop in Northern Germany. An individual cutting time for VV and VP (flowering stage) as well as VS (pod formation) are going to be suitable for the use as whole plant silage. In order to be able to make improved statements, further cultivation years need to be conducted.

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606/219. PRODUCTIVITY OF STOCKED AND PLANT-BASED LEGUME-BASED ORGANIC ROTATIONS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes are fundamental to organic cropping systems as they provide biologically fixed N which supports both livestock production and the following arable cropping phase. In livestock based organic systems, additional N is provided by the manure. In recent years, there has been increasing interest in plant-based systems where the fertility building phase is provided by short-term green manures.

In 1991, the replicated Tulloch organic rotation (02°15'W, 57°11'N) was established. This trial compared 3 years of grass/white clover ley and 3 years of arable cropping (T50) with 4 years of grass/white clover ley and 2 years of arable crops (T67). The arable crop prior to the ley was undersown with the grass/white clover ley. The plots were 0.08 ha, thus allowing for a group of sheep to graze the plots. Each phase of the rotation is present in every year. In 2007, the rotation based on a 4 year grass/white clover ley (T67) was changed to a stockless rotation (T0). This rotation included 5 years of arable cropping and a 1 year grass/red clover green manure. With the exception of potatoes, the arable crops were undersown (u/s) with clover. The beans, barley and wheat were undersown with white clover, while the oats were undersown with red clover. The soil is a sandy loam with approximately 8.3 % soil organic matter (loss on ignition) and pH 6. Long-term average (1992–2021) rainfall is 884 mm per year, and average daily temperature is 8.45°C. All courses of the rotation are present every year. See Willoughby et al. (2022) for more details on the trial. Here, we analyse the productivity of the ley and arable crops in the stocked and plant-based from the Tulloch organic long-term experiment over up to five complete rotations.

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606/220. THE IMPORTANCE OF MANAGEMENT ON THE PRODUCTIVITY OF CEREAL-PEA INTERCROPS

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Summary:

Objectives, Description, Main Results & Conclusions

From a policy perspective, there is growing interest in increasing the production of legumes in European agriculture as they offer opportunities to reduce European greenhouse gas emissions, and emissions associated with land use change from imported soya. However, legumes grown in European agriculture are relatively rare. One option for increasing the production of European legumes is intercropping of cereals and grain legumes as they offer the potential for intensifying crop production, while at the same time minimising the negative impacts on the environment. Low input intercrops managed in the same way as the comparable sole crops typically outperform the sole crops by 20% (Li et al, 2020), thus addressing the need for increased production from existing agricultural land. Nevertheless, cereal-legume intercrops that rely on the legume for the supply of nitrogen yield 20-25% less than the sole crop cereal receiving typical nitrogen inputs. Research has largely focused on comparing intercrops with sole crops; however, for intercrops to become established in farming systems, farmers require more information on how to manage the crop. The management decisions made will be influenced by whether the farmer is growing the crop for grain, either for human or livestock consumption, or for silage. As well as what varieties should be sown, the farmer must make decisions on the relative quantities of the legume and cereal that are sown, and the quantity of nitrogen applied. A systematic literature was conducted to explore the effect of sowing rates and nitrogen application rates on the yield and the components of yield from pea-cereal intercrops under cropping conditions relevant to Europe. Data was extracted from 19, 10 and 6 papers for the barley, oat and wheat intercrops respectively. The presentation will summarise the key findings from the study.

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606/222. WINTER LEGUME-CEREAL INTERCROPPING: A SUSTAINABLE WAY TO INCREASE AGROECOSYSTEM RESILIENCE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Europe largely relies on plant-based protein imports for food and feed, mostly soybean originating from the American continent. Different grain legumes (e.g., pea and faba bean) can be a proper alternative. However, their acreage in European cropping systems still is small. To boost regional protein production, the European Commission funds research programs to raise the cultivation of underutilized legumes in the regional cropping rotations. Although the benefits of legumes, e.g. biological nitrogen fixation and phosphorus mobilization, in crop rotations are well known. There are still barriers limiting their cultivation on a larger scale. The predominant ones are: 1) unstable production depending on the growing season, being larger for spring varieties than winter ones; 2) laborious weed and disease control. Winter-sown crops have agronomic advantages such as enhancement of soil coverage limiting the run-off of nutrients, improvement of water use efficiency, and increment of grain yield. Nonetheless, their output is still variable due to frost damage, higher susceptibility to fungal diseases and flower abortion due to heat stress. The mixed intercropping system with small grain cereal is a viable solution to decrease yield instability and increase regional protein production and agrobiodiversity. The focus of this study is to investigate the yield gap between the summer/winter cultivation of pea and fava beans in pure stands and mixed intercropping systems. Additionally, the potential advantages of intercropping are evaluated compared to monoculture.

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606/239. DEVELOPING GRASS PEA-CEREAL INTERCROPPING SYSTEMS FOR SWITZERLAND AND GERMANY

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Summary:

Objectives

Grass pea is considered one of the most climate resilient crops (Lambein et al., 2019). Intercropping grass pea with cereals may have great potential to minimise lodging and increase yield and yield stability (Bedoussac et al., 2015). It may also allow for a more efficient use of environmental resources and increased nitrogen fixation. The objective of this study is to find suitable grass pea-cereal intercropping systems for production of grass pea in Switzerland and Germany.

Concise description of the work (materials & methods)

A multi-year, repeated intercropping trial (2022 - 2024) will be conducted at different sites in Switzerland (in collaboration with the School of Agricultural, Forest and Food Sciences HAFL and the organic plant breeding association gzpk), and Germany (in collaboration with the Leibniz Centre for Agricultural Landscape Research ZALF).

Experimental sites:

In 2022, the experiment was carried out at an organic farm in Schüpfen (47°2′N, 7°22′E) in the Swiss Canton of Berne and on the trial site of ZALF in Dedelow (53°21′N, 13°48′E). With a mean annual temperature of 13 °C and mean annual precipitation of 352 mm, Dedelow has a warmer and drier climate than Schüpfen (9.1 °C and 1174 mm).

Experimental treatments & design:

Intercrops of grass pea (cv. Fischauer and a commercial variety from Tesoro della Terra, subsequently called PL1 and PL2) with triticale (cv. Impetus, subsequently called T), oat (cv. Lion, subsequently called H1) or naked oat (cv. Patrik, subsequently called H2) were cultivated at two sowing ratios (pea:cereal). The pure stand sowing ratios of these crops were established with 80 (grass pea), 320 (triticale) 340 (oat) and 370 (naked oat) germinable seeds m-2, respectively. The two sowing ratios were based on the pure stands of each crop in %: 80:40 and 95:25. The experimental design was an incomplete block design (alpha lattice design) with ten treatments replicated four times. The experimental plots had a size of 10.5 and 9.45 m2, respectively and comprised of 10 rows each.

Statistics:

Normality of variance was confirmed with a Shapiro-Wilk's test. We used a one-way ANOVA to test for significant differences between intercropping system, location and their interactions on grass pea yield. For significant effects, subsequent pair-wise comparison (Tukey's HSD post hoc test) was used to identify what groups significantly differ from each other.

Main Results

The results show a significant effect of the intercropping system on grass pea yield. The highest grass pea yield resulted from intercropping PL1 with T at a sowing ratio of 95:25, which was significantly higher than the yield of all other intercropping systems. Intercropping with triticale, generally resulted in higher grass pea yields (0.9-1.8 t ha-1) than intercropping with oat (0.35-0.9 t ha-1). The only exception was PL2+T (80:40) which had a similar grass pea yield as PL1+H1 (95:25). A higher ratio of grass pea in the intercrop (95:25) also tends to result in a higher grass pea yield compared to the intercrops with a lower ratio of grass pea to cereal (80:40) (figure 1).

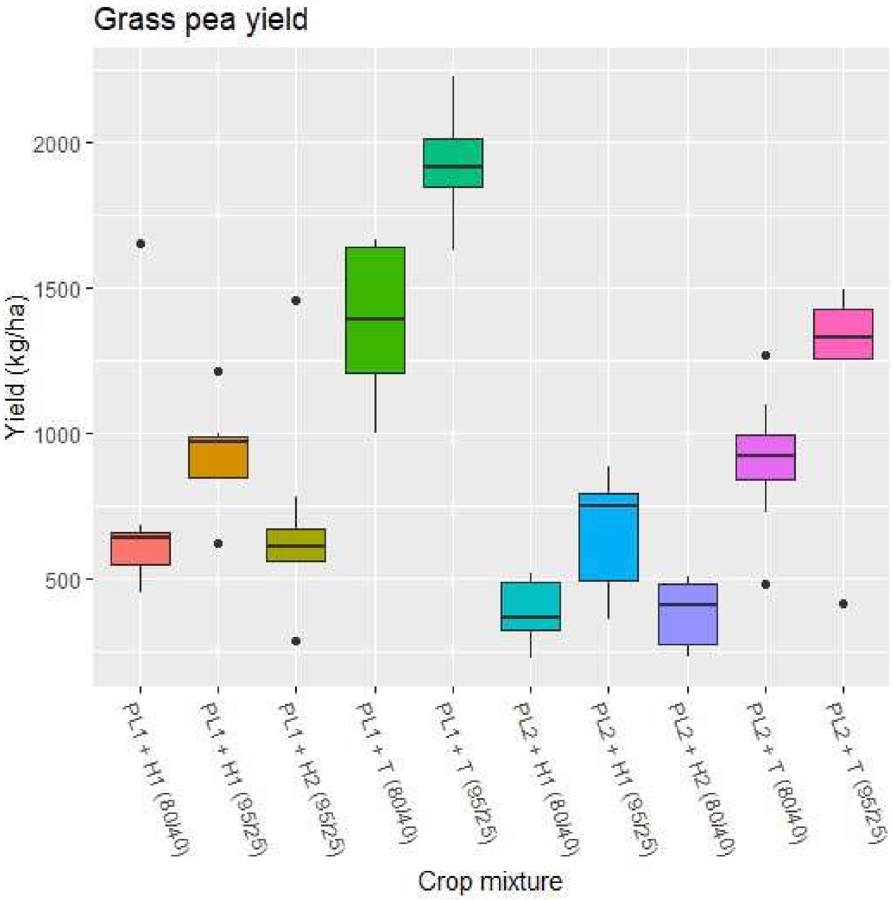

Figure 1. Difference in grass pea yield between different intercropping systems

Conclusions

For the first year, we conclude, that grass pea intercropping with triticale works well and can lead to grass pea yields of up to 2 t ha-1 at a sowing ratio of 95:25. Oat on the other hand has shown to be the dominant partner in mixtures with grass pea at the chosen sowing ratios, leading to low grass pea yields (< 1 t ha-1, figure 1). We further conclude that a sowing density of 80:40 does not seem to be favorable for production of grass pea.

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606/240. INVESTIGATING MIXED CROPPING SYSTEMS WITH PEA AND LENTILS FOR CLIMATE-SMART AND DEMAND ORIENTED AGRICULTURE

Authors:

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Summary:

Objectives

In Switzerland grain legumes cultivation takes up only 3-5% of the arable land, although they contribute significantly to a sustainable transformation of the agricultural and food systems. With more people adopting vegetarian diets, there is an ever-growing demand for plant-based proteins. Moreover, since 2022 ruminants can only be fed with organic-certified fodder produced on Swiss farms (BioSuisse 2022). To up-scale the grain legume production, PROMISE, a national follow-up project of the EU Horizon 2020 project ReMIX, tackles the optimisation of mixed cropping systems (MS) with pea-barley (Haug et al. 2023) and lentil-pea. Cultivation of peas (Pisum sativum L.) and lentils (Lens culinaris Medik.) in pure stands (PS) carries risks of low yield or total yield losses due to lodging, poor weed competition and emerging diseases. Despite their yield potential and higher market value the yield stability across seasons is much lower than for cereals. The objectives of PROMISE are (i) to measure the impact of the two MS on grain and protein yield, and yield stability, lodging and weed tolerance across three seasons, (ii) to assess the root rot disease of the two species in PS and MS, and (iii) to investigate the residual nitrogen for subsequent crops.

Concise description of the work (materials & methods)

Field trials were conducted at two organic certified farms in the Swiss Midlands in three consecutive years (2020, 2021, and 2022). The study comprised three lentil varieties, six up to sixteen (depending on year and experiment) pea varieties and one two-row barley (Hordeum vulgare L.), two treatments (MS, PS) with three replications in a randomised complete block design.

Main Results

Higher grain yields in lentil-pea mixtures in comparison to pure lentil crops in all three years were observed. Land equivalent ratios (LER; Mead, R. & Willey, R. 1980) for total grain yield in 48 out of 54 tested pea-lentil mixtures were above 1.0. Also pea-barley mixtures performed on average better in regards to grain yield in comparison to pure pea crops, which was most pronounced in unfavourable seasons like 2021 with high rainfall. For both systems, we found a strong cultivar effect on mixture performance and genotype x cropping system interaction (Figure 1). Weed incidence and lodging of peas in the pea-barley system, as well as lentils was on average reduced in MS. In pot experiments as well as in the field with same pathogen pressure, symptoms of root rot in lentils was significantly lower than in peas. However, there were no differences in root rot severity between pea grown in MS and PS. Preliminary results showed that the N delivery of the two MS to the following crop were higher than in barley crop (PS). Detailed results across the 2 sites and 3 years will be presented.

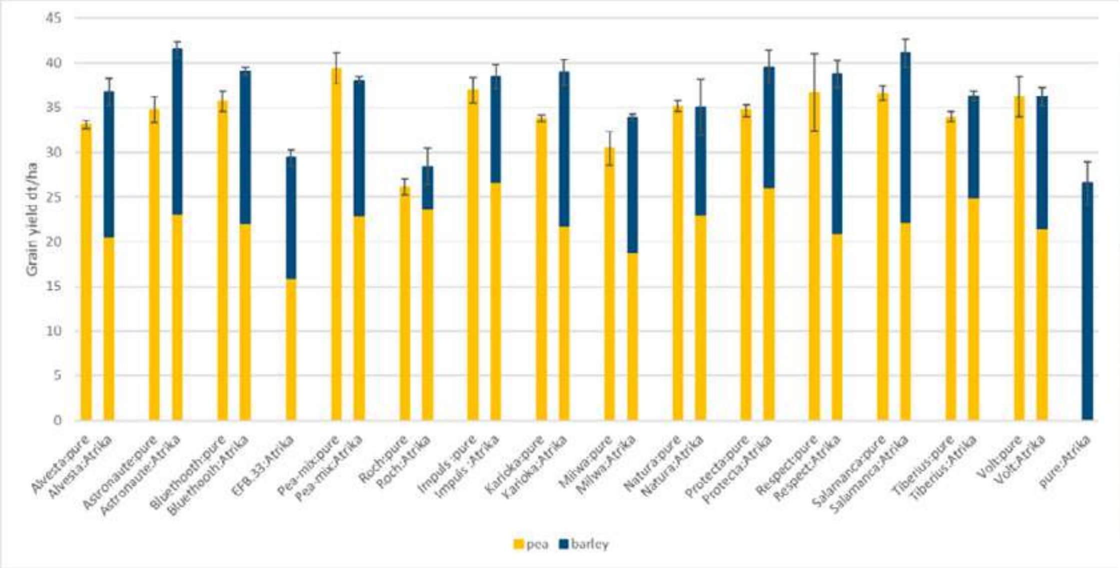

Figure 1 Grain yield with standard error of pea (yellow) and barley (blue) in pure stand and mixture stand for the different cultivars at the farm in Kirchlindach in 2022.

Conclusions

In conclusion, both MS have proven to be resilient cultivation methods, reduce risk of yield losses in years/environments unfavourable for legume cultivation due to the yield of the supporting crop and add values to following crop by enhancing available N and reducing weeds.

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606/248. LONG-TERM LEGUME-BASED CROPPED SYSTEMS FOR 'ECOLOGICAL INTENSIFICATION'

Authors:

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Summary:

Objectives

JHIs Centre for Sustainable Cropping (CSC) is a long-term field-scale legume-based (and stockless) arable cropped-system experiment whose underpinning agronomy is implemented iteratively, and whose ecosystem functions (or 'services') are monitored continuously, to achieve predefined environmental and ecological benefits (Hawes et al., 2021). This long-term study approach aims to identify agronomic practices which can be integrated to better-balance ecosystem functions with yield and economic imperatives.

Concise description of the work (materials & methods)

Running since 2009, the CSC compares regenerative and conventional agronomic practices in a split-large-field design over multiple six-year rotations (potato → winter wheat → winter barley → oil seed rape → faba beans → spring barley →), to allow commercially realistic estimates of costs and benefits. The regenerative system aims to promote soil health, biodiversity, and crop fitness through reduced reliance on chemical interventions (Figure 1). The conventional system is ploughed and receives prescriptive agrichemical applications and prophylactic treatments. Sustainability is assessed by continuous monitoring of system indicators for soil and biodiversity, with indicators for crop quality and economics; to guide further system-design improvements.

Main Results

There are initial trade-offs between enhancing soil health and biodiversity with crop yield in the early stages of transition from intensive to regenerative cropping, though these diminish over time. The length and nature of the transition phase highlights the importance of long-term legume-based trials to identify best-practices.

Future research will involve Life Cycle Assessment (LCA) of the CSC crop rotation supplemented with system function indicators. This insight will be used to develop landscape scale assessment of crop production and ecological functions across scenarios of deployment of CSC's integrated practices, based on the cropping modules within the GOBLIN (General Overview for a Backcasting approach of Livestock Intensification) modelling framework (Duffy et al., 2021).

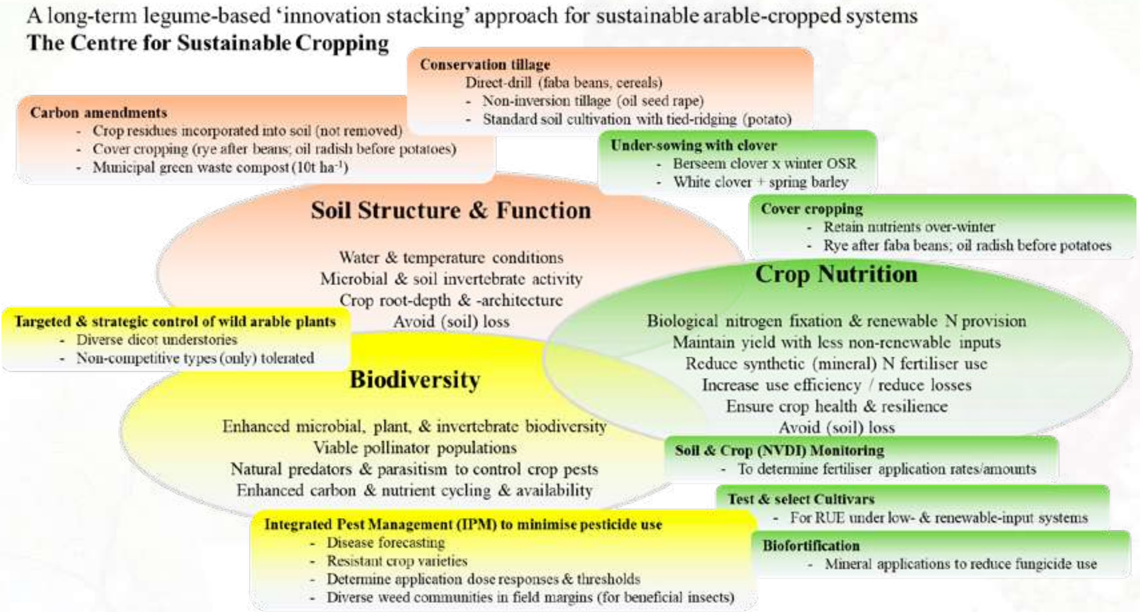

Figure 1. Lists how legumes, as underutilised crops, are deployed and integrated with other practical innovations to deliver multifunctional impacts (Iannetta et al., 2021) that cross-cut sustainability objectives for: 'Soil Structure & Function', 'Crop Nutrition', and 'Biodiversity'.

Conclusions

Short-term studies can overemphasise trade-offs between environment (e.g., biodiversity) and economics (e.g., crop yield). In contrast, long-term studies of legume-based cropped systems highlight the real potential of "Ecological Intensification" (Brooker at al., 2023), and the positive association which can be achieved between ecological and economic goals through stabilisation of crop yields and maintenance of productivity.

Acknowledgements

James Hutton Institute is supported by the Rural and Environment Science and Analytical Services (RESAS), a division of the Scottish Government. This research is also supported by Research & Innovation Actions funded by the European Commission which include 'ReAlising DynamIc vAlue NeTworks for underutilised crops' (www.RADIANTproject.eu, H2020, Grant Agreement Number 101000622); 'ECOlogically sustainable NUTRIent management in agriculture aiming to prevent, mitigate and eliminate pollution in soils, water and air' (www.econutri-project.eu, Grant Agreement Number 101081858. Also, the 'Nitrogen efficient plants for Climate Smart arable cropping systems' (NCS) Project (http://bofin.org.uk/the-ncs-project/, Grant Agreement Number 10043778. which is funded by DEFRA (UK Government's Department for Environment, Food, and Rural Affairs, UK) under the InnovateUK Competition 'Farming Futures R&D Fund, Climate Smart Farming'.

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facilitation of legumes cultivated at bioregional scales is necessary to address the climate-biodiversity-nutrition nexus. Frontiers in Sustainable Food Systems 239. https://doi.org/10.3389/fsufs.2021.692137

606/253. PULSE-OAT MIXTURES: PROSPECTS IN FOOD PRODUCTION AND ENRICHED CROP ROTATIONS

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Summary:

Objectives, Description, Main Results & Conclusions

Modern agriculture faces various challenges that are increasing with changing climate and world population's food demand. Those challengesinclude reduced input availability and requirements of increased tolerance to a/biotic stresses with high expectations towards output and quality. Further, plant protein demand is rising and needs is optimally met in domestic production. With increasing costs of synthetic inputs and raising demand for locally produced and healthy plant based nutrition, these challenges can be tackled at the cultivation level by enriching the crop rotation with pulses and (re) introducing niche crops exhibiting higher nitrogen efficiency and improved nutritional qualities. The CROPDIVA project (Horizon 2020, grant agreement NO 101000847) aims at diversifying the cultivation and diets through the introduction and processing of such niche crops, and further evaluates their socio-economic impact along the value chain.

In particular, this project examines mixed cropping systems of oats with pulses to investigate the contribution to such challenges. The goals are to identify:

- Low-input and high quality mixed cropping systems of lentil-oats and of lupin-oats that do not require additional husbandry work for farmers and yield comparable quality and increased quantity
- the carbon and nitrogen balances of lupin-oat mixtures compared to their monoculture equivalents.

To draw these conclusions, foregoing studies evaluate over 20 pulses-oat mixtures during four seasons in two locations. Initial results will be discussed and conclusions of the aggregate experiments presented.

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606/255. ASSESSING THE IMPACT OF REDUCED PRIMARY INOCULUM CARRYOVER ON THE SPATIOTEMPORAL SPREAD OF ASCOCHYTA BLIGHT IN CHICKPEAS: A SIMULATION MODELLING APPROACH

Authors:

Mohamed Zakeel Mohamed Cassim[1], Susie Sprague[1], Luke Barrett[1]

Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Understanding the persistence of plant pathogen inoculum during periods when host plants are absent is crucial for accurately predicting disease epidemics and optimizing control strategies. In this study, we focus on the necrotrophic fungal pathogen Ascochyta rabiei, investigating the persistence and management of primary inoculum in the landscape. Given the challenges posed by this aggressive pathogen, which has shown resistance to genetic controls, reliance on fungicides has increased, emphasizing the urgent need for effective integrated disease management strategies. Field experiments measuring pathogen load on post-harvest crop residues revealed that primary inoculum levels can decay rapidly and are influenced by in-season management choices. To assess potential management interventions at landscape scales and their impact on disease severity, we employed an epidemiological model parameterized with data from field experiments. The model captured various aspects of dispersal and inoculum decay dynamics, offering insights into the efficacy of landscape deployment strategies and management interventions in reducing inoculum persistence between seasons. Leveraging the outcomes of this study, we aim to develop integrated and area-wide disease management strategies to ensure sustainable control of Ascochyta blight in chickpeas.

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Session 3 Legumes for human and animal nutrition and health

606/8. CAN WE INCREASE THE OIL CONTENT OF COWPEA SEEDS?

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Summary:

Objectives, Description, Main Results & Conclusions

Cowpea (*Vigna unguiculata*) is a key staple crop in West Africa, where it can play a key role for improving nutritional levels and reducing rural poverty in the population. It is also grown in parts of Asia and America and its cultivation is increasing because it provides several agricultural, environmental, and economic advantages to low-income small-holder farmers arowund the world.

Cowpea seeds are a nutritious food source, rich in protein and carbohydrates, containing essential amino acids, and antioxidants. But its oil content is very low . To make cowpeas more nutritious we have increased the oil content in the seeds by introducing a gene cassette with three oil-related genes (oleosin, WRI1 and DGAT1). This transgenic approach has been successful in other crops such as wheat and sorghum, but it was not tested in legumes yet. In cowpea we have selected three transgenic lines that produced on average about 7 times more oil in the seeds than the non-transgenic control. The high oil content seed has lower protein and starch content, but higher soluble sugars, suggesting the carbon flux was regulated by these genes.

To understand the impact of increasing oil on other metabolic pathways, and to identify new targets that could allow further increases in seed oil content without impacting seed composition, we performed a transcriptomic RNAseq analysis on a high-oil transgenic line and the control at four different stages of seed development. We have identified several genes that could be used in future attempts to increase seed oil content not only in cowpeas but in other pulses.

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606/43. NARROW-LEAFED LUPIN (LUPINUS ANGUSTIFOLIUS L.) β-CONGLUTIN SEED PROTEINS POTENTIAL USE IN THE PREVENTION AND TREATMENT OF BREAST CANCER

Authors:

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Work centre:

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Objectives

In BC, the presence of a particular subpopulation of cancer stem cells (CSCs) is related to relapse, metastases, self-renewal capacity and radio-resistance. The technical and medical advances in existing therapies in the last decade had not been able to overcome the fact that between 30 and 50% of patients with BC will develop metastasis. This situation urges the need to find more effective prevention and treatment strategies with the use of plant-based nutraceutical compounds. In this context, considerable interest is focused on legume seed proteins, mainly those from genus Lupinus, and particularly in the "Sweet lupin" group. Interestingly, the seeds of Narrow-leafed lupin (NLL) or

Lupinus angustifolius L. are attracting attention because of their potential use for inflammatory related diseases prevention and improvement. Vicilin family of proteins, and particularly β-conglutin proteins are the most abundant proteins in all lupin species including NLL. Recently, the anti-diabetic, antioxidant and anti-inflammatory properties of β-conglutins have been investigated and, on this basis, NLL β-conglutin proteins has been proposed as new potential functional foods with therapeutic and preventive properties in inflammatory-related diseases like BC. The main goal of the present work was to assess whether those promising nutraceutical compounds could be natural preventive and cytotoxic agents for BC cell lines, mediated by the implication of CSCs related genes and mechanisms.

Concise description of the work (materials & methods)

To achieve it, we purified three different isoforms of NLL β conglutins, β1, β3 and β6, using affinity-chromatography. Then, we evaluated their effectiveness as a treatment at very low concentrations and their mechanisms of action on three different BC cell lines and a healthy one.

Main Results

Results showed lower viability and proliferation, higher apoptotic rates, regulation of ROS levels and DNA damage and anti-stemness properties in BC cell lines. This β-conglutin proteins can become an interesting cytotoxic, natural and anti-stem-cell-like properties treatment for BC, at very low concentrations and preserving the viability of healthy cells. These proteins could act through the regulation of a dual mechanism involving tumorigenic and stemness-related genes such as SIRT1 and FoxO1, depending on the state of p53.

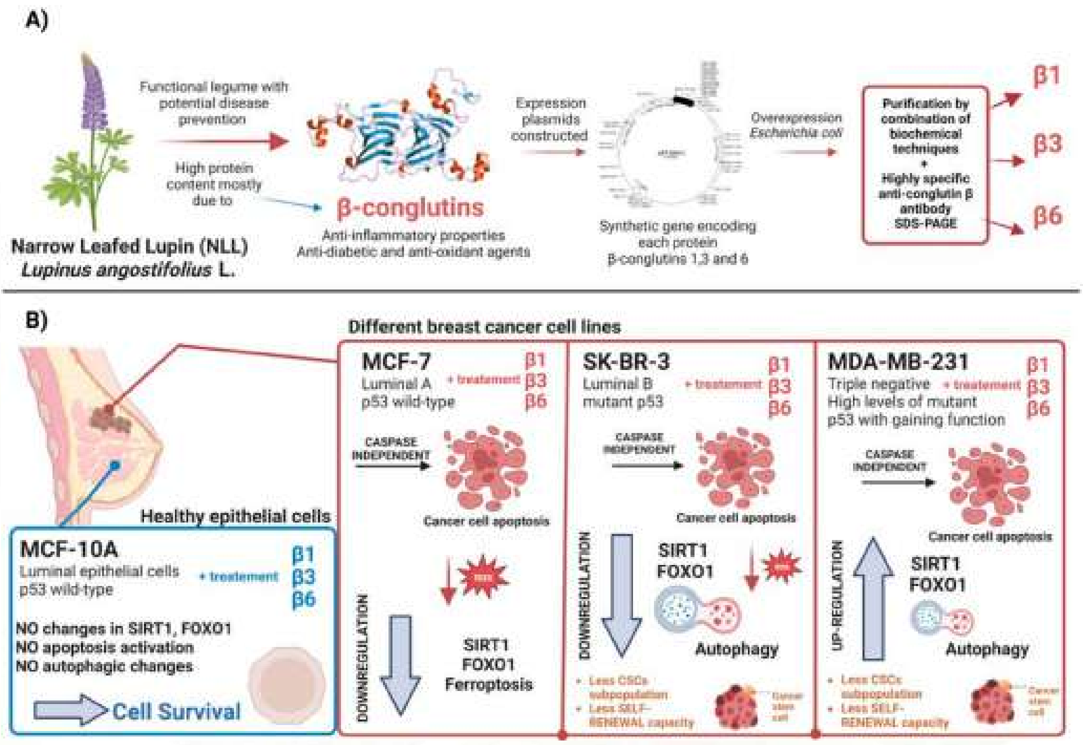

Figure 1. Summary of the research developed in the present study.
Expression, purification, and application of β-conglutin treatment in BC cell lines as a cytotoxic and anti-stemness agent against BC, preserving healthy epithelial cells (A). The regulation of SIRT1/FoxO1 pathway and autophagy in a p53-dependent manner (B). Created with BioRender.com [3].

Conclusions

In conclusion, and for the best of our knowledge, this is the first time that these NLL β-conglutin proteins have been identified as potentially anti-BC agents, with the capability of being used for BC management, making them a unique and novel strategy for the BC treatment.

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606/60. MINING NATURAL DIVERSITY TO DISCOVER THE GENETIC BASIS OF QUALITY TRAITS IN PEA

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

With the rapidly growing market for plant-based foods and proteins, pea has become an essential source of raw materials for many food industries. This places new demands on nutritional and functional traits that are not always present in existing pea cultivars. To identify genetic variation for desired quality traits and discover the underpinning genes, a panel of 220 accessions from the John Innes Pisum germplasm resource was selected across the spectrum of pea genetic diversity. Seeds were analysed for protein composition, resistant starch, phytic acid content, raffinose family oligosaccharides, and the concentration of micronutrients (Fe, Zn, P, Mg and Ca). In addition, the panel was genotyped with a 90K SNP array, and a genome-wide association study (GWAS) was performed. The phenotypic data showed wide variation in all traits. The study identified desirable variants, including lines with a high legumin: vicilin ratio, round-seed lines with significantly higher resistant starch, lines with strikingly reduced concentrations of individual oligosaccharides, and a single line with extremely low phytic acid content. GWAS analysis revealed significant associations between the abundance of legumin, vicilin, lipoxygenase and albumin and the R locus, a mutation in starch-branching enzyme I. However, several other loci associated with abundant seed proteins, including convicilin, annexin-like protein and late embryogenesis abundant protein, were also identified. Moreover, a novel locus on chromosome 6 harbouring inositol-pentakisphosphate 2-kinase was significantly linked with both phytic acid and phosphorous content. In addition, F2 and RIL populations were deployed to map two independent QTLs associated with low phytate content. These findings will contribute to developing pea cultivars with superior agronomic and quality traits based on genomic tools.

606/78. IN VIVO INTERNALIZATION DYNAMICS OF SOYBEAN PROTEASE ISOINHIBITORS, IBB1 AND IBBD2, OF THE BOWMAN-BIRK FAMILY IN HT29 COLORECTAL CANCER CELLS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

IBB1 and IBB2D are major soybean protease Bowman-Birk inhibitors (BBIs) with ability to inhibit either trypsin/chymotrypsin or trypsin activities, respectively, that are currently being investigated as chemopreventive agents of colorectal cancer in humans. In order to gain insight about the action mechanism of BBIs and their potential therapeutic targets, the in vivo internalization dynamics of IBB1 and IBBD2 proteins, in their active and chemically inactive forms, was monitored in human colorectal adenocarcinoma HT29 cells by fluorescence microscopy. For this purpose, a new fluorescent labeling protocol recently developed in our laboratory was used. We observed that the internalization process started after a few minutes. At short times of culture (< 3 h), HT29 cells showed a green fluorescent signal derived from CyDye2-labeled BBI proteins, forming patches randomly distributed across the cytoplasm. The fluorescent signal intensity gradually increased throughout the culture, indicating that BBIs cross the plasma membrane of HT29 colon cancer cells and accumulate inside them. The internalization process was independent of the inhibitory activities of BBIs since both active

and chemically inactivated proteins were internalized. In multiplex experiments, we found that the red fluorescent signal from the endosome marker FM 4-64 clearly overlapped with labelled BBI proteins in the cytoplasm, giving a yellowish fluorescence. These results suggest that BBIs proteins are internalized into the cytoplasm of HT29 cells through one of the existing endocytic pathways. At longer times of culture (>12 h), the fluorescent signal of BBI proteins overlapped with the Hoechst 33342-labeled nucleus, indicating that these proteins are also capable to internalize into the cell nucleus.

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606/115. VARIATION IN RAFFINOSE FAMILY OLIGOSACCHARIDE PROFILE IN PEA SEEDS IMPACTS THE HUMAN GUT MICROBIOTA AND FERMENTATION METABOLITE COMPOSITION

Authors:

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Work centre:

(1) John Innes Centre, (2) Quadram Institute Bioscience

Summary:

Objectives

The aim of this work was to understand the effects of RFOs from pea seeds on plant development and agronomic traits, and on the modulation of the structure and function of the human gut microbiota.

Concise description of the work (materials & methods)

We used two Pisum sativum lines: Cameor, a commercial cultivar, and the mutant line, FN 1551, derived from the JI 2822 fast neutron mutant population developed previously in the group (Domoney et al., 2013) which lacks a major raffinose synthase gene (rfs) and has been backcrossed to Cameor (BCFN1551).

The flours from dry seeds of Cameor and BCFN 1551, along with individual RFOs and precursors, were used in batch fermentation seeded with a human faecal sample.

Untargeted metabolomics and LC-MS targeted analysis of short-chain fatty acids (SCFA) were carried out on the supernatant from the batch fermentation assays.

Main Results

We showed that RFOs play a major role in protecting pea plants from drought stress. Under controlled glasshouse conditions, withdrawing watering for nine days during the vegetative state led to significant alterations in plant development and seed yield for both genotypes, while a pronounced significant difference in seed yield and dry weight above soil was observed for the mutant line (p value <0.0001). This effect was accompanied by a significant increase in verbascose in the seeds from water-deficit Cameor, compared to bench-watered Cameor, as determined by hydrophilic interaction chromatography coupled with mass spectrometry (HILIC-MS). These data support the influence of RFOs in tolerance to water-stress. Supplementation with individual raffinose and pea flour from Cameor led to an increased Bifidobacterium population, associated with health-promoting properties (O'Connell et al., 2013), and reduced abundance of Alistipes onderkonkii, a gut microbe associated with emerging detrimental clinical implications of gut inflammation and mental signs of depression (Caso et al., 2021). We also confirmed the ability of the main Bifidobacteria species, B.longum and B.adolescentis, to utilise RFO in vitro. An increase in 12-ketolithocholate was observed in the BCFN 1551 fermentation, which is a secondary metabolite involved in bile acid metabolism, as compared to Cameor. No major differences in total SCFA or acetate, the main SCFA produced by the human gut microbiota, was observed between the two genotypes, which may be due to sucrose being significantly increased in the mutant.

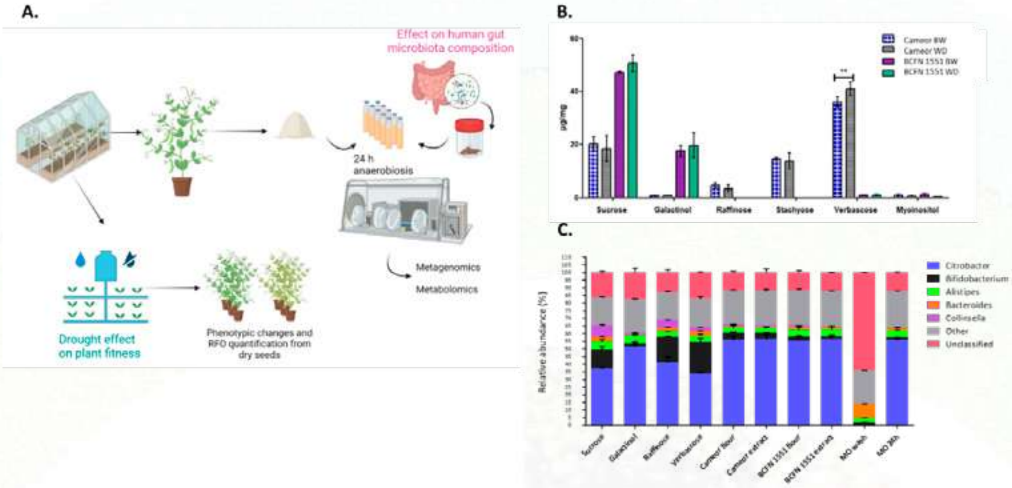

Figure. Impact of genetic and environmental factors on RFO production in pea seeds and effect of fermentation on human gut microbiota. A. Infographic summary of the experiments. B. HILIC/LC-MS results of RFOs and precursors from the seeds of water-deficit (WD) and bench-watered (BW) Cameor and BCFN 1551 plants. C. Metagenomic analysis from batch fermentations showing increased relative abundance of Bifidobacterium following supplementation by individual metabolites, seed flour and seed extracts.

Conclusions

These results showed that modulation of RFOs within pea seeds influences the human gut microbiota composition and metabolic profile after fermentation. Further work is ongoing to produce TILLING mutants with different RFO contents.

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606/157. CREATING SAPONIN-FREE YELLOW PEA SEEDS BY CRISPR/CAS9-ENABLED MUTAGENESIS ON B-AMYRIN SYNTHASE

Authors:

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Work centre:

(1) University of Calgary. Calgary. Canada, (2) National Research Council. Canada

Summary:

Objectives, Description, Main Results & Conclusions

Dry pea (Pisum sativum) seeds are valuable sources of plant protein, dietary fiber, and starch, but their uses in food products is restricted to some extent due to several off-flavor compounds. Saponins are one class of these off-flavor compounds that impart bitter, astringent, and metallic tastes to pea products (Heng et al., 2006). Here, in an elite yellow pea cultivar (CDC Inca), we used CRISPR/Cas9 genome-edit to induce mutations in the entry-point enzyme in saponin biosynthesis, β-amyrin synthase (PaBAS) (Morita et al., 2000). The efficiency of CRISPR/Cas9 was systematically optimized in pea hairy roots with variables in rhizogenes strain, promoters for gRNA/Cas9, temperature of tissue culture. The best performing construct and culture conditions were applied to stable transformations of pea for targeted mutations in PaBAS. As results, two types of mutant alleles (deletion and inversion) were generated in PaBAS coding sequences in the T1 plant, and the segregating bi-allelic PaBAS mutants in the T2 population were selected. In addition, transgene-free T2 individual plants were selected. The resulting PaBAS mutant without transgenes showed 99.8% removal of saponins in pea seeds. This work demonstrated that, using an optimized CRISPR/Cas9 system, flavor-improved traits can be readily introduced in any pea cultivar of interest.

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606/167. COMBINATION OF THREE NULL MUTATIONS IN PISUM SATIVUM L. IMPACTS POSITIVELY ON DIGESTIBILITY

Authors:

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Summary:

Objectives

Legume crops are a great choice as food crops to fulfil many needs, including nurturing the human population with plant-derived foods while also reducing the impact of agriculture on the planet, mostly through biological nitrogen fixation in roots as a consequence of bacterial symbiosis. While pea seeds are generally considered a high-quality plant-based protein source, some of its proteins have been documented as being poorly digested by farm animals or to interfere directly with digestion through inhibition of digestive enzymes. Three groups of albumins fall into this group: pea albumin 2 (PA2) and lectin, both of which are associated with poor digestion (Le Gall et al., 2007), and the inhibitors of trypsin (TI) and chymotrypsin (Clemente et al., 2005).

Concise description of the work (materials & methods)

We have used high-throughput genetic screens to generate lines lacking all three proteins. In this study, seeds from TI and triple mutant and control lines (BC6_F4 TI mutant and BC6_F4 wild-type segregant lines; triple null combination at F6) were used to investigate the impact of the mutations on seed protein and digestibility traits.

Main Results

The seed protein profile of the selected mutant and wild-type lines showed very similar patterns apart from the proteins (PA2, lectin and TI) which are absent from the mutants (Figure). Measurement of overall trypsin and chymotrypsin inhibitory activities of control and mutant seed protein extracts confirmed the absence of any inhibitory activity in mutants. Seed meals were subjected to in vitro digestion following the INFOGEST protocol (Brodkorb et al., 2019). The protein hydrolysis (%DH) showed significant differences in the intestinal phase when the three genotypes are compared. The triple null mutant showed the highest DH at over 75%, whereas the single TI mutant showed around 60% of protein hydrolysis and the control around 50% (Figure). SEC analysis of the molecular distribution of the soluble fraction of the three digestion phases revealed a broadly similar profile for the control and two mutant pea samples analysed, but with quantitative differences in peak heights. The free amino acids quantified from digestion of the three pea seed samples supported the greater digestibility of the mutant samples, with the mutants providing higher amounts of most amino acids, including the sulphur-containing amino acids, methionine and cysteine. The analysis of seed yield from the variant lines grown under greenhouse conditions suggests that there is no penalty associated with the mutations.

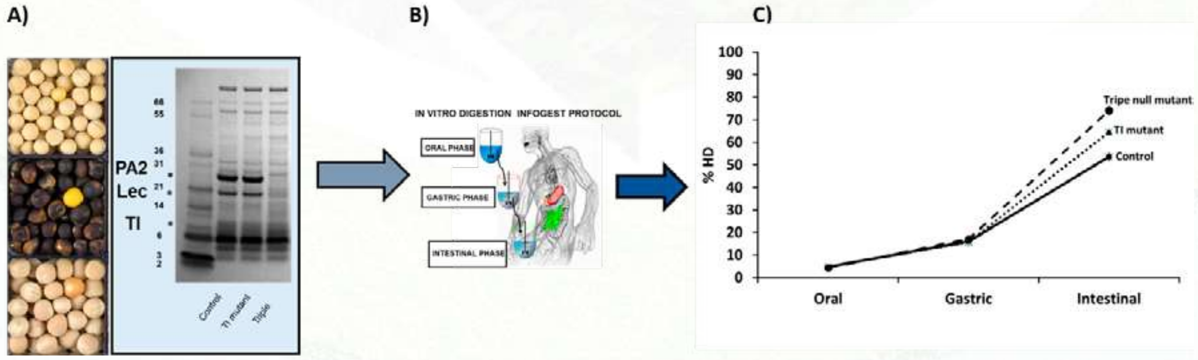

Figure. A) Albumin profile of control and mutant seeds selected B) *In vitro* ***digestion protocol C) Protein hydrolysis after*** *in vitro* ***digestion (HD).***

Conclusions

Thus the combination of an elevated seed protein concentration, loss of inhibitors and a potential allergen, together with improved digestibility and amino acid bioavailability makes these mutants an attractive proposition to improve protein bioavailability in pulse crop.

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606/173. EFFECTS OF PARTIAL SUBSTITUTION OF GRASS SILAGE WITH RED CLOVER SILAGE ON MILK PRODUCTION AND METHANE EMISSION IN DAIRY COWS

Authors:

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Work centre:

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Summary:

Objectives

Red clover (*Trifolium pratense*) silage is known for high milk-production potential, and in some studies, it has reduced ruminal CH4 emissions (Vanhatalo and Halmemies-Beauchet-Filleau, 2020; Bica et al., 2022), but results are inconsistent (Gidlund et al., 2017). The objective of this study was to compare milk production and CH4 emissions of cows fed either grass or red clover-grass silage.

Concise description of the work (materials & methods)

The study was conducted with 19 Nordic Red cows averaging (± SD) 109 ± 52 days in milk and producing 35.4 ± 4.5 kg of milk/d. Dietary treatments were grass silage (GS; *Phleum pratense*, festuca pratensis) and 2:1 mixture of red clover and grass silages (CGS). The experiment was switch-back design with 4-week periods of treatments in sequence GS, CGS, and GS. Cows were fed partial mixed rations ad libitum and concentrate pellets (4.7 kg dry matter (DM)/d). Partial mixed rations consisted of silage (75%), faba bean (13%), oats (6%) and barley (6%). Pellets consisted of oats, barley, rapeseed meal, and minerals. Cows were milked with Lely milking robot and enteric CH4 and H2 production was measured with GreenFeed system integrated in the robot. Milk composition was analysed for all cows, while total-tract nutrient digestibility was measured for 11 of the experimental cows. All data were analysed with SAS 9.4 Mixed procedure.

Main Results

Final proportion of the concentrate in the diet was 40.6% for GS and 38.8% for CGS. Dietary crude protein (CP) concentration was 17.3% in GS and 19.7% in CGS and neutral detergent fibre (NDF) concentration 40.2% in GS and 34.0% in CGS. Dry matter intake was on an average 24.4 kg/d, and it was 0.9 kg greater ($P = 0.01$) for CGS than for GS. Digestibilities of DM, CP, and NDF were greater for GS than for CGS ($P < 0.02$). Average milk yield was 31.8 kg/d, and it was 1.9 kg greater ($P < 0.01$) for CGS than for GS. However, cows on CGS had lower ($P < 0.01$) milk protein and fat concentration than on GS and therefore yields of energy corrected milk (ECM), protein, and fat were not affected ($P > 0.10$) by treatment. Methane production was unchanged between the treatments ($P > 0.10$) being on average 496 g/d, 14.1 g/kg of ECM or 20.5 g/kg of DM intake, respectively. Hydrogen production was on average 1.01 g/d, and it was 0.27 g/d greater ($P < 0.01$) for CGS than for GS.

Conclusions

Increased DM intake and milk yield for CGS rather than for GS as well as unchanged ECM are in line with several previous experiments (Vanhatalo and Halmemies-Beauchet-Filleau 2020). Present results suggest that increased DM intake compromised CH4 mitigation potential of CGS, despite the reduction of dietary NDF concentration.

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606/209. CHEMOMETRIC ANALYSIS OF GRASS PEA (*LATHYRUS SATIVUS L.*) USING ATR-FTIR SPECTROSCOPY: PREDICTIVE MODELS FOR ODAP AND HOMOARGININE CONTENTS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Grass pea (*Lathyrus sativus L.*) is a resilient cool-season legume that tolerates environmental and biotic stresses. Neuro lathyrism, a neurological disorder, is associated with the presence of a neurotoxin called β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) in grass pea seeds [1]. ODAP exists in two isomeric forms (α and β), with the β isomer being the toxic variant, accounting for 95% of total ODAP [1]. Grass pea is also a unique source of homoarginine, a nonproteinogenic amino acid involved in the production of nitric oxide (NO), a vasodilator that prevents cardiovascular and renal dysfunctions. This study aimed to develop chemometric prediction models for grass pea ODAP and homoarginine contents using a rapid spectroscopic FTIR screening method coupled with mass spectrometry and supported by statistical analysis. Dried grass pea seeds, harvested over three years under similar conditions, were milled, and resulting flours analysed using HPLC-ESI-MS/MS [2]. For quick screening, Attenuated Total Reflectance – Fourier Transformed Infrared Spectroscopy, ATR-FTIR (Thermo Nicolet 6700 spectrometer with an ATR crystal diamond) was performed on the samples. Partial least square regression multivariate analysis was conducted using Unscrambler X 10.4.1 software to explore the correlations between spectral data and quantified ODAP isomers and homoarginine. Calibration models were designed using flour samples from the three harvesting years, with selected spectral regions based on relevant literature. The models were validated through random cross-validation, achieving good correlation (above 0.5) between predicted and observed values for most parameters. Further experiments are ongoing to increase data variability explanation and reduce the root mean square error of the models. ATR-FTIR, combined with multivariate analysis, offers an alternative method for accurately quantifying parameters that impact health positively (e.g., homoarginine) or pose challenges to incorporating grass pea into the diet (e.g., ODAP).

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606/224. PsSULTR4 IS THE ONLY VACUOLAR SULFATE TRANSPORTER IN PEA AND A KEY DETERMINANT OF SEED PROTEIN COMPOSITION EVEN UNDER SULFUR-SUFFICIENT CONDITIONS

Authors:

Fanélie Bachelet[1], Myriam Sanchez[2], Delphine Aime[2], Christine Le Signor[2], Florence Naude[2], Nadia Rossin[2], Vanessa Vernoud[2], Fabrice Neiers[3], Markus Wirtz[3]

Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

The role of sulfur (S) in both drought tolerance and seed quality makes it important to understand the trade-off between these two traits with respect to S utilization (Bonnot et al. 2023). This is particularly important for legumes, whose seeds contribute to the human requirement for S-amino acids. To investigate the contribution of S stored as sulfate in the vacuole to seed protein quality in pea, we focused on the only vacuolar sulfate transporter (SULTR4) present in pea (PsSULTR4). Its simulated 3D structure appeared to be conserved with that experimentally determined for its Arabidopsis homolog AtSULTR4;1 (available on PDB, https://www.rcsb.org/structure/7LHV). This observation, together with its experimentally confirmed tonoplast localization, suggests that it is the functional homolog of AtSULTR4;1, which mediates sulfate efflux from the vacuole (Kataoka et al. 2004). Five mutant and wild-type lines were phenotyped under S-sufficient and S-deficient conditions. One mutation in the cytosolic domain (E568K) reduced seed yield under S deficiency to a similar extent as a nonsense mutation, highlighting this residue as essential for sulfate transport activity. Interestingly, mature seeds of these two mutants developed under S-sufficiency showed changes in seed protein composition characteristic of S-deficiency, with less S-rich storage proteins. This was associated with reduced sulfate utilization within the seed. Gene expression studies and sulfate measurements in developing seed tissues revealed the importance of vacuolar sulfate remobilization in the embryo-surrounding tissues for the synthesis of S-rich seed proteins. These data will be presented together with perspectives on the contribution of vacuolar sulfate to seed quality.

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606/6. PEA GRAIN YIELD, PROTEIN, AND MINERAL NUTRIENTS AFFECTED BY ENVIRONMENT, GENETICS, AND AGRONOMIC PRACTICES

Authors:

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Work centre:

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Summary:

Objectives

Pea (*Pisum sativum*) protein represents an attractive source of plant-derived protein due to its non-genetically modified status, high digestibility, gluten-free nature, neutral flavor profiles and low allergenic risk [1]. In addition to protein, dry pea grains are excellent sources of dietary fiber, complex carbohydrates, resistant starch, vitamins and minerals [2]. Montana is the largest producer of field peas in the USA with over 448,000 acres of peas harvested in 2021 accounting for more than half of the total acres harvested in the USA [3]. The objective of this study was to investigate the yield and nutrient concentrations affected by genetics, environments, and management practices.

Concise description of the work (materials & methods)

Seven pea cultivars including five yellow peas (AC Earlystar, CDC Saffron, Delta, Jetset and Nette 2010) and two green peas (Hampton and Majoret) were planted at seven locations with diverse environmental conditions for two years. Four replications were planted for each entry and trials were arranged in a randomized complete block design. Yield, protein, and mineral concentrations were measured. GxE biplot was used to display GxE interactions. Pea yield and nutrient concentrations were related to environment and soil nutrients.

Main Results

Pea yields were significantly affected by location in both growing seasons, by cultivar in one and by cultivar x location interactions in both years ($P<0.01$). GGE biplot analysis identified Nette2010 as the winning cultivar for six of the 13 environments and as the most environmentally responsive cultivar (Figure1). Nette2010 is also the highest yielding of the seven tested cultivars. Cultivar and location both affected seed protein levels in both years ($P<0.01$). There were no significant cultivar x location interactions observed for seed protein. Results indicate that high yielding environments do not negatively influence seed protein levels in a strictly defined manner. Seed mineral concentrations were significantly affected by cultivar and location (P<0.05) in both growing seasons for all minerals evaluated with the exception iron in one year. Cultivar by location interactions were observed for all but one mineral (Boron) in one year and six of 10 minerals evaluated in another year. Location generally affects seed mineral levels more than cultivar. Examination of seed mineral contents across cultivars revealed that the green pea cultivars Hampton and Majoret are high in P and Zn. Of the yellow peas, cultivar Delta can be distinguished from the others by higher levels of seed K, S and Cu.

Pisum sativum, Protein, Mineral, Genetics, Environment, Agronomy

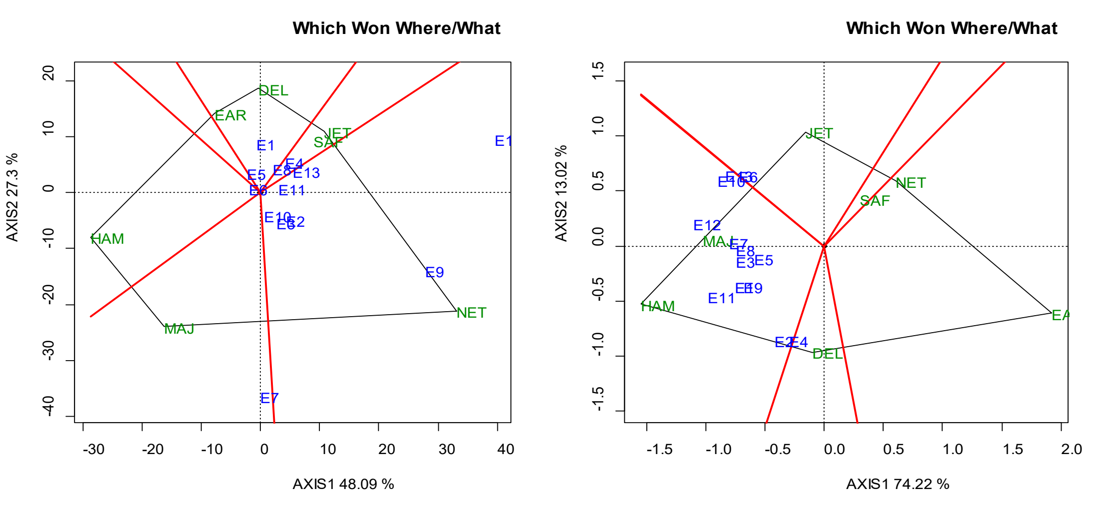

Figure1. Pea seed yield and protein affected by cultivar and environment

Conclusions

Pea yield, protein, and mineral concentration were greatly affected by genetics and environments. Yield was affected more by environment than genetics, while protein concentration is controlled more by genetics and more predictable. High protein cultivar generally produced higher protein concentrations across all environments. Mineral concentrations were affected by locations and most of the macronutrients in seed were correlated to soil mineral concentrations.

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606/13. MUNG4FE; IRON ENRICHED MUNGBEANS FOR EAST AFRICA

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Mungbean [*Vigna radiata* (L.) R. Wilczek var. radiata], also known as green gram, is an important legume crop in Asia, expanding rapidly to other parts of the world, including sub-Saharan Africa. The global area covered by mungbean is about 7 million ha. It is a good source of protein, carbohydrates and important micronutrients like iron and zinc. Mungbean is consumed as whole seed or as split seeds, flour, or as sprouts. The crop is a central income generating agro-enterprise in semi-arid Kenya, Uganda and Tanzania. Improved mungbean lines (26) developed from NM 94 x CN 9-5, CN 9-5 x NM 94, and Harsha x NM94 crosses were evaluated for yield and micronutrient composition across different locations in East Africa (Uganda, Kenya, and Tanzania). The parental lines CN 9-5 and Harsha showed high content of iron (8.7mg/100g and 8.1mg/100g respectively) in a previous study (Nair et al., 2015). Significant genotype effects were observed for most of the traits, except for seed yield per plant and dry matter content ($P<0.05$). Genotype X Environment interaction was significant for all traits, except for dry matter and iron content. Twelve lines (AVMU 1682, AVMU 1686, AVMU 1680, AVMU 1696, AVMU 1698, AVMU 1683, AVMU 1679, AVMU 1694, AVMU 1687, AVMU 1695, AVMU 1697 and AVMU 1684) recorded high iron content (7.31 to 8.06 mg/100g). Further evaluation of these lines will be conducted for potential release as varieties as well as utilizing these lines as donors for mungbean improvement program.

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606/20. EFFECT OF EXTRUSION PROCESS ON FUNCTIONALITY AND STARCH DIGESTIBILITY OF PULSE FLOURS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

The objective of this study was to investigate the effect of extrusion process on functionality and starch digestibility of pulse flours. Green lentil, navy bean and yellow pea flours were fed through a twin-screw extruder with addition of 5% or 10% moisture at barrel temperature of 120°C, 140°C or 160°C. The extrudates were milled into flours with a laboratory Buhlur mill. Chemical and functional properties of the extruded flours were determined according to the published methods. Protein content of the extruded navy bean flour reduced as moisture decreased or barrel temperature increased. Increasing temperature increased starch content of the extruded navy bean flour. Reducing extrusion moisture and temperature increased water hydration capacity of the navy bean flour and oil emulsion capacity of all flour types. Oil absorption capacity (OAC) of the extruded yellow pea flour increased with reducing moisture and increasing temperature. OAC of lentil flour was increased by adding 5% moisture and heating at 160°C. Increasing moisture reduced resistant starch (RS), but increased rapidly digestible starch (RDS) and slowly digestible starch (SDS) of lentil. An increase in temperature resulted in the breakdown of the RS of lentil to more digestible starches. Increasing moisture and temperature reduced SDS, but increased RDS of yellow pea. Functionality and starch digestibility of pulses could be improved by selecting appropriate extruding conditions. Future market for pulses could be expanded through value-added processes such as extrusion to pulse ingredients and products.

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606/27. EFFECTIVENESS OF DRY-FRACTIONATED PEA PROTEIN CONCENTRATE TO PREPARE GLUTEN-FREE FOCACCIA FLATBREAD WITH OPTIMAL SENSORY, TEXTURAL AND NUTRITIONAL PROFILE

Authors:

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Summary:

Objectives

The aim of this work was to formulate a gluten-free focaccia flatbread fortified with pea protein concentrate (55 g/100 g protein content), and optimize its sensory and nutritional profile. Focaccia is a traditional Italian garnished flatbread, widely appreciated throughout the entire country, prepared with wheat flour, vegetable oil, yeast and salt (Pasqualone et al., 2022). This bakery product has not been formulated in a gluten-free version yet, despite the increased request of gluten-free foods. Gluten-free bakery products are often characterized by a poorer nutritional composition compared to the conventional counterpart, being rich in lipids and poor in proteins. Pulses are characterized by high content of proteins, fibers, micronutrients and phytochemicals. The pea protein concentrate was obtained by dry fractionation, a more sustainable technology of protein enrichment compared to wet fractionation.

Concise description of the work (materials & methods)

A simplex-centroid mixture design with seven formulations and three replicates helped to study how the flour ratios influenced the physical and sensory properties of dough and breads.

Main Results

The special cubic model significantly described all the responses determined in the dough and flour mixes, and most of those determined in the focaccia. The addition of pea protein concentrate led to an increase of water absorption index (WAI) and a decrease of water solubility index (WSI), thus influencing the pasting properties of the flour mixes with a decrease of apparent viscosity, probably due to the starch dilution as the protein content increased. The midpoint of the experimental domain (focaccia containing 5 g/100 g of pea protein concentrate and 40 g/100 g of a 50:50 blend of rice and corn flours) appeared to be the optimal focaccia formulation (De Angelis et al., 2023) (Figure 1). This level of pea protein concentrate, indeed, allowed to avoid the typical discolorations of pea. The color coordinates a* and b* accounted for 11.97 and 31.86, respectively, corresponding to a pale orange. Moreover, crumb hardness and chewiness accounted for 9.11 N and 4.83 N, respectively, and legume odor and flavor were moderate (5.6 and 5.3 c.u. in a 0-9 scale, respectively).

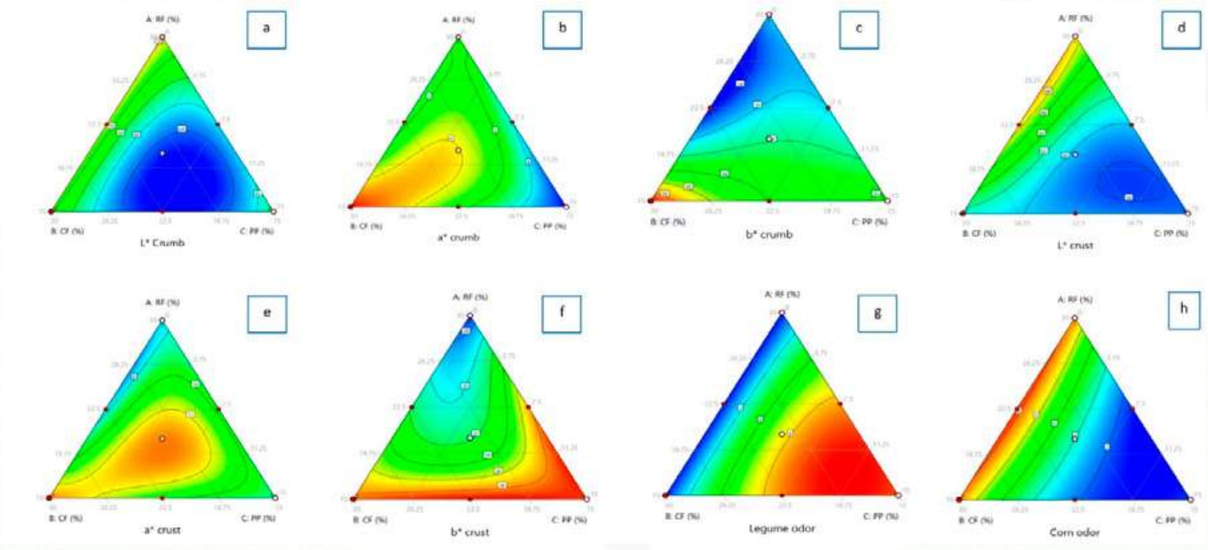

Figure 1. Contour plots depicting the color and odor of focaccia samples prepared with different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). (a) = L index of crumb; (b) = a* index of crumb; (c) = b* index of crumb; (d) = L* index of crust; (e) = a* index of crust; (f) = b* index of crust; (g) = legume odor; (h) = corn odor. Color variation from blue to red indicates an increase of the considered parameter.*

Conclusions

The optimized focaccia could be labelled as "source of protein" (energy value provided by proteins >12%), "source of fiber" (fiber >3 g/100g), and "low-fat" (fat <3 g/ 100 g).

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606/42. MICRONUTRIENT FORTIFICATION OF LENTIL USING HOT EXTRUSION TECHNOLOGY

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Work centre:

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Summary:

Objectives

Micronutrient deficiency or "hidden hunger" is a worldwide public health problem. Micronutrients such as iron, zinc, vitamin A, vitamin D, thiamin, pyridoxine, riboflavin, niacin, folate, and vitamin B12, are essential for all life forms to sustain optimal physiological function. Lentils are an excellent source of protein, fibre, Fe, Zn, Se, Ca, and folate compared to most other grain legumes (Thavarajah et al., 2011). Fortification with highly bioavailable micronutrients can significantly improve humans' micronutrient status, and it delivers essential micronutrients to vulnerable populations in both developed and developing countries at low cost without change in dietary habits (Keats et al., 2019). Extrusion technology is the easiest approach for implementing multiple micronutrient fortification (MMF) to achieve the recommended micronutrient intake levels. The objectives of this study were to develop acceptable multiple micronutrients fortified lentil analogs using hot extrusion technology (HET) and to determine if HET-fortified lentils are acceptable to consumers compared to unfortified lentils.

Concise description of the work (materials & methods)

We developed fortified lentils by mixing micronutrient premixes with lentil flour and emulsifiers with a specific nutritional and dietary design. We assessed the total nutritional profile of fortified lentils, protein digestibility, colorimetric changes, and stability of micronutrients after six months and one year of storage in retail market environment conditions. The sensory acceptability of fortified lentils was assessed with 160 lentil consumers in Bangladesh using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely). The protein and starch hydrolysis kinetic during gastric and intestinal phases of digestion and bioaccessibility of fortified lentils' minerals will be determined. We also planned to assess uncooked multi-fortified lentil's cost and its acceptability to both domestic and international lentil consumers.

Main Results

Micronutrient analysis results showed that beta carotene, vitamin A, vitamin D3, folic acid, vitamin B1, B3, B6, B12, iron, and zinc concentration in fortified lentils were increased by 885%, 140%, 4300%, 1724%, 312%, 800%, 355%, 843%, 264%, and 170%, respectively, compared to the control lentil. Fortification didn't have any significant effect on protein content and its digestibility. No significant differences in colorimetric changes and sensory quality were observed among the cooked fortified and control samples. Results from the protein and starch hydrolysis kinetic during gastric and intestinal phases of digestion and bioaccesibility of fortified lentils' minerals will be presented during our presentation.

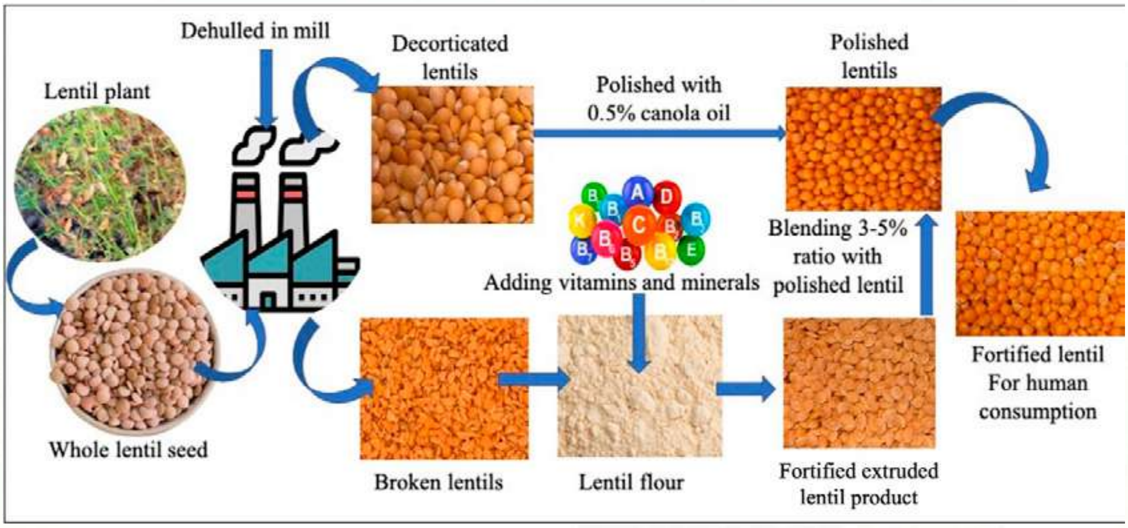

Figure 1. Workflow for the multiple micronutrient fortification streams for lentils using hot extrusion technology.

Conclusions

Lentils fortified with multiple essential micronutrients would address iron deficiency anemia and other major nutritional anemias such as vitamins A, B1, B2, B6, B12, D, folates, and zinc. This range covers the significant nutrient needs of nutritionally vulnerable groups such as vegans, vegetarians, refugees, and populations in low-income countries.

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606/79. BIFIDOGENIC PROPERTIES OF NON-FRUCTOSYLATED GALACTOOLIGOSACCHARIDES FROM PEA (*PISUM SATIVUM*) USING INFANT FECAL HOMOGENATES

Authors:

Maria del Carmen Marin-Manzano[1], Oswaldo Hernandez-Hernandez[2], Marina Diez-Municio[2], Cristina Delgado-Andrade[1], Javier Moreno[2], Alfonso Clemente[1]

Work centre:

(1) Estación Experimental del Zaidin (EEZ-CSIC). Granda. Spain, (2) Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC). Granada. Spain

Summary:

Objectives, Description, Main Results & Conclusions

The interest for naturally-occurring oligosaccharides from plant origin having prebiotic properties is growing, with special focus being paid to supplemented products for infants. Currently, non-fructosylated alfa-galactooligosaccharides (alfa-GOS) from peas have strong interest due to their prebiotic activity in adults and their mitigated side-effects on gas production from colonic bacterial fermentation. In this study, commercially available non-fructosylated alfa-GOS from peas and beta-galactooligosaccharides (beta-GOS) derived from lactose were fermented using fecal homogenates from children aged 11 to 24 months old during 6 and 24 h. The modulatory effect of both GOS on different bacterial groups and bifidobacteria species was assessed; non-fructosylated alfa-GOS consumption was monitored throughout the fermentation process and the amounts of lactic acid and short-chain fatty acids (SCFA) generated were analyzed. Non-fructosylated alfa-GOS, composed mainly of manninotriose and verbascotetraose and small amounts of melibiose, were fully metabolized and presented remarkable bifidogenic activity, similar to that obtained with beta-GOS. Furthermore, non-fructosylated alfa-GOS selectively caused an increase on the population of Bifidobacterium longum subsp. longum and Bifidobacterium catenulatum/pseudo-catenulatum. In conclusion, non-fructosylated alfa-GOS could be used as potential ingredient in infant formula supplemented with prebiotic oligosaccharides.

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606/80. GLYCATION AFFECTS DIFFERENTLY THE MAIN SOYBEAN BOWMAN-BIRK ISOINHIBITORS, IBB1 AND IBBD2, ALTERING THEIR ANTIPROLIFERATIVE PROPERTIES AGAINST COLON CANCER CELLS

Authors:

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Summary:

Objectives

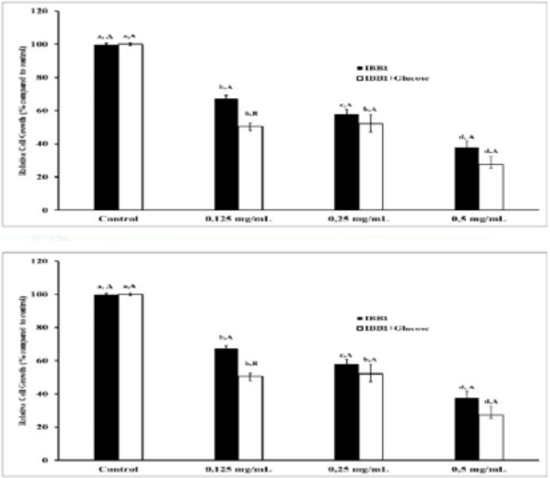
Naturally-occurring serine protease inhibitors of the Bowman-Birk (BBI) family exert their potential chemopreventive and/or therapeutic properties via protease inhibition. Processing of soybeans for human consumption has been proposed as responsible for the loss of positive bioactivity due to the glycation of the proteins during this process. The aim of this work was to understand the effects of glycation of the two major BBI isoinhibitors from soybean, IBB1 and IBBD2, on their functional properties measured as trypsin and chymotrypsin inhibitory activity and antiproliferative effect on HT29 human colorectal adenocarcinoma cells.

Concise description of the work (materials & methods)

Proteins react with reducing sugars at lysine and arginine residues in early stages of the maillard reaction. In soybean BBI isoinhibitors, IBB1 and IBB2, lysine and arginine are key residues in their inhibitory domains. Soybean BBI were purified and subjected to glycation using glucose at high temperature.

Main Results

Glycation pattern of both soybean BBI isoinhibitors was analysed by MALDI-TOF spectrometry and the peptides obtained were analyzed in silico. Both isoinhibitors showed remarkable heat stability. IBB1 maintained 100% of its inhibitory activity after 90 minutes at 95 °C, losing only 25% of its trypsin inhibitory activity after 120 minutes of heat treatment. IBBD2 was less thermostable and after 120 minutes at 95 °C its activity was less than 50% of the control. In the presence of glucose, IBBD2 lost most of its inhibitory activity while IBB1 maintain the same activity as in absence of sugar. Glycation of both isoinhibitors was confirmed by MALDI-TOF spectrometry since glucose adducts were identified. In an attempt to identify the glycated amino-acid residues, the peptides obtained by MALDI-TOF-TOF were analyzed in silico using the program FindPept, a software tool that identifies masses resulting from unspecific proteolytic cleavage normally happening in glycated samples. Apparently the lysines residues responsible for the trypsin inhibitory activity of IBB1 are less prone to be glycated than the arginine resides in the inhibitory domains of IBB2. Previous studies in our group have proven the capacity of BBI to inhibit the growth of human colon cancer cells HT29 in a dose-dependent manner (Clemente et al., 2010).



Effects of glycation over the anti-proliferative effect of BBI isoinhibitors, IBB1 and IBBD2, on the in vitro growth of HT29 human colorectal adenocarcinoma cells. IBB1 and IBBD2 were previously heated for 90 minutes in the presence or absence of glucose. Controls have no inhibitor and cell viability was considered 100%. (A) Percentage of cell growth treated with IBB1 (closed bars) and glycated IBB1 (open bars). (B) Percentage of cell growth treated with IBBD2 (closed bars) and glycated IBBD2 (open bars). Growth media were supplemented with protein in the concentration range 0–61 mM and cells harvested after a period of 96 h. Values are means of at least three independent experiments with five technical replicates, bars represent standard deviations. Mean values with different letters were significantly different (P <0.05; Bonferroni's test). Lower case letters are used to compare relative cell growth with different concentrations of protease inhibitor; uppercase letters are used to compare relative cell growth after treatment with glycated and non-glycated protease inhibitors.

Conclusions

As a result of the differential glycation process, the anti-proliferative properties of IBBD2 against HT29 colon cancer cells were significantly diminished whereas IBB1 was unaffected (Figure) (Olias et al., 2019).

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606/82. THE POSSIBILITY OF INCREASING THE PROTEIN CONTENT OF FABA BEAN SEEDS

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes are the main source of protein for many people all over the world. On average, the protein content in faba bean seeds is 25...30% (DM). The object of the present study was to evaluate the effect of different rates of nitrogen fertilizer on faba bean seeds protein content.

The trial was carried out on the fields of METK in growing season 2020. Before sowing the trial area was fertilized by Yara Mila NPK 7-20-28 + S B Mg, 300 kg ha-1 (N-21, P-18.5, K-62.3 kg ha-1). The trial was established on 10 m^2 plots using NNA randomized design in four replications. The sowing rate is 30 germinated seeds per m^2. The faba bean varieties Bioro and Jõgeva were used for testing. Ammoniumnitrate (nitrogen content 34.4%) was used as the top-fertilizer. Two diferent nitrogen rates: + 10 (variant A) and + 10 + 10 kg ha-1 (variant B) and two application times were used: A) plant heght was 15 cm (BBCH 16) and B) A+ just before the start of flowering (BBCH 59).

The protein content in seeds decreased in the case using extra nitrogen: Bioro control variant – 28.8%, variant B – 28.5%; Jõgeva control variant – 29.8%, variant B – 28.8% (table). Seed yield increased in case using extra nitrogen: Bioro control – 3821, A – 4177 (+ 356 kg), B – 4441 (+ 620 kg) kg ha-1; Jõgeva control – 2356, A – 2542 (+ 186 kg), B – 3101 (+ 745 kg) kg ha-1. The increase in grain yield compensates for the decrease on protein, and the total protein yield increases with the use of additional nitrogen. The results indicate that the highest rate of added nitrogen (21+10+10 kg ha-1), variant B, produces higher crude protein yield on faba bean seeds.

606/95. GENETIC IMPROVEMENT OF PEA TO ENHANCE NUTRITIONAL VALUE

Authors:

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Work centre:

(1) John Innes Centre. Colney. UK, (3) iDNA Genetics. UK

Summary:

Objectives, Description, Main Results & Conclusions

As the demand for plant-based proteins grows and the desire to reduce soybean imports increases, the need for sustainable home-grown protein in the UK is essential. Pea (*Pisum sativum*) seeds provide a good source of dietary protein and carbohydrate but this is considered inferior to soybean by the animal feed industry in terms of protein concentration, digestibility and amino acid profile. Pea seed protein is mainly composed of the globular storage proteins, legumins and vicilins. Legumins contain sulphur-containing amino acids (cysteine and methionine) which are essential for animal and human nutrition, whereas vicilins generally have very few of these amino acids. Vicilins have been associated with biological/defence functions but are also associated with allergenic responses in humans. Vicilins and legumins are encoded by multiple gene families. Sequencing has revealed the range of gene copy number at the different vicilin loci to be between one and eleven, although some genes are predicted to be non-functional. Using a fast neutron mutagenized population (Domoney et al., 2013), we have identified deletion mutations for five classes of vicilin genes, which have been inter-crossed to produce a quintuple null line lacking the majority of vicilin proteins. Removing vicilins alters the seed protein profile with compensatory increases in other proteins. We have assessed the impact of these changes on seed protein content, peptide and metabolite profile, and on agronomic traits such as germination, seed production and insect predation. Overall, the novel pea lines offer potential an extremely valuable alternative to soybean.

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606/97. EFFECT OF CONVENTIONAL vs ORGANIC FARMING SYSTEM ON THE SEED COMPOSITION OF COMMON BEAN (*Phaseolus vulgaris L.*)

Authors:

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Work centre:

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Summary:

Objectives

Organic farming can be defined as having no synthetic inputs (pesticides or fertilizers) and does not use transgenic plants. It looks for minimizing the impact on the environment by maintaining biodiversity, reducing the pollution and eutrophication of water, and degradation of soil quality. This work aims to investigate the effect of farming systems (organic vs. conventional) on the nutritional and functional composition of common bean seeds.

Concise description of the work (materials & methods)

Two homozygous lines classified in the market class 'fabada' were used: line A25 derived from the landrace 'Andecha' and the breeding line A4804 obtained from A25. A completely randomized design was used with four replicates per farming system (conventional vs. organic) and cultivars (A25 and A4804). The conventional trial was developed in soil intensively used in bean crops for the last 20 years. The organic trial was performed in soil with a rotation system of common bean-ryegrass during the last 8 years. Dry pods were manually harvested, threshed, and 50 g of seeds per line and plot were ground. The proximate composition included moisture, ash, fat, protein, and total carbohydrate. Moisture was determined by drying at 105º C, ash by incineration at 550º C, crude protein by the Kjeldahl method and total fat by Shoxlet method. Total carbohydrate (%) was estimated using the difference method. Fatty acid profiles were analyzed by GC-MS according to Rodríguez Ruiz et al. (1998), and soluble sugars [sucrose and raffinose family oligosaccharides (RFO)] were extracted according to Gangola et al. (2014) and the determination in on HPLC system. Significant differences in composition were detected using a two-factor ANOVA (factors: genotypes, and farming systems).

Main Results

Analysis of variance showed significant differences related to the farming system for protein and total carbohydrate content. The protein content was significantly higher in organic farming systems while carbohydrates were higher in samples from conventional farming (Figure 1A). No significant differences were detected by proximate composition between genotypes. The fatty acid profile was established considering saturated and unsaturated (mono- and polyunsaturated) fatty acids. Significant differences were detected between genotypes for saturated and unsaturated fatty acids. The content of saturated and unsaturated fatty acids did not show significant differences for the farming system. However, it was detected a significantly higher accumulation of polyunsaturated fatty acids in the conventional crop, and a higher content of monounsaturated fatty acids in the organic beans (Figure 1B). Concerning soluble sugars, sucrose was the major soluble sugar, and the organic crops showed significantly higher values. Significant differences were also detected between genotypes for ROF and sucrose, and the values were significantly higher in the assays with line A25 (Figure 1C). In sum, the seeds from the organic farming trial had a higher protein content, monounsaturated fatty acids, and sucrose while conventional crop systems showed higher content of polyunsaturated fatty acids. Finally, both genotypes showed significant differences in the fatty acid profile and the soluble sugars evaluated.

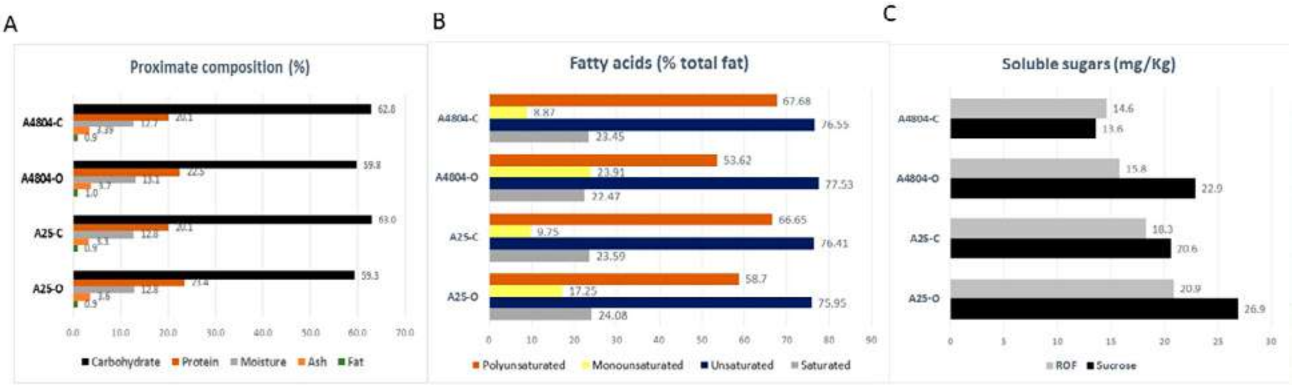

Figure 1. Bar charts showing the proximate composition, fatty acids, and soluble sugars in two bean cultivars (A25 and A4804) cultivated in two farming systems (A25-C, A4804-C, conventional; A25-O, A4804-O, organic)

Conclusions

This study revealed both the impact on seed composition of organic vs conventional farming systems and the variation between genotypes, even within very close genotypes as evaluated in this study.

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606/107. PANNONIAN (*VICIA PANNONICA*) AND HAIRY VETCH (*VICIA VILLOSA*) AS PIG FEED

Authors:

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Work centre:

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Summary:

Objectives

Roughage enriches the environment, reduces aggressive and stereotypic actions and has positive effects in the digestive tract of pigs. Its use is obligatory in organic pig husbandry (EC 2018/848). Silage derived from whole vetch plants is a potentially protein-rich roughage (up to 250 g crude protein per kg DM) that might contribute to closing the amino acid gap in organic farming. However, ingredients with anti-nutritional effects can lead to a decreased acceptance and negative impacts on the fattening performance and carcass quality. The aim of the study was to investigate, if vetch silage is readily ingested by fattening pigs and if it affects fattening performance and carcass quality.

Concise description of the work (materials & methods)

The litters of six sows ((Large White x Norwegian Landrace) x Piétrain) were available. At trial start with a mean body mass of 28 kg, pigs were sorted into two feeding groups containing 20 animals, which were housed in pens containing 10 pigs each. Silage derived from Pannonian vetch (P) and hairy vetch (H) was fed as roughage each to one of two groups. All animals were fed a farm-made feed mixture (crude protein in 88% DM: pre-fattening: 163.9 g kg-1; medium-fattening: 159.4 g kg-1; final fattening: 116.5 g kg-1) and the respective roughage until slaughter (mean final body mass of 121 kg). Silage was initially fed semi ad libitum. However, consumption increased so much that a restriction to 0.75 kg silage per animal per day became necessary. In the medium fattening period (50 - 75 kg body mass), each pig received 1.00 kg and in the final fattening period 1.25 kg of silage per day. Body mass development (weekly), feed consumption and disease incidence were recorded. On defined days, silage residues present in the outdoor runs were collected 5 hours after manure removal before re-littering and their dry matter content was determined. In addition, video observations of the pens were made. At the abattoir, dressing percentage and lean meat content were recorded. Simple linear regressions and linear mixed models with the fixed factor feeding group and the random factors sex and dam were used for the statistical analysis.

Main Results

The silages from P and H contained an average of 19.5 and 16.7% dry matter with 15.7 and 18.6% crude protein and 2.0 and 2.9% crude fat. Both silages attracted great interest from the animals at the time of feeding. Video observations showed that the P and H racks were emptied within 1.6 and 4.6 hours on average. Regarding the collection of silage residues, it must be noted that a reliable separation of silage, manure and straw residues was not possible, so that an underestimation of the intake tended to occur. Recollections were higher at the beginning than at the end of fattening. Of the dry matter presented an average of 40% of P and 25% of H were recollected. This suggests that although the animals quickly pulled P completely out of the rack, it was less attractive outside of the rack than H. There were no significant differences in fattening performance and carcass quality (which were comparable to previous studies on the farm) between the two vetch species (Figure).

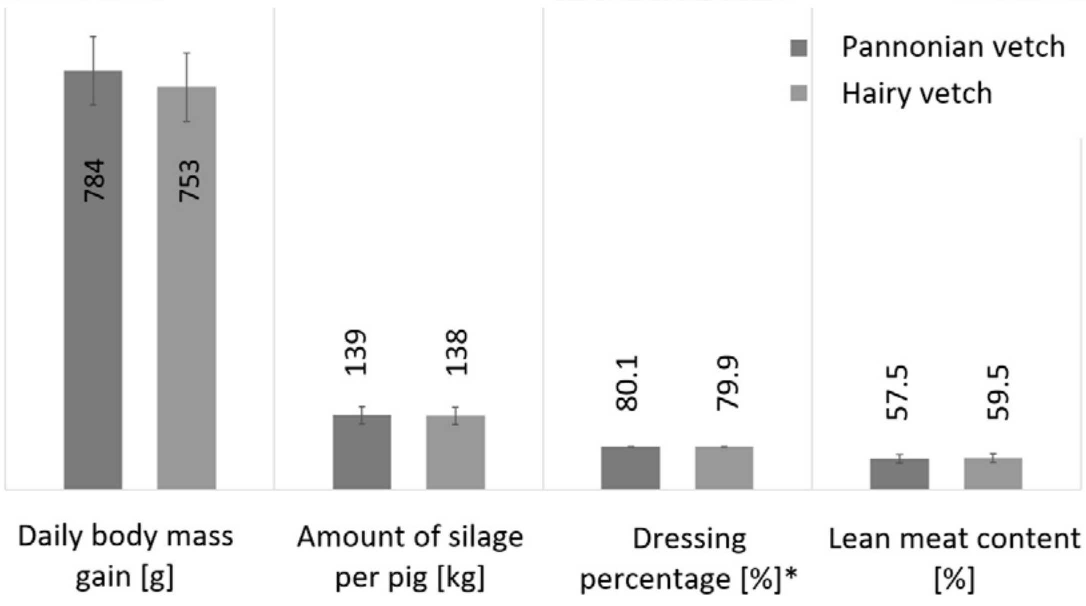

*Figure. Fattening performance and carcass quality parameters of pigs presented with vetch silage. Results derived from linear mixed models (lmer); *simple linear regr. (lm), n.s.>0.05.*

Conclusions

No negative effect of vetch silage on animal health and performance was observed. Vetch silage can thus be used as roughage in pig fattening with satisfactory fattening performance and carcass qualities. Since acceptance is given, trials based on these preliminary trials will follow to determine digestibility and compare vetch silage with clover grass silage and straw as roughage. The determination of roughage intake needs to be improved.

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606/109. EVALUATION OF THE NUTRITIONAL, ORGANOLEPTIC AND BIOACTIVE PROFILE OF CHICKPEA BASED BISCUITS AFTER *IN VITRO* GASTROINTESTINAL DIGESTION

Authors:

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Summary:

Objectives

Biscuits are a very popular and versatile snack food due to their convenience, diverse taste, easy availability, long shelf life and reasonable cost. The biscuit market is in constant evolution due to the current consumers' demands, pushing producers into designing new formulations replacing the traditional cereal flours by other options accepted as healthier or intended for specific population groups (Mesias et al., 2019). The purpose of this investigation was the design of functional gluten-free biscuits with 30% chickpea flours of different varieties (2 commercials and 2 non-commercials).

Concise description of the work (materials & methods)

Biscuits were organoleptically characterized, as well as in their composition and antioxidant profiles. The INFOGEST in vitro gastrointestinal digestion method (Minekus et al., 2014) was applied to establish protein digestibility and amino acid bioaccessibility, and to evaluate selected bioactive properties at physiological conditions.

Main Results

The external appearance of chickpea biscuits was darker than the shortbread biscuit (control), with decreased L* and a* values, especially in that made with a dark purple non-commercial seed. The three-point bending test showed that inclusion of chickpea flour improved the breaking strength, a positive aspect compared to the shortbread cookie brittleness. The nutritional profile was also enhanced, with a higher protein, amino acid and total polyphenolic (TPC) contents, as well as the antioxidant activity, which was even better by the inclusion of non-commercial seeds. After gastrointestinal digestion the protein digestibility of chickpea biscuits was the same for all the samples (15%) except for one of the non-commercial lines (20%). Amino acids such as arginine, phenylalanine, tyrosine and leucine exhibited the highest bioaccessibilities for all the biscuits but the one made with one of the commercial lines (Fuentesauco). The digestion process greatly improved the antioxidant activity of biscuits, once again standing out the cases of non-commercial chickpeas, where the iron reducing ability power (FRAP) was 5-6 times higher than that detected in the shortbread cookie (24.4 and 37.1 μmol TEAC/g digested sample for non-commercial seed vs. 5.7 μmol TEAC/g digested sample in the shortbread biscuit). Bioaccessible total polyphenols in those samples were double than in the control one, p-coumaric and ferulic acids, rutin and resveratrol being predominant. As an approximation to the possible antihypertensive action of the chickpea biscuits, the angiotensin converting enzyme (ACE) inhibitory activity was analyzed in the bioaccessible fractions of digested cookies. Biscuits including non-commercial seeds exhibited lower IC50 than those made with commercial seeds, therefore a higher antihypertensive potential could be expected.

Biscuit	**ABTS** TEAC^1 (μmol/g)	**FRAP** TEAC^1 (μmol/g)	**TPC** GAE^2 (μmol/g)	**ACE inhibitory activit** IC_{50} (μg prot/ml)
Control	138.23 ± 3.02	5.66 ± 0.12	27.90 ± 1.00	^{3}
Commercial seed 1	122.82 ± 7.00	12.90 ± 0.67	40.29 ± 0.61	716.85 ± 44.38
Commercial seed 2	108.36 ± 4.23	18.27 ± 0.04	44.25 ± 0.52	692.48 ± 63.18
Non-commercial seed 1	164.25 ± 5.71	24.36 ±0.42	53.76 ± 1.46	604.15 ± 38.09
Non-commercial seed 2	141.53 ± 10.18	37.11 ± 2.79	49.17 ± 2.05	597.95 ± 64.35

1TEAC defined as Trolox-equivalent antioxidant capacity. 2GAE defined as Gallic acid-equivalent. 3The control biscuits was not included in this analysis due to absence of protein.

Conclusions

In conclusion, the introduction of chickpea flours in the recipe of shortbread biscuits resulted in improved organoleptic, nutritional and bioactive profile after gastrointestinal digestion. Data obtained for non-commercial chickpea varieties were even better than in commercial ones, pointing to a possible commercialization of those seeds to provide consumers with added-value products.

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606/127. COVERAGE OF DAILY MINERAL REQUIREMENTS OF SOME WATER STRESS TOLERANT BEAN LINES AFTER SOAKING AND BOILING

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Common beans (*Phaseolus vulgaris*) are a healthy dietary source of protein, dietary fiber, minerals, and vitamins. However, they contain anti-nutritional factors, such as phytates, tannins and lectins, which can limit their nutritional value by hindering the bioaccessibility of iron and zinc; so, it's necessary to reduce the amount of antinutrients by processing methods. Soaking and cooking are the most common preparation practices to improve nutritional and organoleptic qualities of legumes. Heat treatments can modify the composition of foods, especially minerals and vitamins which can be lost due to chemical modifications, or by leaching into the cooking water (1). Within the international project BIO-BELIEF, four bean lines carrying traits of nutritional interest or water stress tolerance, Meccano (commercial line), hip1 (experimental lpa line), BAT 93 and BAT 881 (control genotypes), were studied for the content of minerals before and after cooking. Samples were soaked for 17 hours in distilled water and boiled in the CREA Research Centre for Food and Nutrition experimental kitchen. Minerals (P, Mg, K, Ca, Mn, Fe, Zn) were determined in raw and cooked samples by ICP-Plasma, and the amount provided by 100 g of cooked beans was compared to the Dietary Reference Values (DRV) for the Italian population (LARN 2014). Results demonstrated that K is the mineral that suffers the greatest loss in all samples, followed by Mn and Mg. The highest loss of Fe occurred in BAT 881 (-72.53%), while their best retention occurred in BAT 93, followed by hip1. Instead, BAT 93 suffers a high loss of Zn (-71.41%). Despite the decrease in mineral concentrations, 100g of cooked BAT 881 covers the 24% (men) and the 13% (women) of DVR for Fe, and BAT 93 provides 13% (women) and 10% (men) of the recommended daily amount of Zn.

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606/143. GASTRONOMIC VALORIZATION OF BITTER VETCH FOR HUMAN CONSUMPTION IN MADRID

Authors:

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Work centre:

(1) Centro de Innovación Gastronómica CIGCM. IMIDRA. Madrid. Spain, (2) Centro de Innovación Gastronómica IMIDRA. Madrid. Spain

Summary:

Objectives

In the current environmental situation, the legumes growth with low requirements emerges as an opportunity. The bitter vetch (Vicia ervilia (L.) Willd.) is a Mediterranean grain legume minor crop, highly adapted to arid drylands. Cultivated mainly for animal feed, its human consumption has been scarce, mainly in stews and flour mixtures in ancient and famine times (De la Rosa et al., 2022). The aim of this work is its morphological, nutritional and culinary characterization and the development of attractive gastronomic proposal for consumer reconsideration.

Concise description of the work (materials & methods)

Samples were obtained both from a local farmer of Madrid (Moro commercial crops 2021 and 2022) and from experimental IMIDRA farm "La Chimenea" (Moro and Taranto varieties crop 2022). Amelia variety chickpeas were used as reference. Seeds size, weight, coat percentage, and absorption capacity after soaking (16 hours, deionized water) were measured for all samples. The proximal composition was determined by an independent agrifood laboratory. Seeds were cooked using a standardized model and the hardness was evaluated as maximum force with a MiniKramer probe in a TA.XTPlus texturometer (Martínez-Maqueda & De Lorenzo, 2019).

Main Results

Moro commercial sample (2022) showed seed size and weight significant larger. Comparing to chickpea, the soaking-related absorption was greater in most of bitter vetch samples (124.10-140.75% dry weight) (115.10%), except for 2022 Moro commercial (112.20%). Seed coat proportion followed the previous pattern in dry conditions, although no differences were observed between bitter vetch and chickpea after soaking (10.60-12.37% total weight). Greater protein content (21.55±0.71% dry weight), lower fat percentage (0.85±0.71%), and a growing trend in dietary fiber contribution, was found in bitter vetch compared to chickpea. Texturometry showed lower hardness values for bitter vetch at 2 h (66.83 vs 112.02 N chickpea), so minor culinary treatment will be required. The elimination of its bitterness constitutes one of our working lines with important advances (data not shown). Different gastronomic proposals such as sprouts, curries, hummus or salads have been developed (Figure), highly accepted by consumers.

Figure. Bitter vetch (Vicia ervilia (L.) Willd) and related gastronomic proposals. (A) Dry seeds, (B) sprouts, (C) Mediterranean salad, (D) curry, (E) hummus.

Conclusions

In conclusion, the observed techno-culinary and nutritional bitter vetch properties, plus an attractive gastronomic offer, represent the opportunity to recover for human consumption an agro-environmental and gastro-diversity promissory legume.

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606/144. COOKABILITY OF TWO DOZEN PEA ACCESSIONS

Authors:

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Work centre:

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Summary:

Objectives

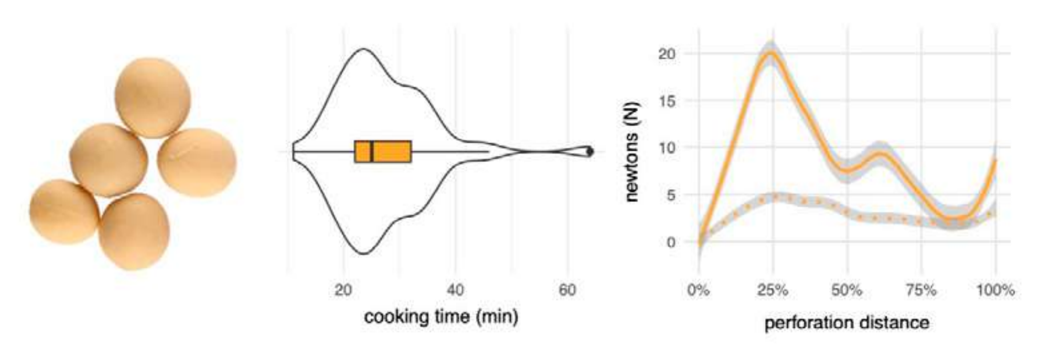
Cooking quality, also called cookability, of dry legume seeds (aka pulses) is a key trait since pulses are staple foods in many countries and pulses are bound to play an important role in our transition toward less resource-demanding diets. What determines cookability in pulses is still an open question (Wood 2017).

Concise description of the work (materials & methods)

To attempt answer this question, we tested two dozen (24) diverse pea accessions for cooking time and cooking evenness using a Mattson cooker. We determined their protein, amylose, amylopectin, and phytate levels, calculated their water absorption capacity, relative area gained (after imbibition), and coefficients of variation (CV) for size, and measured seed hardness with a penetrometer to obtain seed-coat hardness. All these variables were then used in a correlation analysis to infer potential correlations with cooking time and cooking evenness.

Main Results

We found that accessions with wrinkled seeds (due to a mutation in the starch-branching enzyme I gene; Yu et al. 2021), behaved markedly different from all the other 19 accessions in terms of cooking time, seed-coat hardness, and water absorption capacity. Measures on seed area correlated well with cookability, especially the CV for the size of dry seeds. When excluding the accessions with wrinkled seeds from the correlation analysis, seed-coat hardness and relative area gained also scored high as potential cookability predictors.



Seeds from a single accession with boxplot/violin plot from Mattson cooker data and graph from penetrometer data (solid line for soaked seeds and dashed lines for soaked and cooked seeds).

Conclusions

Cooking evenness is an overlooked aspect of cookability and the vast majority of studies on cookability in pulses focus on cooking time. Boxplot information (Figure) provides a robust means for deciphering both cooking time (median) and cooking evenness (IQR), and we therefore advocate for implementing this information in future cookability studies. The CV for the size of dry seeds could be a potential predictor for cookability of any accession type (smooth, dimpled, and wrinkled) but needs further testing over multiple years and environments. However, if this predictor of cookability is proven stable, it could serve as a tool for pea cultivar development, as breeding materials with improved cookability could be screened for without actually cooking the seeds.

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606/154. STATUS OF HEAVY METALS AND POTENTIALLY TOXIC ELEMENTS IN THE FRENCH BEAN IN THE MARKET OF THE CITY OF ZAGREB

Authors:

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Work centre:

(1) University of Zagreb Faculty of Agriculture. Department of plant nutrition. Croatia (2) University of Zagreb Faculty of Agriculture. Croatia (3) University of Zagreb Faculty of Agriculture. Department of vegetable crops. Croatia

Summary:

Objectives, Description, Main Results & Conclusions

The goal of this paper is to determine the status of heavy metals and potentially toxic elements (Cd,Cr,Cu,Ni,Pb,Zn,Mo,Co) in French bean (Phaseolus vulgaris L.) in the markets of the city of Zagreb. Samples were collected on October 17, 2022, in three retail chains, three stores with organic products and three markets. After digestion with concentrated HNO3 and HClO4, the content of heavy metals and potentially toxic elements in the samples was determined by an atomic absorption spectrometer. The dry weight (DW) of the French bean varied from 6.65 to 12.62%. The determined average amounts of heavy metals and potentially toxic elements were in the following ranges: cadmium 0.019-0.025 mg Cd/kg DW and 0.0017-0,0024 mg Cd/kg fresh weight, chromium 0.02-0.25 mg Cr/kg DW and 0.001-0.025 mg Cr/kg fresh weight, copper 7.75-8.74 mg Cu/kg DW and 0.73-0.82 mg Cu/kg fresh weight , nickel 2.48-4.87 mg Ni/kg DW and 0.24-0.36 mg Ni/kg fresh weight, lead 0.61-0.82 mg Pb/

kg DW and 0.05- 0.09 mg Pb/kg fresh weight, zinc 27.30-41.02 mg Zn/kg DW and 2.53-3.80 mg Zn/kg fresh weight , molybdenum 1.45-3.18 mg Mo/kg DW and 0.12-0.34 mg Mo/kg fresh weight, cobalt 0.21-0.28 mg Co/kg DW and 0,020-0.24 mg Co/kg fresh weight. The highest content of copper, nickel, cadmium and cobalt were determined in samples from retail chains, zinc and chromium in samples from organic product stores, and the highest amounts of lead and molybdenum were determined in samples from the market and did not exceed the maximum allowable concentration in any sample.

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606/162. CHEMICAL COMPOSITION (MAIN INGREDIENTS AND ANTI-NUTRITIONAL FACTORS) OF COMMON VETCH (Vicia sativa L.) VARIETIES

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Common vetches are rich in protein, have good protein quality and are therefore very interesting as protein source in organic farming. On the other hand, common vetches contain various secondary plant metabolites that can act as antinutritive factors (ANF) such as pyrimidine glucosides (vicine & convicine) and cyanoalanine toxins (γ-glutamyl-ß-cyanoalanine (GCA) & ß-cyanoalanine (BCA)). To test the influence of the variety on the chemical composition, a field trial with five varieties of Vicia sativa L. (Berninova, Ina, Jaga, Slovena, Toplesa) was conducted at the Thünen Institute of Organic Farming in Northern Germany. The chemical composition in the grains of the varieties were analyzed directly after harvest. In order to test whether different treatments have an effect on the chemical composition of the seeds, especially on the ANF, the seeds were also germinated and ensiled. The protein contents in the varieties varied between 31.9 and 33.8% and increased to 33.2 and 35.0% during germination while ensiling had no effect. The pyrimidine glycosides, which are among the most important ANF (Enneking and Wink 2000), showed contents between 6.7 and 8.2 g/kg for vicine and 0.23 to 1.1 g/kg for convicine. The ensiling led to the reduction of vicine to 60 % and of convicine to 71% of the initial level. Germination only degraded vicine, but not convicine. The contents of GCA in the tested varieties ranged between 9.6 and 12.9 g/kg and for BCA between 0.03 and 0.06 g/kg. The ensiling process resulted in a strong degradation of the GCA- and the BCA-contents to 40-50% of the initial level. Germination led to marginal effects on the toxins.

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606/163. NUTRITIONAL COMPOSITION, IN VITRO GASTROINTESTINAL BEHAVIOUR AND BIOACTIVITY OF LENTIL GERM, A LENTIL PROCESSING BYPRODUCT

Authors:

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Summary:

Objectives

Lentil is an important crop that contributes 15% to global pulse production. Lentils are marketed directly to consumers in the form of whole seeds, dehulled footballs, or dehulled splits. In the process of lentil dehulling, seed coat, and germ are generated as a byproduct. Lentil byproducts have limited economic value, however, reincorporating them into the food processing chain may be a useful strategy for valorization and contributing to the circular economy. Therefore, the aim of this work was to evaluate the nutritional and protein composition of red lentil germ, the in vitro gastrointestinal (GI) behavior of proteins, and the peptide and bioactivity profiles of digesta, as a first attempt at valorization of this lentil side-stream.

Concise description of the work (materials & methods)

Lentil germ was subjected to simulated GI using the static INFOGEST method (Brodkorb et al. 2019). Protein digestion was monitored by gel electrophoresis (SDS-PAGE), and the analysis of free peptides and amino acids was carried out to determine the distribution of the nitrogen fractions after gastric and intestinal digestion. Peptides resistant to GI digestion were characterized by HPLC-tandem mass spectrometry

Main Results

Proximate composition showed that red lentil germ is a good source of protein (43.6%) and fiber (30.9%, soluble to insoluble fiber ratio of 3:1). Moreover, lentil germ was characterized by a small content of starch (5.7%) and significant amounts of antinutrients including phenolic compounds (10.92 mg gallic acid equivalents/g, soluble to bound phenolics ratio of 2:1), phytic acid (1.71 g/100 g) and trypsin inhibitors (19.13 trypsin inhibitory units/g). Vicilin (61% of total protein content) and convicilin (18% of total protein content) were predominant in the protein fraction of lentil germ and were resistant to gastric digestion. Contrarily, they were readily hydrolyzed into free amino acids and peptides of molecular weight below 3 kDa and from 3 to 10 kDa during intestinal digestion, comprising 22.5% and 40.9%, and 21% of the total protein content, respectively. The profile of free amino acids released during digestion supports lentil germ proteins as an excellent source of Thr, Lys, Ile, Leu, Val, and Phe to be used as alternative protein ingredients. GI digestion promoted the release of bioactive peptides causing the increase in the antioxidant and ACE inhibitory activities of lentil germ in the time course of digestion (Table). The peptidomic analysis of the intestinal digesta identified a total of 57 digestion-resistant peptides, 4 of which were found to be potentially bioactive according to in silico analysis.

Digestion phase	ACE inhibitory activity (IC$_{50}$, mg/mL)	ORAC (mM TE/g)	ABTS (mM TE/g)
U	1.10 ± 0.00^{c}	177.50 ± 16.24^{a}	66.72 ± 3.78^{a}
G	0.37 ± 0.01^{b}	249.36 ± 16.28^{b}	85.69 ± 1.37^{b}
I	0.19 ± 0.00^{a}	304.98 ± 29.96^{c}	132.93 ± 5.01^{c}

Table. ACE inhibitory activity, ORAC and ABTS scavenging activity in lentil germ at different phases of gastrointestinal digestion.

Data are the mean ± standard deviation of three replicates. Different lowercase letters within a column indicate statistical differences among different digestion phases ($P < 0.05$; Bonferroni's test). Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; ACE, angiotensin I-converting enzyme; G, end of gastric digestion (120 min); I, end of digestion in the small intestine (240 min); ORAC, oxygen radical absorbance capacity; U, undigested sample.

Conclusions

The results of this study prove the nutritional value and the health-promoting potential of lentil germ and support its reuse and recyclability for plant protein food innovation.

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606/166. PREPROCESSING OF FAVA BEANS ENHANCES PROTEIN DIGESTIBILITY AND THE FORMATION OF RESISTANT BIOACTIVE PEPTIDES IN EXTRUDED FLOURS

Authors:

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Work centre:

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Summary:

Objectives

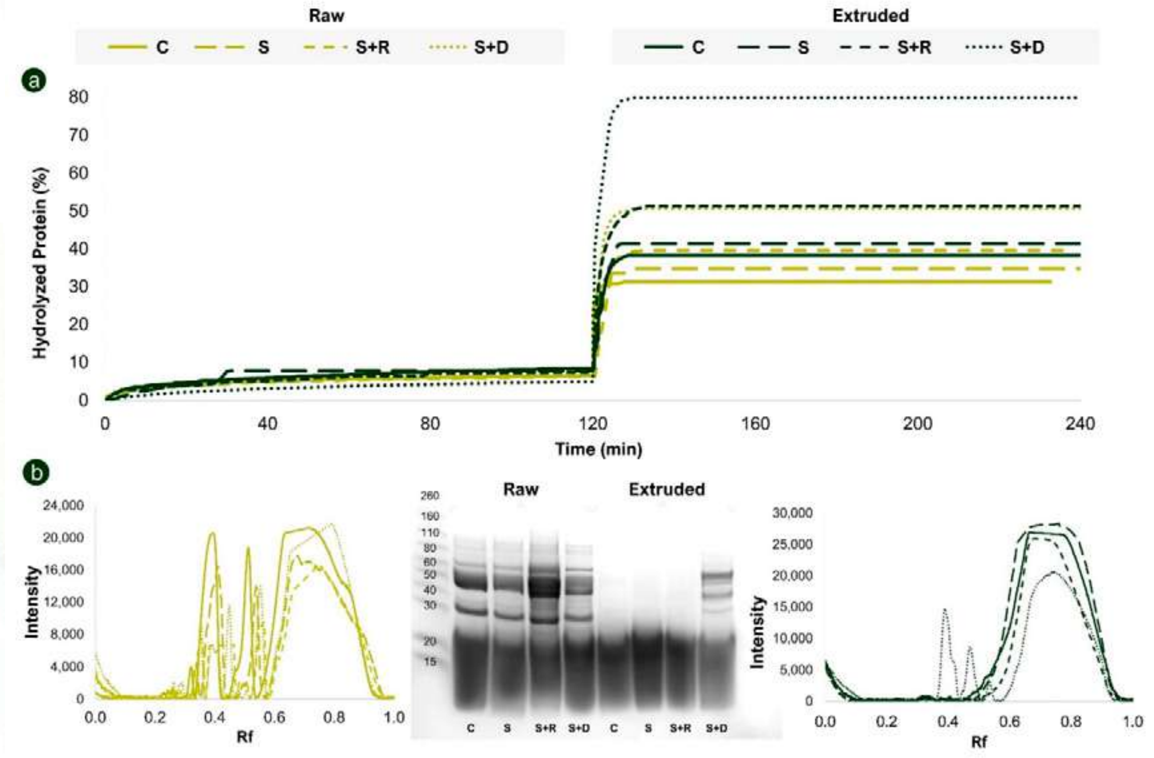
Understanding the effect of preprocessing techniques and extrusion-cooking on protein digestion and release of resistant bioactive peptides (BPs) remains an underexplored area in pulses. Preprocessing techniques, like soaking and dehulling, enhance antinutrients reduction. However, they can alter the concentration and arrangement of bean components affecting the extrusion-cooking impact on protein denaturation and digestibility. This study evaluated how preprocessing affects protein digestion and bioactivity in extruded fava bean flours.

Concise description of the work (materials & methods)

Three treatments were made: soaking (S), soaking and reconstitution (seed coat and cotyledon ground separately, then reassembled) (S+R), and soaking and dehulling (S+D), compared to a control flour (C) before cooking in twin-screw extruder. Protein hydrolysis (PH) was monitored during digestion of raw and extruded flours by the analysis of soluble proteins, peptides (PEP), and free amino acids (FAA). The antioxidant, antidiabetic, and anti-hypertensive activities of intestinal digests were investigated using in vitro assays. Peptidomic (HPLC-MS/MS) and in silico analysis (BIOPEP database) of intestinal digesta of selected sample were conducted to identify bioactive fragments.

Main Results

In raw and extruded flours, antinutrients content decreased, and PH increased as preprocessing techniques were applied (Figure a). S eliminated soluble antinutrients (tannins and polyphenols) and, after extrusion, slightly increased PH compared to C. S+R and S+D to a greater extent, enhanced the effects of extrusion on heat-sensitive antinutrient removal (trypsin inhibitors, tannins, and polyphenols), increased PH during intestinal digestion, and release of digestion-resistant peptides. The molecular weight distribution analysis of proteins (SDS-PAGE) revealed the presence of resistant proteins in the gastric digests of S+R (20-50 KDa) and S+D (50-70 KDa) (Figure b). S+D released more PEP (22.51%) and FAA (15.50%), especially methionine, lysine, and isoleucine, in the intestinal phase compared to the other treatments. In terms of bioactivity, preprocessing decreased the antioxidant activity measured by ORAC and DPPH assays; however, increased ABTS radical scavenging (Figure c), antidiabetic (Figure d and e), and anti-hypertensive (Figure f) activities. S+D displayed the highest dipeptidyl peptidase (DPP)-IV (IC50=13.20 µg/mL), α-amylase (IC50=8.59 mg/mL), and angiotensin I-converting enzyme (ACE) (IC50=1.71 mg protein/mL) inhibition, being selected for peptidomic analysis. A total of 24 BPs were identified, all containing fragments with DPP-IV and ACE inhibitory activities; 6 also had antioxidant fragments. VIPAGYPVAIK and GLTETWNPNHPEL emerged as the BPs with the best antidiabetic and antioxidant potential. Previous studies identified the same antioxidant peptides in hydrolyzed (Samaei et al., 2020) and germinated (Bautista-Exposito et al., 2021) fava bean.



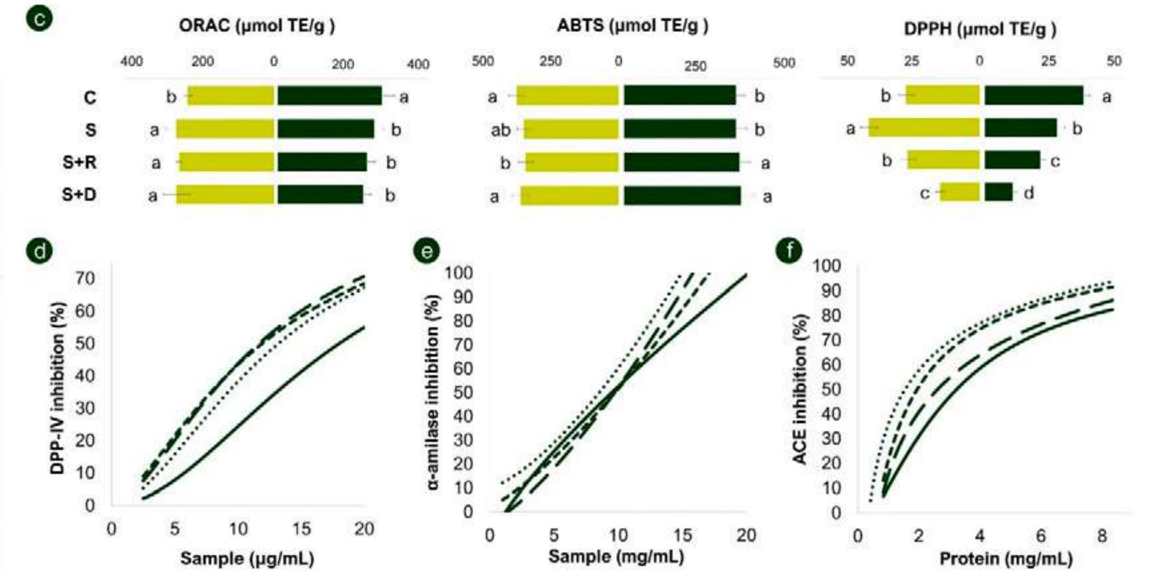


Figure. Effects of preprocessing on (a) PH and (b) SDS-PAGE of gastric digest of extruded and raw fava bean flours, and (c) in vitro antioxidant, (d,e) antidiabetic, and (f) anti-hypertensive activity of the intestinal digests. C = control, S = soaked fava bean (24h at 21°C, water:bean ratio (v/w)=10:1), S+R = soaked and reconstituted fava bean (20% hull+80% cotyledon), and S+D = soaked and dehulled fava bean. Extrusion cooking set at: T1=70°C, T2=90°C, T3=120°C, T4=150°C; screw speed 150 rpm; feed ratio 300 g/min, die diameter 4.8 mm. Results are means of three replicates, bars represent standard deviations. Different letters indicate significant differences (P<0.05; Tukey's test).

Conclusions

Our findings demonstrated that physical preprocessing techniques (S+D) combined with thermoplastic-extrusion, increase fava bean protein digestibility while favoring the release of digestion-resistant BPs in the intestine.

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606/216. THE IMPACT OF PLANT NUTRIENTS ON THE PERFORMANCE AND QUALITY OF LEGUMES

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Summary:

Objectives

Legume crops such as peas (Pisum sativum L.) and faba beans (Vicia faba L.) have traditionally been grown for animal feed or for improving soil fertility and ensuring optimal nutrient availability for subsequent crops. This role is strongly driven by the capacity of legumes to conduct biological N2 fixation (BNF), which minimizes the requirement of external nitrogen fertilizer inputs. In recent decades, the production of legumes for human consumption has increased due to their potential as protein-rich raw materials and ingredients in plant-based diets. This transition places legumes in the centre of future sustainable agri-food systems as high-value crops, but with this novel role follows strict requirements regarding food quality and safety.

Concise description of the work (materials & methods)

The yield stability and quality of legumes is highly dependent on the nutritional status of the plant during the growth season. Plants require 17 elements of the periodic table in order to complete a lifecycle. These include carbon, oxygen and hydrogen, which are derived from CO2 and soil water as well as 6 macronutrients (nitrogen, phosphorus, potassium, magnesium, calcium, sulphur) and 8 micronutrients (iron, chloride, manganese, zinc, copper, boron, molybdenum, nickel). The macro- and micronutrients are all taken up by plant roots if being available in the right chemical form at the right time. In high-yielding agricultural systems fertilization strategies are carefully designed and managed to ensure optimal plant nutrient availability at critical time-points in the growth season. However, due to the ability of legumes to conduct BNF, optimization of fertilization strategies to legumes is rarely considered. It is thus very likely that many legume species are nutrient deficient, which potentially reduces the performance and quality of legume crops.

Main Results

Plant nutrients are the building block of all primary and secondary metabolites in plant tissue. However, the links between plant nutrition and food quality in legumes are poorly understood. Harvest yield and food quality parameters are rarely positively correlated and superior food quality may be obtained at nutrient supplies that are suboptimal or excessive for harvest yield (Figure).

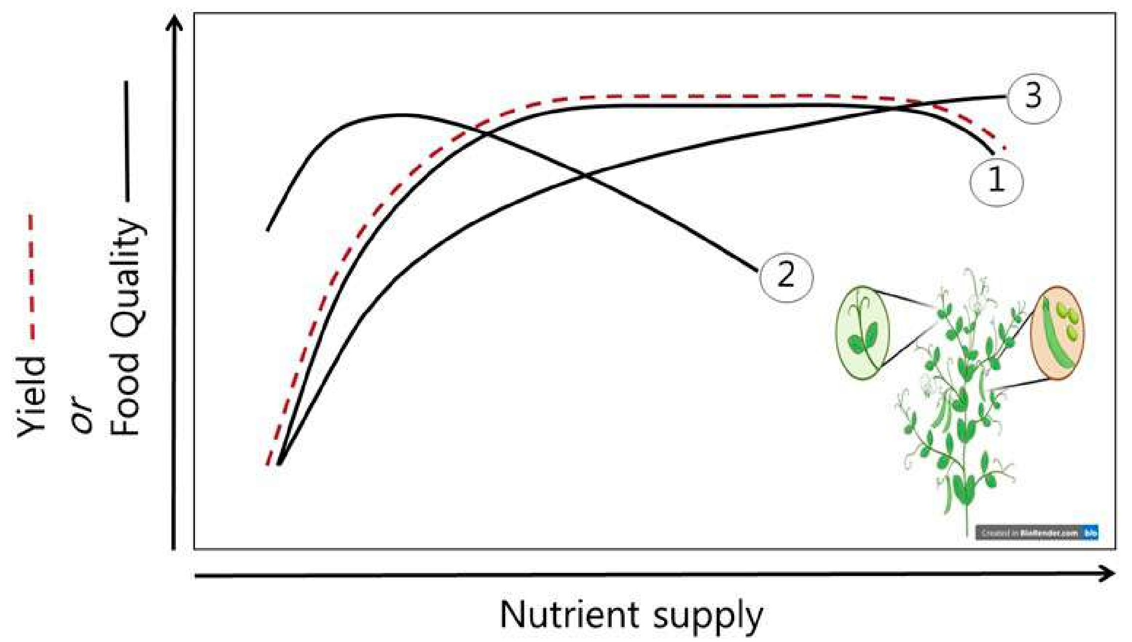

Figure. Plant nutrient supply versus harvest yield (dotted curve) and food quality (curves 1, 2 and 3). Superior food quality is rarely obtained at nutrient supplies optimal for high harvest yields (1). It may instead be obtained at nutrient supplies that are suboptimal (2) or excessive (3) for harvest yield.

Conclusions

At the conference, results from ongoing research projects will be presented and the impact of plant nutrients and different fertilization strategies on legume performance and quality will be discussed.

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Session 4 Biodiversity and genetic resources exploitation

606/58. EFFECTS OF CLIMATE CHANGE ON THE DISTRIBUTION OF LENS GENUS: A MODELLING APPROACH FOR IDENTIFYING CONSERVATION PRIORITIES

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Summary:

Objectives

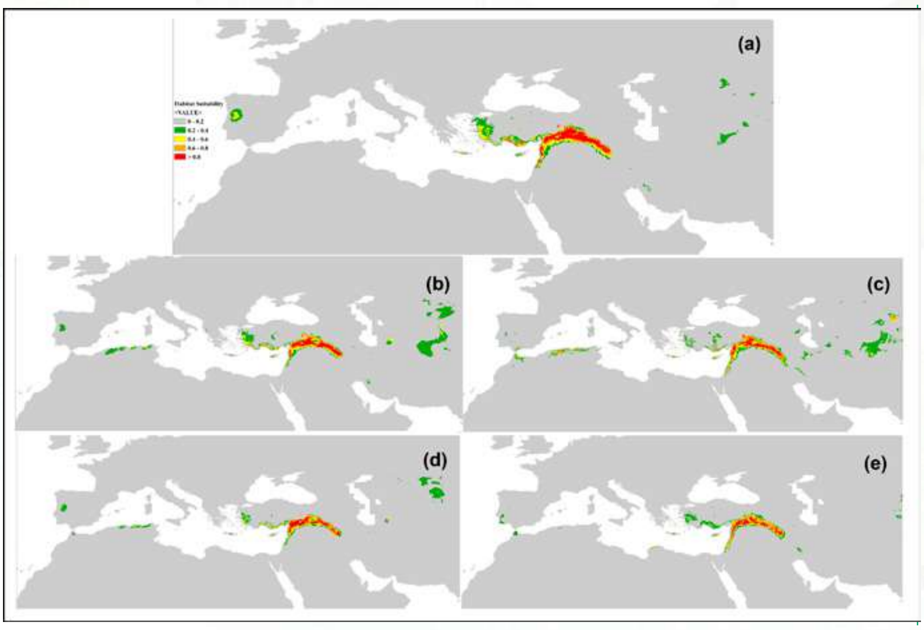
The objectives of this study were to (i) assess the impact of climate change on the potential distribution pattern of wild lentil species (Lens orientalis, Lens ervoides, Lens nigricans, Lens. lamottei, Lens odemensis and Lens tomentosus) under different scenarios and (ii) determine the conservation status and level of protection for the diversity within the Lens genus, both in situ and ex situ.

Concise description of the work (materials & methods)

For this study, occurrence records were obtained from GBIF (https://www.gbif.org/) and Genesys (https://www.genesys-pgr.org/) data sources. Twelve variables were selected to model the current and future distribution of six wild lentil species under the SSP2-4.5 and SSP5-8.5 scenarios in 2050 and 2070, using a species distribution modeling approach MaxEnt (Phillips et al., 2006). Extinction risk was evaluated based on IUCN criterion B, and the conservation score was assessed using the GapAnalysis method (Khoury et al., 2019).

Main Results

The precipitation of the coldest quarter (bio19) and temperature annual range (bio6) are the most important variables influencing the distribution of wild lentil species. A high potential habitat covering 177,011.7 km2, with a probability of occurrence greater than 0.5, was recognized for L. tomentosus. Under the SSP5-8.5 scenario, this habitat area is projected to decrease by 25% by 2070 (Figure 1). The Final Conservation Score (FCS) varied between 17.85 and 37.55, highlighting three wild lentil species (L. ervoides, L. nigricans, and L. tomentosus) as high priority for conservation strategies, and three species (L. orientalis, L. lamottei, and L. odemensis) as medium priority. According to the risk assessment, L. tomentosus is categorized as a vulnerable species, while the other five species are considered to be of least concern or near threatened. Approximately 40 to 50% of wild lentil species are currently facing the threat of extinction.



Prediction of potentially suitable habitat for L. tomentosus under current and future climate change scenarios SSP2-4.5 and SSP5-8.5 in 2050 and 2070. The suitability was classified into very high (>0.8), high (0.6 – 0.8), moderate (0.4 – 0.6), low (0.2 – 0.4) and unsuitable (0 – 0.2).

Conclusions

This study highlights the potential impacts of climate change on the lentil wild relatives species distribution patterns. It provides information on the critical conservation priorities within the Lens genus, emphasizing the need for comprehensive conservation strategies to protect the genetic diversity offered by lentil wild relatives. The findings underscore the vulnerability of L. tomentosus and the significant proportion of wild lentil species facing the risk of extinction. Urgent action is required to safeguard the diversity and ensure the resilience of the Lens genus in the face of changing environmental conditions.

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606/70. THE UPDATED FABA BEAN REFERENCE GENOME AND PROSPECTS FOR GENBANK GENOMICS TO PAN-GENOMICS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicia faba L.) is one of the first domesticated plants at the dawn of agriculture. Its high yield potential and higher grain protein content hold promises for sustainable regional protein supply in temperate regions. The giga-size chromosomes (~13 Gb) bloated by repetitive sequences had hampered its study and crop improvement. With international collaborative effort, we generated the first high-quality annotated reference genome for faba bean (Jayakodi et al. 2022). Recently, we further maximized the quality of the reference genome and gene annotation using optical map and full-length transcripts. With a revolutionary new low-cost high accurate long-read sequencing and a high-quality chromosome-scale physical map in hand, it is now possible to undertake investigations into the pan-genome of faba bean. Further, to capture the global genome diversity efficiently, we characterize the faba bean germplasm collections comprising plant genetic resources in genebanks and modern elite lines (~ 2000 accessions) using a cost-effective genotyping-by-sequencing (GBS) optimised for faba bean. We select a representative genotypes to develop chromosome-scale genome assemblies using this diversity space. The faba pan-genome expect to catlogue global genomic variations and enable improved trait mapping and serve as the permanent genomic resource for sustainable breeding and research.

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606/72. LEVERAGING LANDRACE DIVERSITY: BRIDGING THE GAP BETWEEN KEY TRAITS AND GENOMIC LOCI FOR BREEDING IN COMMON BEAN

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Common bean (Phaseolus vulgaris L.) is a crucial source of plant proteins and essential nutrients for human consumption. Its cultivated forms originated from two independent domestication events in Mesoamerica and the Andes. Large collections of germplasm of this species are currently held in institutes across America as well as in Europe. Primarily consisting of landraces and still harboring significant untapped diversity, these collections have great value for breeding. The advent of next-generation sequencing-based genotyping has revolutionized the genetic characterization of germplasm, offering a great tool to achieve a more rational and efficient use of genetic resources. Genome Wide Association Study (GWAS) capitalize on vast genotyping and phenotyping datasets for the identification of molecular targets for breeding. Here we report the result of GWAS performed on a common bean diversity panel maintained at the genbank of DSA3 (FAO code ITA 363); composed of 192 genotypes, it represents a quite great portion of the European diversity of this species (Caproni et al. 2019). The panel was genotyped by means of a double digest Restriction-site Associated DNA sequencing that resulted in 106,072 polymorphic loci (Raggi et al. 2019). Phenotypes related to plant morphology, phenology, nutritional characteristics and tolerance to abiotic stress were also collected that allowed the identification of several meaningful candidate genes involved in their control. Among the others, significant associations for salt stress tolerance were detected for the first time in this species with candidate genes involved, among others, in the vesicular trafficking machinery, plant developmental processes and abiotic stress responses. Hopefully, this platform will help the selection of new common bean cultivars able to better cope with the challenges posed by the effects of climate change.

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606/134. NUTRITIVE VALUE OF WILD LEGUME SPECIES GROWN IN A GRASSLAND AGRICULTURAL SYSTEM

Authors:

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Work centre:

(1) Swedish University of Agricultural Sciences. Sweden

Summary:

Objectives

Generally comprised of red clover (Trifolium pratense L.) and various forage grass species, leys (grassland agricultural systems utilized in rotation with annual crops) are the primary source of forage in northern Sweden. Due to issues with red clover persistence, ley productivity and nutritive value decrease following the second production year (Frankow-Lindberg et al., 2009). A previous study recommended wild forage legume species native to northern Sweden as promising additions to leys in the region to provide a legume component following the loss of red clover (Micke & Parsons, 2023). This study evaluates the nutritive value of red clover and three native legume species, Lathyrus pratensis L. (LP), Vicia cracca L. (VC), and Vicia sepium L. (VS), when grown in mixed stands with timothy and harvested under varying frequencies.

Concise description of the work (materials & methods)

A field experiment was established in northern Sweden in 2021 with treatments composed of varying seed mixtures and harvest frequencies. Seed mixes included a control of timothy and red clover, two mixes with either VC or LP sown with timothy, and a maximum diversity mix with all three native legume species sown with timothy. Plots were harvested according to either a one-, two-, or three-cut system in 2022. Samples of the legume component were analysed for nutritive value and analysed using a general linear mixed model procedure with harvest and species as fixed effects, as well as their interaction.

Main Results

The mean estimate of CP for the effect of species was significantly lower for red clover than the native legume species. CP concentrations were 170, 212, 213, and 229 g kg-1 DM for red clover, VS, LP, and VC, respectively. The CP concentration of red clover from this study is considerably lower than values reported by variety trials at the same site where a three-cut system is utilized. Additional data is needed to determine the cause of the low CP concentration of red clover in this study, as the impact of harvest frequency on red clover CP is not clear in this study. The aNDFom (amylase-treated, ash-free neutral detergent fiber) concentrations of VC and LP were significantly higher than red clover and VS in all harvests apart from the one-cut system (Table 1). All species had varying aNDFom concentrations across harvests, with the highest aNDFom in harvests from the one-cut system and lowest in the three-cut system. LP had a significantly lower VOS (våm-vätskelöslig organisk substans = ruminal fluid) digestibility than the other legume species in all harvests. Additionally, the digestibility of VS was significantly higher than that of VC in all harvests. The digestibility of red clover had larger variation between harvests, but was not significantly different from VS in any harvest except for the one-cut system.

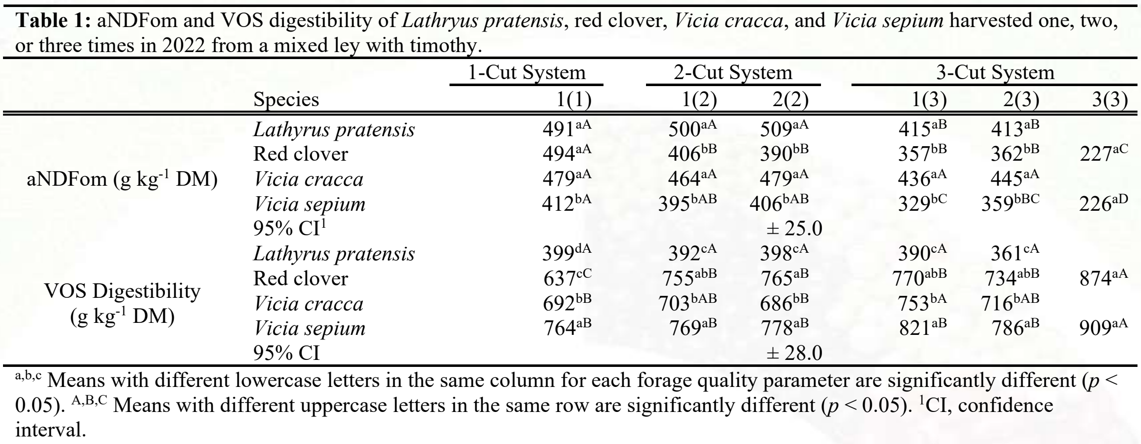

Table 1: aNDFom and VOS digestibility of *Lathryus pratensis*, red clover, *Vicia cracca*, and *Vicia sepium* harvested one, two, or three times in 2022 from a mixed ley with timothy.

		1-Cut System	2-Cut System		3-Cut System		
	Species	1(1)	1(2)	2(2)	1(3)	2(3)	3(3)
aNDFom (g kg^{-1} DM)	*Lathyrus pratensis*	491^{aA}	500^{aA}	509^{aA}	415^{aB}	413^{aB}	
	Red clover	494^{aA}	406^{bB}	390^{bB}	357^{bB}	362^{bB}	227^{aC}
	Vicia cracca	479^{aA}	464^{aA}	479^{aA}	436^{aA}	445^{aA}	
	Vicia sepium	412^{bA}	395^{bAB}	406^{bAB}	329^{bC}	359^{bBC}	226^{aD}
	95% CI[1]			± 25.0			
VOS Digestibility (g kg^{-1} DM)	*Lathyrus pratensis*	399^{dA}	392^{cA}	398^{cA}	390^{cA}	361^{cA}	
	Red clover	637^{cC}	755^{abB}	765^{aB}	770^{abB}	734^{abB}	874^{aA}
	Vicia cracca	692^{bB}	703^{bAB}	686^{bB}	753^{bA}	716^{bAB}	
	Vicia sepium	764^{aB}	769^{aB}	778^{aB}	821^{aB}	786^{aB}	909^{aA}
	95% CI			± 28.0			

[a,b,c] Means with different lowercase letters in the same column for each forage quality parameter are significantly different ($p < 0.05$). [A,B,C] Means with different uppercase letters in the same row are significantly different ($p < 0.05$). [1]CI, confidence interval.

Table 1: aNDFom and VOS digestibility of Lathryus pratensis, red clover, Vicia cracca, and Vicia sepium harvested one, two, or three times in 2022 from a mixed ley with timothy.

Conclusions

These results demonstrate that VS has superior or comparable nutritive value to red clover under all harvest frequencies. Though VC had a significantly higher fiber concentration than red clover, its CP concentration and digestibility demonstrate its potential as a forage crop. The poor digestibility reported for LP is of concern, and additional work is needed to determine what factors contribute to its indigestibility. An additional year of data collection is underway to assess the nutritive value of these forage legume species in the second production year.

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606/151. GENETIC BASIS OF POD TRAITS RELATED TO DOMESTICATION AND CONSUMER PREFERENCE IN COMMON BEAN

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Common bean (Phaseolus vulgaris L.) is an important legume crop grown for both 1) protein-rich seeds and 2) green “snap” beans with edible pods. The domestication of common bean and subsequent selection of snap bean varieties both entailed critical changes to pod structure. In the case of domestication and range expansion, this included a reduction in pod shattering, which is important for seed dispersal of wild beans. During the comparatively recent breeding of snap beans, there was a further and drastic reduction in pod fiber. We have identified several major genes and mutations related to these changes. These include a single base pair substitution in the active site of PvPOD-DEHISCENCE1 (PvPDH1), a gene responsible for regulating pod shattering in the early evolution of cultivated common bean (1). We subsequently identified that mutants at this locus are found at highest frequencies in arid conditions, where shattering is most problematic. We then identified a major locus responsible for the loss of fibrous pod "strings" during the evolution of snap green beans. In snap beans, a tandem direct repeat of PvINDEHISCENT (PvIND) and a Ty1-copia retrotransposon insertion between the repeats is responsible for a loss of fibrous pod strings (2). These sequence features are associated with a dramatic increase in PvIND expression. Intriguingly, snap beans revert to the ancestral fibrous state at a frequency of approximately 1%. The tandem duplication and intervening transposable element are lost during pod reversion, likely due to high-frequency unequal crossing over, paralleling patterns of the Bar gene in Drosophila. Our results give insight into the deployment of exotic genetic resources and aridity tolerance in common bean. Furthermore, they shed light on reversible gain-of-function mutations and possible genetic solutions to reversion.

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606/169. LEGUME AND HERB BASED SPECIAL SEED MIXTURES FOR GREENING THE TRAM TRACKS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

During more than 30 years of collecting activities about 5 500 accessions of legumes, herbs and grasses have been collected in the territory of the Czech Republic. These seed samples represent a vast part of domestic plant diversity. Most of collected samples have been evaluated, regenerated and stored in the national gene bank. The main aim of the project No. SS01020023: "Germplasm for cities and landscape", financed by Technology Agency of the Czech Republic (TACR) is to select and evaluate domestic genetic resources of legumes, herbs and grasses suitable for greening of cities, transport constructions, tram and train tracks, revitalisation of damaged areas and apply them practically in special seed mixtures for urban conditions. Selected genotypes are tested on physiological limiting factors and survival under extreme conditions in growing chambers. Nine special seed mixtures using resilient genotypes were compiled and since 2021 have been evaluated in trials performed on tram tracks under traffic. Five experimental localities in Prague were selected for the evaluation. Urban conditions are characterized by multiple stressors influencing the vegetation, like severe drought, high temperatures, salinisation, intensive direct insolation or deep shading on the other hand. Based on the results it can be concluded, that only limited number of plants species and their genotypes could successfully resist in this inhospitable environment. Grasses cope much better with the extreme conditions of urban climate than legumes and dicots. Legumes and dicots are very valuable due to their aesthetic value and as feed sources for insects and pollinators. Three most promising seed mixtures are now proposed for legal protection in the form of utility models, and their use will be directed for the climatic conditions of Central European cities.

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606/170. CULTIVAR DIFFERENCES IN ROOT DEVELOPMENT AND DEPTH OF FORAGE LEGUMES

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Summary:

Objectives

Forage legumes contribute to the sustainable development of agricultural systems by providing high-protein feed and improving soil fertility. Concurrently, they deliver ecosystem services, including cropping system resilience to climate

change (Lüscher et al. 2014). The root system development and depth are instrumental for improved resource use efficiency as well as the agronomic and environmental performance of crops. However, grassland legumes are mainly bred for aboveground traits and little attention has been given to the belowground part (Marshall et al. 2016). The cultivar differences in root system development and depth remain poorly understood. Thus, to increase knowledge on this we investigated three important grassland legumes; red clover (Trifolium pretense L.), white clover (Trifolium repens L.), and lucerne (Medicago sativa L.).

Concise description of the work (materials & methods)

Cultivars of lucerne (cv. Cigale, Mezzo and Musette), red clover (cv.Callisto, Elara and Amos) and white clover (cv. Brianna, DLT TRF 3536 and Silvester) were studied in a rhizotron tube experiment at the University of Copenhagen, Denmark. A detailed screening of differences in the early establishment, root architecture, and rooting depth was made in 0.5 and 2m tall transparent acrylic rhizotron tubes. The root development over time was monitored by measuring rooting depth, and imaging roots followed by image analysis by the AI-based software RootPainter.

Main Results

The first-year results from the rhizotron experiment showed differences in root growth between both crop species and cultivars (Fig. 1).

Lucerne had initially a faster root growth, but this did not last during the growing season. After 45 days, red and white clover had significantly more dense and deeper roots, compared to lucerne ($p<0.05$). Interestingly, Silvester, one of the white clover cultivars, was found to have the deepest rooting depth of 1.82 m followed by the red clover cultivar Amos (1.75 m) measured 118 days after transplanting.

The highest planar root length density (pRLD) determined from the images was found in red and white clover cultivars at soil depths between 0.3 and 1.2 m ($p<0.001$). Initially, red clover (cv. Amos), lucerne (cv. Musette) and white clover (cv. DLF-TRF) showed greater pRLD in the top 0-0.3 m soil depth. The red clover cultivars Elara and Callisto had greater pRLD between 0.3 and 1.2 m depth, whereas the white clover cultivar Silvester showed greater pRLD below 1.2m soil depth.

Figure 1: The roots of lucerne (LU), red clover (RC) and white clover (WC) cultivars recovered 28 days after transplanting (DAT) from 0.5m rhizotron tubes (a), and root images taken 118 DAT in the 60-90 cm soil depth, with the highest planar root length density determined in RC and WC cultivars and the LU cultivar Mezzo, in 2m rhizotron tubes.

Conclusions

Contrary to the general perception, our results showed deep root growth of white clover. Lucerne is well-known to have deep roots, but was shown to have a slower root development in the establishing year. The results provide new insights into the root development of three important grassland legumes, especially in deep soil layers. The knowledge on root development will have implications for future breeding for improved resource use efficiency, cost-effective and sustainable means of protein-rich biomass production, and climate resilience. The rhizotron study is currently validated in the field as well as in 4 m tall rhizoboxes including also root function studies based on tracer uptake.

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606/175. GENETICS OF DOMESTICATION IN COMMON BEAN (PHASEOLUS VULGARIS L.): AN APPROACH FOR THE ANALYSIS OF CANDIDATE GENES VIA TILLING-BY-SEQUENCING

Authors:

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Summary:

Objectives

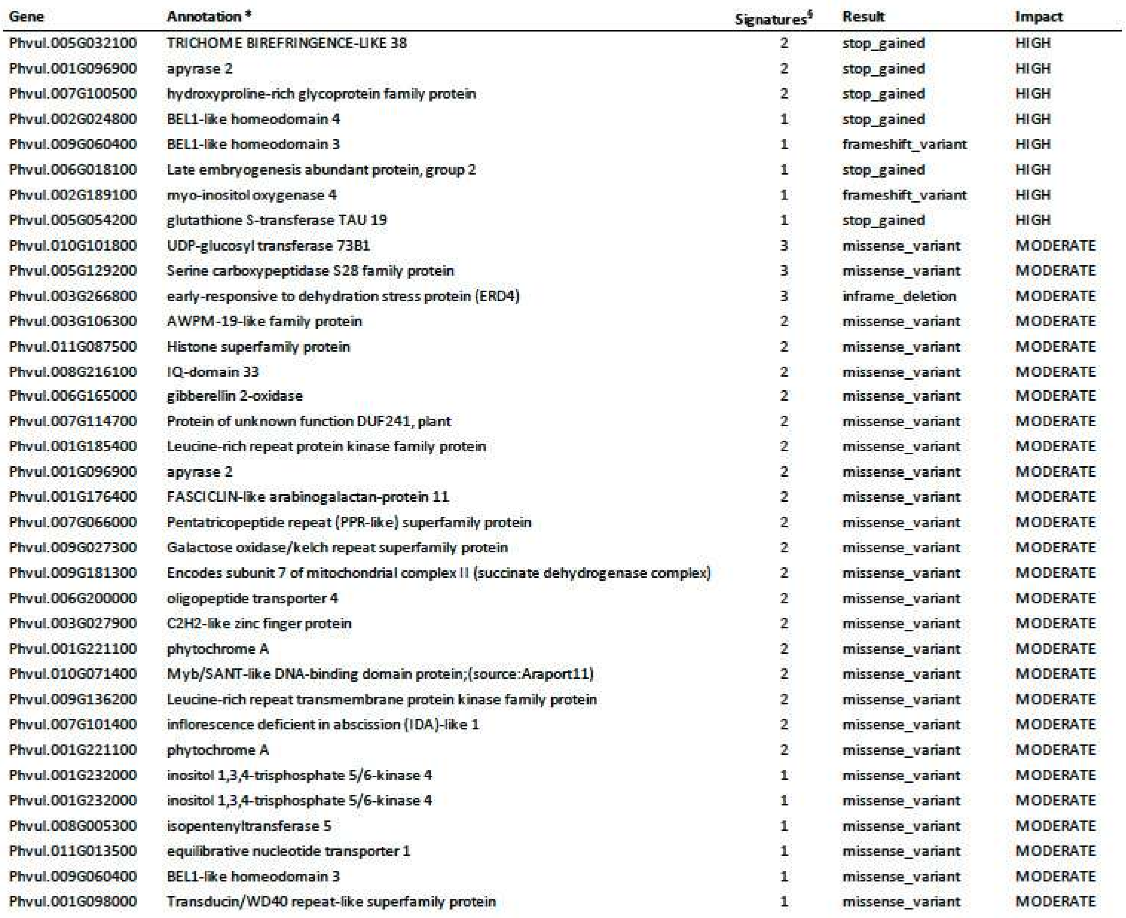
According to Charles Darwin, domestication can be thought as a great model to study convergent evolution. Among crops, common bean (Phaseolus vulgaris L.) represents a unique example of multiple parallel independent domestications: wild common bean is organized in two geographically isolated and genetically differentiated wild gene pools (Mesoamerican and Andean) that diverged from a common ancestral wild population, then independently domesticated in Mexico and in South America nearly 8,000 years ago. These processes resulted in morphological changes (e.g. seed and leaf sizes, seed coat color, growth habit, photoperiodic responses) that distinguish culturally adapted classes of beans. In addition to the interest emerging from its domestication history, common bean also carries a pivotal agronomic value: it is one of the most important grain legumes for human consumption and, as a legume, it also has a role in sustainable agriculture owing to its ability to fix atmospheric nitrogen. To gain deeper insights into the genetic basis of common bean domestication we used an available TILLING population, obtained by chemical EMS-mutagenesis of the Mesoamerican genotype BAT 93 (1), and developed a TILLING-by-sequencing pipeline starting as a method for the identification of mutants of candidate domestication genes.

Concise description of the work (materials & methods)

DNA was extracted from seeds of 1728 M4 individuals of an EMS-mutagenized population developed in the Mesoamerican genotype BAT93 [1]. Samples were combined using a 3D pooling system (Figure1) and sequenced by Illumina at High Coverage (50x) after enriching target regions using custom capture probes. Probes were designed to cover a total of 719 genes of interest (approx. 491 Mb), based on the presence of: i) one or more signals of domestication or ii) differential expression between the Andean genotype and Mesoamerican genotype, and iii) known involvement in the phenomenon of shattering, in seed traits, in the cytokinin hormonal pathway and in phytic acid biosynthesis and metabolism.

Main Results

Sequencing data were aligned on the BAT 93 reference genome. Variant calling from 3D pools was performed using four variant callers and low frequency variant calling was performed by combining different algorithms (NGSEP v4.1.0, GATK Mutect2 v4.2.0, VarScan v2.4.2, CRISPR v0.2): 144 variants were identified on 128 candidate genes. Data validation on the same DNA samples was performed via PCR and Sanger re-sequencing: 84 variants were confirmed (65,6%) and were carried by a total of 78 lines, along 103 of the screened genes. Among these variants, 8 had a high inferred impact (gain of stop codon or frameshift), 50 had a moderate inferred impact (non-synonymous variants) while the remaining did not produce any amino acid change. Selected M4 (35) lines carrying high (8) and medium impact (27) mutations are being genotyped and reproduced to obtain M5 homozygous individuals; phenotypical analysis of selected lines will be also carried out (Table 1).



Gene	Annotation *	Signatures§	Result	Impact
Phvul.005G032100	TRICHOME BIREFRINGENCE-LIKE 38	2	stop_gained	HIGH
Phvul.001G096900	apyrase 2	2	stop_gained	HIGH
Phvul.007G100500	hydroxyproline-rich glycoprotein family protein	2	stop_gained	HIGH
Phvul.002G024800	BEL1-like homeodomain 4	1	stop_gained	HIGH
Phvul.009G060400	BEL1-like homeodomain 3	1	frameshift_variant	HIGH
Phvul.006G018100	Late embryogenesis abundant protein, group 2	1	stop_gained	HIGH
Phvul.002G189100	myo-inositol oxygenase 4	1	frameshift_variant	HIGH
Phvul.005G054200	glutathione S-transferase TAU 19	1	stop_gained	HIGH
Phvul.010G101800	UDP-glucosyl transferase 73B1	3	missense_variant	MODERATE
Phvul.005G129200	Serine carboxypeptidase S28 family protein	3	missense_variant	MODERATE
Phvul.003G266800	early-responsive to dehydration stress protein (ERD4)	3	inframe_deletion	MODERATE
Phvul.003G106300	AWPM-19-like family protein	2	missense_variant	MODERATE
Phvul.011G087500	Histone superfamily protein	2	missense_variant	MODERATE
Phvul.008G216100	IQ-domain 33	2	missense_variant	MODERATE
Phvul.006G165000	gibberellin 2-oxidase	2	missense_variant	MODERATE
Phvul.007G114700	Protein of unknown function DUF241, plant	2	missense_variant	MODERATE
Phvul.001G185400	Leucine-rich repeat protein kinase family protein	2	missense_variant	MODERATE
Phvul.001G096900	apyrase 2	2	missense_variant	MODERATE
Phvul.001G176400	FASCICLIN-like arabinogalactan-protein 11	2	missense_variant	MODERATE
Phvul.007G066000	Pentatricopeptide repeat (PPR-like) superfamily protein	2	missense_variant	MODERATE
Phvul.009G027300	Galactose oxidase/kelch repeat superfamily protein	2	missense_variant	MODERATE
Phvul.009G181300	Encodes subunit 7 of mitochondrial complex II (succinate dehydrogenase complex)	2	missense_variant	MODERATE
Phvul.006G200000	oligopeptide transporter 4	2	missense_variant	MODERATE
Phvul.003G027900	C2H2-like zinc finger protein	2	missense_variant	MODERATE
Phvul.001G221100	phytochrome A	2	missense_variant	MODERATE
Phvul.010G071400	Myb/SANT-like DNA-binding domain protein;(source:Araport11)	2	missense_variant	MODERATE
Phvul.009G136200	Leucine-rich repeat transmembrane protein kinase family protein	2	missense_variant	MODERATE
Phvul.007G101400	inflorescence deficient in abscission (IDA)-like 1	2	missense_variant	MODERATE
Phvul.001G221100	phytochrome A	2	missense_variant	MODERATE
Phvul.001G232000	inositol 1,3,4-trisphosphate 5/6-kinase 4	1	missense_variant	MODERATE
Phvul.001G232000	inositol 1,3,4-trisphosphate 5/6-kinase 4	1	missense_variant	MODERATE
Phvul.008G005300	isopentenyltransferase 5	1	missense_variant	MODERATE
Phvul.011G013500	equilibrative nucleotide transporter 1	1	missense_variant	MODERATE
Phvul.009G060400	BEL1-like homeodomain 3	1	missense_variant	MODERATE
Phvul.001G098000	Transducin/WD40 repeat-like superfamily protein	1	missense_variant	MODERATE

*List of selected genes carrying high or medium impact variants identified with TILLING-by-sequencing. * annotations are according to Arabidopsis homologs; § domestication signatures set are: A, genes related to gene pod-shattering (PS); B, differentially expressed genes between domesticated and wild genotypes; C, PS genes in the Mesoamerican pool according to [2]; D, PS genes in the Mesoamerican pool according to [3].*

Conclusions

The TILLING-by-sequencing approach adopted here (three-dimensional DNA pooling strategy coupled with a High Coverage Illumina sequencing after enriching target regions using custom capture probes) allowed us to identify 53 target genes carrying variants with high/medium impact and whose phenotypic effects are currently under evaluation on 31 of them. Considered the relevance of these results and to provide the scientific community a structured platform to look for mutants in common bean, the development of a new TILLING population in the Meccearly dwarf borlotto type variety (Blumen) has recently started and 3516 M1 plants are currently under reproduction

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606/182. CHARACTERIZATION OF A FABA BEAN DIVERSITY PANEL: PHENOTYPIC ANALYSIS AND GENOME-WIDE ASSOCIATIONS FOR AGRONOMIC AND SEED QUALITY TRAITS

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Summary:

Objectives, Description, Main Results & Conclusions

Around the world, faba bean (Vicia faba) is a crop that is cultivated in diverse climate zones, including the Northern latitudes. However, increasing the faba bean production from the current relatively small acreages in the Nordic region could contribute to establishing a more sustainable food system. Access to early high-yielding cultivars with a seed quality that meet the demands from feed and food producers, is a prerequisite for this. In this work, we characterized a diversity panel of faba bean consisting of 220 accessions, including gene bank material, landraces, and old and modern varieties. These were genotyped using DArTseq and agronomic traits were characterized in field trials in Sweden. The results revealed a large variation in eleven agronomic and seed quality traits including starch and protein content. Seed yield was positively correlated to plant height, number of seeds per plant, and time to maturity, according to pairwise correlations between traits. Further, early flowering accessions had a higher susceptibility to bean weevil damage, and protein content was negatively correlated to starch content. By aligning the genotype data to the newly sequenced faba bean genome (Jayakodi et al. 2023) we identified 6607 SNPs which were used in a genome-wide association study. This led to the discovery of 55 markers that were significantly associated with six of the characterized traits. Our results showed that while earlier improvements in faba bean breeding have resulted in larger yields, they have also led to a selection for a delayed onset of flowering and maturity.

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606/208. EARLY LEGUME DOMESTICATION FAVORED FAST GROWTH AND RESOURCE-CAPTURE

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Summary:

Objectives

Extant crop biodiversity is largely made up by pre-industrial landraces. These derive from two different albeit intertwined processes: crop domestication and diversification under cultivation. Since all we have is the net result of both, it is often difficult to establish what traits were selected at each step. For instance, although yield has been historically thought of as the primary breeding target of the first farmers, increasing evidence indicates that domestication might have given priority to taxa that developed better under agricultural settings, and that improved yield may have been a later product of human control over crop reproduction (Abbo et al., 2014). Crop fields present striking ecological and demographic differences from natural populations, were interspecific density is much lower and diversity higher. Therefore, crops have to develop under higher competition for space and resources than their wild relatives (Denison, 2016; Milla, 2023). Here, we investigated whether initial crop development might have indeed focused on traits related to competition and resource acquisition by comparing differences between wild relatives and domesticated species and between wild and crop forms of the latter.

Concise description of the work (materials & methods)

We used common garden experiments to establish reproductive and vegetative differences under three different competition regimes in 28 accessions of lentil (Lens sp.), grasspea (Lathyrus sp.) and vetch (Vicia sp.). We measured architectural traits related to competition, resource and spatial acquisition: plant height and lodging, growth rate and total aerial and root biomass.

Main Results

Although taxonomic differences were always important and every genus had idiosyncratic patterns, our results showed that wild relatives consistently differ significantly from wild populations of crops in their competition and resource acquisition traits, producing smaller plants, with much smaller roots and with lower growth rates. Competition level also had a significant effect on all traits, which led to significant differences in how plants responded to low vs. high population density. Conversely, the effect of selection under domestication on the measured treats was much more subtle, often non-significant or even, in some cases, associated to reversals (i.e., leading to seemingly less competitive plants).

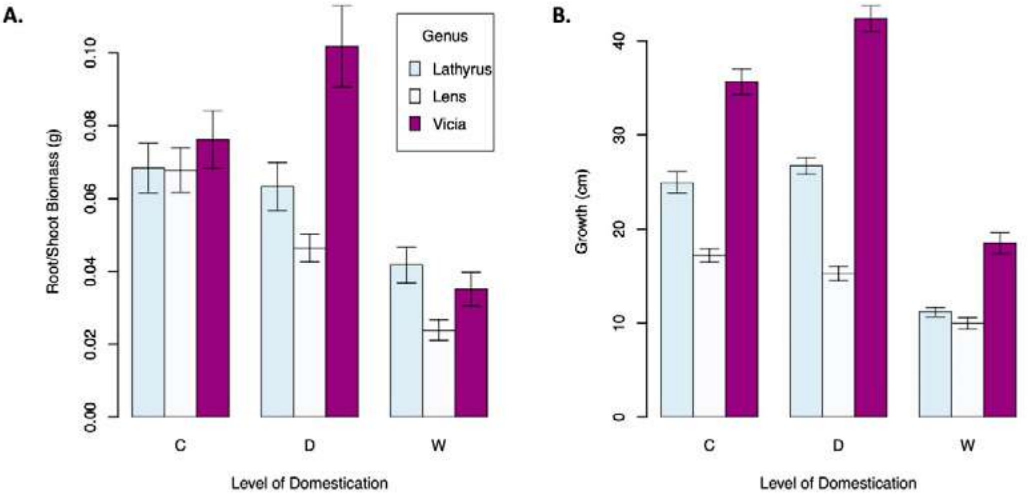

Figure 1. Differences in acquisition traits and relative growth rate across three levels of domestication in Fabeae. A. Root/Shoot is significantly different across taxa and levels of domestication. Domesticated taxa have a significantly higher allocation to roots than their wild relatives. Error bars represent 95% CI. B. Differences in Relative Growth Rates: wild relatives grow more slowly than domesticated taxa. C: crop accessions (landraces) of Lathyrus sativus, Lens culinaris & Vicia sativa; D: wild accessions of the same crops; W: wild (i.e., never domesticated) taxa (Lathyrus amphicarpos, Lens nigricans & V. amphicarpa, respectively).

Conclusions

These results indicate that early domestication might have indeed favored taxa that were particularly competitive and more effective in resource capture. At the same time, our findings may suggest that the effects of selection under domestication are complex, and that existing landraces are likely reflective of different selection pathways across taxa and cultivars.

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606/19. RNA-SEQ ANALYSIS OF THE ASIATIC VIGNA TO EXAMINE DIFFERENTIAL GENE EXPRESSION DURING GERMINATION AND EARLY PLANT DEVELOPMENT

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Summary:

Objectives, Description, Main Results & Conclusions

Seed germination strategies varies between plant that place cotyledons above ground in epigeal germination or reserve them belowground in hypogeal germination. The type of cotyledon emergence is usually fixed for many plant families; however, for Fabaceae species (also known as the legumes), such as those in the genus Vigna, both epigeal and hypogeal germination are observed. In this study, we examined phenotypic variation of these germination traits, as well as differentially expressed genes (DEGs) during early seedling development among Vigna species in the subgenus Ceratotropis ("Asiatic Vigna") using RNA sequencing. Thousands of DEGs were detected among multi-species comparisons, reflecting numerous genetic factors influencing timing of gene expression during early development of Vigna species. Gene ontology enrichment analyses further revealed functional variation associated with the germination strategies. Possible key functions and select genes further differentiating the Asiatic Vigna species, were also identified. These functions included, but were not limited to, stress and defense responses, signal transduction, and photosynthesis, reflecting trade-offs associated with differing germination types. Such trade-offs during seedling development have important agroecological implications in terms of crop productivity, crop diversity, and a plant's response to environmental conditions. While there remains a need for a more in depth genetic analysis of seedling morphological traits among the Asiatic Vigna, results of this study do provide preliminary research into functions influencing gene expression during early plant development in this genus. The results further highlight the need for increased plant genomic resources, particularly a genus-wide pan-genome.

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606/61. CHARACTERIZATION OF SPANISH CHICKPEA GENOTYPES (CICER ARIETINUM L.): PROXIMATE, MINERAL COMPOSITION AND PHENOLIC COMPOUNDS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The aim of this study was to assess the nutritional value, the mineral composition, and the phenolic compound profiles of ten Spanish chickpea genotypes. Seed morphological characteristics were determined as useful traits for analyzing plant biodiversity and genotypic discrimination. Most of these advanced lines and/or recombinant inbred lines (RILs) derived from intraspecific crosses among kabuli-type chickpeas, then showing the morphological characteristics established for kabuli chickpeas. The variety Kasin and two RILs: 5-RIL-33 and 5-RIL-92 shared the same parental genotypes, one of them from India (WR315) of desi-type. These three genotypes were resistant to both ascochyta blight [Ascochyta rabiei (Pass.) Labr.] and Fusarium oxysporum f. sp. ciceris race 5. Only one genotype (5-RIL-33) presents coloured-grains and pink flowers (desi-type traits). The protein content of all genotypes was higher than 20% with some outstanding lines having >25%. Other nutritional components as crude fat, crude fibre and carbohydrates were broadly uniform across the studied material. Similar assertion can be made in relation to mineral composition. The analysis of the phenolic compounds on methanolic seed extracts reveals common features as the presence of gentisic and 4-hydroybezoic acids, beside L-glutamic, citric, and succinic organic acids. In contrast, some compounds as gallic acid, gallocatechin and rutin are exclusively present in the coloured 5-RIL-33 in addition to the reference black chickpea variety. Based on seed traits two subgroups can be distinguished among kabuli type genotypes, those of round shape and absent ribbing of medium size and, a second one named BT- for their big size which shared intermediate shape and strong ribbing.

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606/100. SELECTION OF SNAP BEAN GENOTYPES FOR EUROPEAN ORGANIC FARMING BASED ON YIELD STABILITY

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Summary:

Objectives

Snap beans are a group of common bean (Phaseolus vulgaris L.) cultivars whose immature pods are consumed as green vegetables. The stability of a genotype is usually assessed by the variability of yields across environments. This study aims to identify the best snap bean genotypes adapted to European organic production using stability indices.

Concise description of the work (materials & methods)

A set of 311 snap bean lines (Snap Bean Panel; García-Fernandez et al 2022) was evaluated in three European localities with different climatic conditions during the years 2019, 2020, and 2021: Villaviciosa-Asturias-Spain; Bacau-Romania, Paterno, Potenza, Italy. A randomized design with 2-3 plots per line, year, and locality was used. A plot included 8–10 plants per line distributed in 1 m linear. The yield was measured as the 'weight of dry pods' harvested per plot (g). The outliers were removed and lines with data for more than five plots were considered. The coefficient of variation and eleven yield stability indices per genotype were estimated with the help of the R package toolStability (Wang et al 2023). Genotypes with low indices are considered stable except in the index 'Environmental variance'. Genotypes with low values in the stability indices are usually considered stable. Hierarchical K-means Clustering on Principal Components (HCPC) analysis was led with the stability indices to identify the main groups of genotypes.

Main Results

A total of 271 lines with results between 5 and 15 plots were retained. The production ranged between 1202 and 137 g per plot. The lines with indeterminate growth habit had the highest yields than determinate lines (685 vs. 412 g). Harvest averages were higher in the Spanish trials (643 g) followed by Italian (457 g) and Romania fields trials (293 g). ANOVA showed significant differences among 'Genotypes' and in the interaction 'Genotype x Locality'. Yield stability indices had a wide variation, showing significant correlations among many of them (Figure 1A). HCPC analysis revealed two main dimensions that explained 73.6% of the variance (Figure 1B). The 271 lines were grouped into 5 main clusters. Group '4' with 21 lines had the lowest values for yield stability indices, indicating high stability. The '4' group included lines with determinate (5) and indeterminate growth habits (16) and wide variations of phenotype in pod colour (13 green; 6 yellow; 2 mottled green), pod size (4, very long; 4 long; 9 medium; 4 short) and cross-section (17 flat; 4 pear shape).

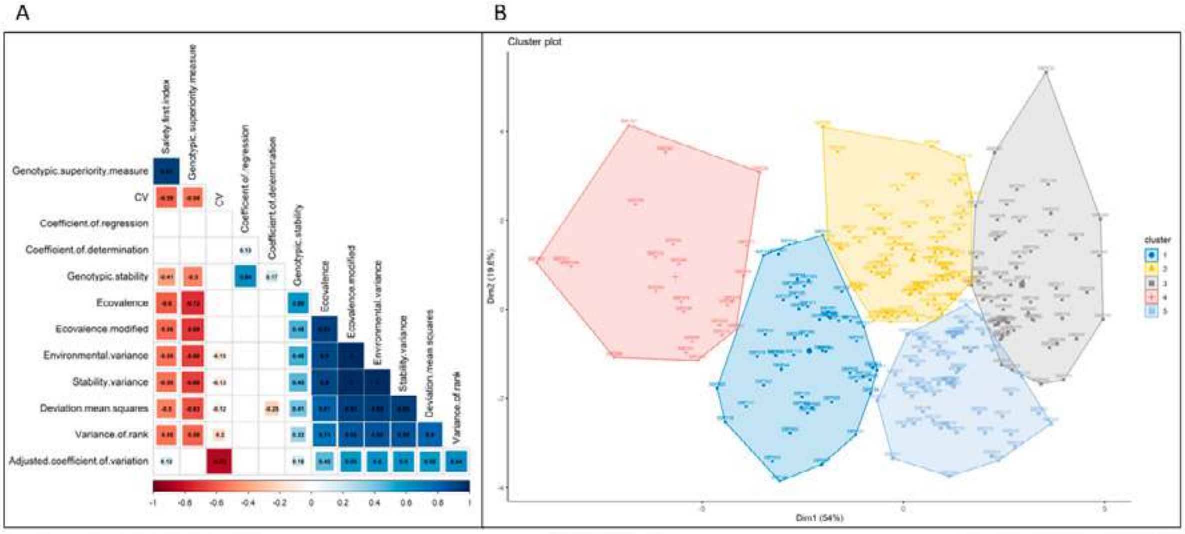

Figure 1. A/ Corrplot showing the Pearson correlation among the 12 yield stability indices: Adjusted coefficient of variation, Coefficient of determination, Coefficient of regression, Deviation mean squares, Ecovalence, Environmental variance, Genotypic stability, Genotypic superiority measure, Stability variance, Variance of rank and Safety-first Index and Coefficient of variation. B/Biplot shows the results of the HCPC analysis and the main five clusters identified.

Conclusions

Based on 12 yield stability indices, 21 high-stability snap bean lines were identified from developed field trials in three contrasting European localities. Also, within this set were identified specific lines for each locality showing higher yields (>1000g/plot): six lines in the Spanish trials, three in the Romanian trials, and two in the Italian trials.

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606/101. APPLIED GENOMICS FOR IDENTIFICATION OF CAUSATIVE MUTATIONS IN SOYBEAN

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The Genome-wide association study (GWAS) is a powerful method to identify a causative mutation (CM) that underlies a phenotype change. GWAS has led to successful discoveries in many cases but failed to predict traits of complicated genetics or high complexity. As a statistical method, GWAS is critically dependent on the data set size, the genotype quality and the phenotype frequency. For soybean, there is a great amount of publicly available genomic data already gained from independent studies that could be utilized for CM identification and thus, accelerate soybean improvement. The hypothesis of our applied genomics approach is that the existing genotype data can be leveraged in an empowered GWAS strategy to identify CM with higher confidence, even for complicated traits. We developed an applied genomics methodology for CM identification encompassing three novel concepts: Synthetic Phenotype, Accuracy Calculation and Curated Panel of Diverse Soybean Resequenced Accessions. As a result, we created a Synthetic phenotype to CM strategy (SP2CM; Škrabišová et al., 2022) and developed a set of supportive genomic tools that are available either online (soykb.org/tools) or can be downloaded for standalone use. The tools enable an easy-to-use creation of a Synthetic Phenotype from user-provided genotype data or the curated panel of accessions (AccuCalc); the analysis of direct correspondence (AccuTool); the exploration of haplotype context (SNPViz) and the exploration of natural variation (Soybean Allele Catalog). By utilizing the SP2CM strategy, we validated CMs for a palette of cloned genes that represent the most common scenarios of phenotype frequency. Importantly, we discovered new genes/CMs involved in seed coat pigmentation, soybean cyst nematode resistance and seed protein content. We created a hub for soybean applied genomics (soykb.org/soyhub/) that enables CM identification for providing more precise and thus accelerated soybean breeding and improvement.

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606/111. DIVERSITY EXPLORATION AND APPLICATION POTENTIAL OF BOWMAN-BIRK PROTEASE INHIBITORS IN SUB-SAHARAN ACCESSIONS OF VIGNA UNGUICULATA (L.) WALP

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Bowman-Birk protease inhibitors (BBIs) are a little class of small proteins identified by a conserved structure with 14 cysteines residues and two independent inhibitory domains. BBIs are important as they protect the plant from insect attacks, but they also behave as bioactive compounds for human health, exerting chemopreventive and anti-cancer actions (Clemente and Domoney, 2006, Panzeri et al., 2020). The aims of this work were: explore and understand natural genetic diversity of a trypsin-trypsin BBI (TT-BBI) and a trypsin-chymotrypsin BBI (TC-BBI) of Vigna unguiculata accessions (do-

mesticated and wild) from Sub-Saharan Africa, evaluate the evolutive pressures that are acting on the two genes and assess in silico the most promising BBI proteins in the interaction with trypsin and/or chymotrypsin. We screened the TT and TC-BBI genes sequences from 399 samples of 168 accessions. We clarified haplotype relationships and assessed selective pressures on the two genes with phylogenetic and network approaches, retrieving a total of total of 24 TT-BBI and 29 TC-BBI haplotypes. The deduced isoforms were, respectively, 13 and 19, while mature proteins 7 and 10. Selection analyses showed that the two genes are experimenting selective pressures, especially negative. Molecular dynamics and minimisation energies were considered to appraise the energetic interaction of the BBIs with physiological targets, allowing us to identify potentially better BBIs in the interaction with physiological targets and, consequently, to cancer markers. This knowledge could be useful for future agricultural programmes without overlooking bioactive compounds as novel elitary traits.

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606/122. PHENOTIPING FABA BEAN LANDRACES FOR AGRONOMIC TRAITS AND BROOMRAPE RESISTANCE UNDER ANDALUSIAN ENVIRONMENT

Authors:

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Objectives, Description, Main Results & Conclusions

The purpose of this work was to recuperate and value Andalusian faba bean landraces kept at the Legume Germplasm Bank of The Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) in the Alameda del Obispo Centre (Córdoba, Spain). Due to the importance of this rich genetic heritage, the concern was to make awareness of the characteristics and values they present. This will allow their use or their inclusion in breeding programs of the species which are needed to promote the use of this legume. Twenty-four faba bean accessions were characterised and evaluated during the seasons of 2020-2021 and 2021-2022 in fields located at the Alameda del Obispo Centre (Córdoba). Among them, a set of eleven genotypes specifically originated from the Alto Guadalquivir Region, were also assessed during both seasons in a locality belonging to this region (El Carpio, Córdoba). The commercial faba bean cv Volantín was included as control. The experiment was conducted in a randomised block design with three repetitions, with a plot size of 15 m2 with rows of 4 m and a distance between rows of 0.5 m. The average yield (kg/ha) per accession and per location was calculated. The morphological characterisation was carried out considering eighteen traits described in the International Board for Plant Genetic Resources (IBPGR, 1985) for faba bean crop. Evaluation for Orobanche crenata resistance was tested in a field naturally infested with seeds of this parasitic plant. During both years studied, genotypes Vf-1019 and Vf-1030 were the most productive in the locality of Córdoba, meanwhile in El Carpio was Vf-911. Landrace Vf-911 was also the most resistant cultivar to O. crenata in the evaluation carried out for this parasitic plant.

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- *Acknowledgements:This work was supported by European Agricultural Fund for Rural Development (FEADER)-Rural Development Programme 2021–2027(CRF.CRF2022.001).*

606/124. ACCUCALC: AN EASY-TO-USE PYTHON PACKAGE FOR SOYBEAN GENOMICS AND BREEDING

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Soybean is one of the most important commodities worldwide. Therefore, a lot of genotyping data was produced that can serve as a model for the development of genomic analyses, strategies, and tools for more precise breeding and accelerated improvement. Genome-wide association study (GWAS) is a statistical method that associates a genotype with a phenotype and thus, can identify candidate genes. Even though it is a widely used method, its potential to discover a causative mutation (CM) is overestimated. This is caused by the fact that GWAS is data set size sensitive and quality of input data dependent. Also, the complexity of multi-genic traits complicates GWAS results interpretation. To improve GWAS efficiency in identifying a true CM, we developed an empowered “GWAS to Genes” strategy that we named Synthetic phenotype to causal mutation, SP2CM. To enable the utilization of SP2CM for other crops and species, we developed an easy-to-use Python package called AccuCalc [1]. AccuCalc formats Synthetic phenotype from a user-provided genotype data in variant calling format (vcf) or HapMap format (hmp) and thus, serves as a valuable tool for direct correspondence (Accuracy) calculation between the Synthetic phenotype and every variant position in the genotype data. AccuCalc can also calculate Accuracy for any selection of binary phenotype that a user provides. Further, a user-provided GWAS result in a tab-delimited format can be used as an additional input file and thus, the AccuCalc analysis output enriched by p-values can be visualized as an Accuracy accentuated Manhattan. AccuCalc Python package facilitates an easy-to-use GWAS results evaluation and easier interpretation and is freely available on GitHub.

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606/185. GENETIC BASIS OF PORTUGUESE COMMON BEAN (Phaseolus vulgaris L.) METABOLOMIC PROFILE FOR ENHANCING NUTRITIONAL QUALITY

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Summary:

Objectives, Description, Main Results & Conclusions

Common bean (Phaseolus vulgaris L.) is a widely consumed grain legume known for its edible seeds and pods, which provide essential nutrients such as dietary protein and bioactive beneficial compounds with antioxidant, anti-carcinogenic, and/or anti-inflammatory properties (Thirumurugan et al., 2018). Portugal, as part of the Iberian Peninsula, is recognized as a secondary centre of genetic diversity for common bean, where traditional landraces are still cultivated. To assist the development of common bean varieties with improved quality, we associated the metabolite content in dry seeds of a collection of 100 Portuguese accessions previously measured by LC-Mass spectrometry methodologies (Mecha et al., 2022), harvested in two locations with contrasting climate (warmer in Cordoba, Spain, and milder in Cabrela, Portugal), with the genotypic screening of the same accessions using SNP-based arrays. The analysis of the metabolites by untargeted metabolomics was obtained by Orbitrap high-resolution mass spectrometry, whereas, for the quantification of targeted phenolic compounds, UPLC-Q-TOF-MS was used. In this previous study, 70 compounds were annotated, most of them belonging to phenylpropanoids, polyketides, lipids, and lipid-like molecules superclasses. Total phenolics and flavonoids contents (TPC and TFC) were determined by Folin-Ciocalteu and aluminum chloride colorimetric methods. Linear mixed models accounting for the genetic relatedness among accessions were tested for marker-trait associations. The SNP associations with the strongest -log10P-values (>4) were identified for TPC, kaempferol, catechin, t-ferulic acid, and sinapic acid. The proportion of variance explained by these associations varied from 6 to 29%. The candidate genes and metabolic pathways underlying the associations are currently being explored. This study provides valuable genomic targets for the development of molecular selection tools, supporting the utilization of plant metabolomics and association mapping as powerful approaches to improve the nutritional quality of common bean.

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606/195. VULNERABILITY OF CHICKPEA LANDRACES TO INCREASED VIRULENCE IN DIDYMELLA RABIEI POPULATIONS

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Summary:

Objectives

Ascochyta blight (Didymella rabiei) is a key chickpea production constraint in many countries. Changes in virulence of the pathogen population threatened the production of popular resistant chickpea varieties and develop resistance to commonly used fungicides in some countries. Chickpea landraces and wild relatives are key resistance sources to combat emerging virulent pathogen populations. The kabuli chickpea breeding program at the International Centre for Agricultural Research in the Dry Areas (ICARDA) has been using virulent pathotypes to phenotype breeding lines and germplasm accessions since early 1980's. The aim of this study was to assess if the existing chickpea germplasm holding at ICARDA gene bank carries good levels of resistance to emerging virulent D. rabiei pathotypes.

Concise description of the work (materials & methods)

The Focused Identification of Germplasm Strategy (FIGS) is one of the approaches used for mining adaptive trait within many accessions kept in gene bank (Stenberg and Ortiz, 2021). Using the FIGS approach, 200 chickpea accessions (FIGS set) originated from 14 countries were used in this study. Two resistant (ICC12004 and FLIP94-090C) and one susceptible (ILC263) genotype were included as checks. The accessions and checks were evaluated for their resistance to Pathotype I and IV. Separate experiments were done for each Pathotype in two with randomized complete block design under glasshouse condition at Lebanese Agricultural Research Institute. Seedlings were inoculated with 10 days old culture with 1X105 spores/ml. Inoculated seedlings were incubated for 72 h under high humidity. Each seedling was evaluated using 1-9 rating scale where 1= health seedlings and 9= dead seedlings (Chen et a. 2004).

Main Results

None of the accessions and checks were free from infections by both Pathotypes (Fig). Only 5% of the FIGS set showed good levels of resistance to Pathotype-I but all accessions and resistant checks were susceptible to Pathotype-IV. This study showed that chickpea accessions at ICARDA genebank do not have enough levels of resistance to virulent pathogen population to be used in the breeding program.

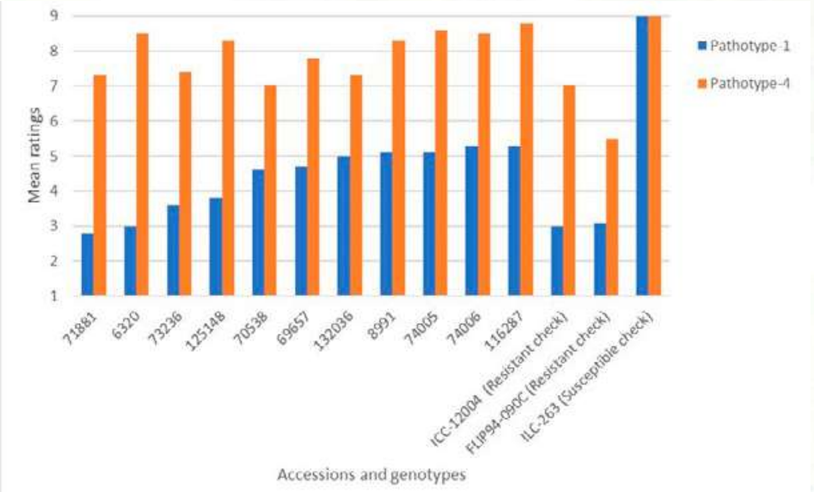

Figure. Rating values for each accession are means of two experiments (8 plants/experiment). Accessions with <4 rating are considered resistant; 4-5 as moderately resistant; and >5 moderately susceptible to highly Susceptible. Pathotype-I and Pathotype-IV were reported in 1980s and 2004 in Syria. The resistant checks are widely used for crossing purposes at ICARDA chickpea breeding program.

Conclusions

Pre-breeding efforts are required to transfer resistance genes from wild relatives in the primary gene pool. Increased pathogen virulence can also cause genetic erosion on landraces that can be used to develop breeding lines with other adaptive traits in the breeding program.

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606/221. ALLELE MINING OF GERMPLASM COLLECTIONS IN COWPEA: PROGRESS AND CHALLENGES

Authors:

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Summary:

Objectives

Plant genetic resources are the raw material for crop improvement. Cowpea (Vigna unguiculata L. Walp.) is a very diverse crop, and diverse germplasm, including locally adapted landraces and wild relatives, is stored in germplasm collections worldwide. These collections harbor useful allelic variation for adaptation to abiotic and biotic stresses, but they are often too large for their effective utilization in breeding programs. Subsets of these collections, including core and mini-core sets, have been developed for cowpea. One of these mini-core collections is the "UCR Minicore", a collection of 368 highly-homozygous domesticated cowpeas from 50 countries that derives from the University of California Riverside (UCR) cowpea collection (Muñoz-Amatriaín et al., 2021). This mini-core, which is also available at the U. of León (Spain), has been genotyped at a high density, and its phenotypic characterization for a variety of traits in multiple locations has yielded loci and genes involved in herbivore resistance, flowering time, seed size, and pod shattering, among others.

Concise description of the work (materials & methods)

Allele mining of larger collections involving thousands of accessions is more challenging due to the difficulties associated with their phenotypic evaluation. A relatively novel approach called genome-environment association or envGWAS (Rellstab et al., 2015), can be used to identify genetic variants associated with adaptation to abiotic stresses, including heat and drought. These are major environmental constraints influencing yield that will be aggravated by climate change. EnvGWAS associates genome-wide SNPs with climatic variables based on the sampling location of the accession, namely latitude and longitude. Therefore, having accurate geographical information within the passport data of landraces and wild relatives is crucial for the approach´s success.

Main Results

In a current project funded by the Foundation for Food and Agriculture Research (ICRC20-0000000032), we are mining the cowpea core collection of the International Institute of Tropical Agriculture (IITA Core), one of the largest cowpea collections in the world, to identify beneficial alleles for heat tolerance. Over 600 cowpea landraces and wild relatives from Africa possessing geographic coordinates were used for extracting historical climatic data available at WorldClim v2 (http://www.worldclim.com/version2). A detailed exploration of the passport information, both manual and automated via the tool ITALLIC (Onsongo et al. 2022), revealed inconsistencies in almost 200 accessions. These inconsistencies were mostly related to geographic coordinates not matching the detailed location information available for the accessions. Most accessions within the IITA genebank were collected before the advent of global positioning systems (GPS), and efforts to improve databases, including geolocation data, could have resulted in undesired errors. Luckily, germplasm collectors were often detailed in their descriptions of the collecting sites (roads, distance from cities, village names, etc.). In a coordinated effort between IITA genebank curators and a Geographic Information System (GIS) specialist, GPS coordinates have been confirmed or updated for over 100 accessions (Figure), improving the precision of our envGWAS.

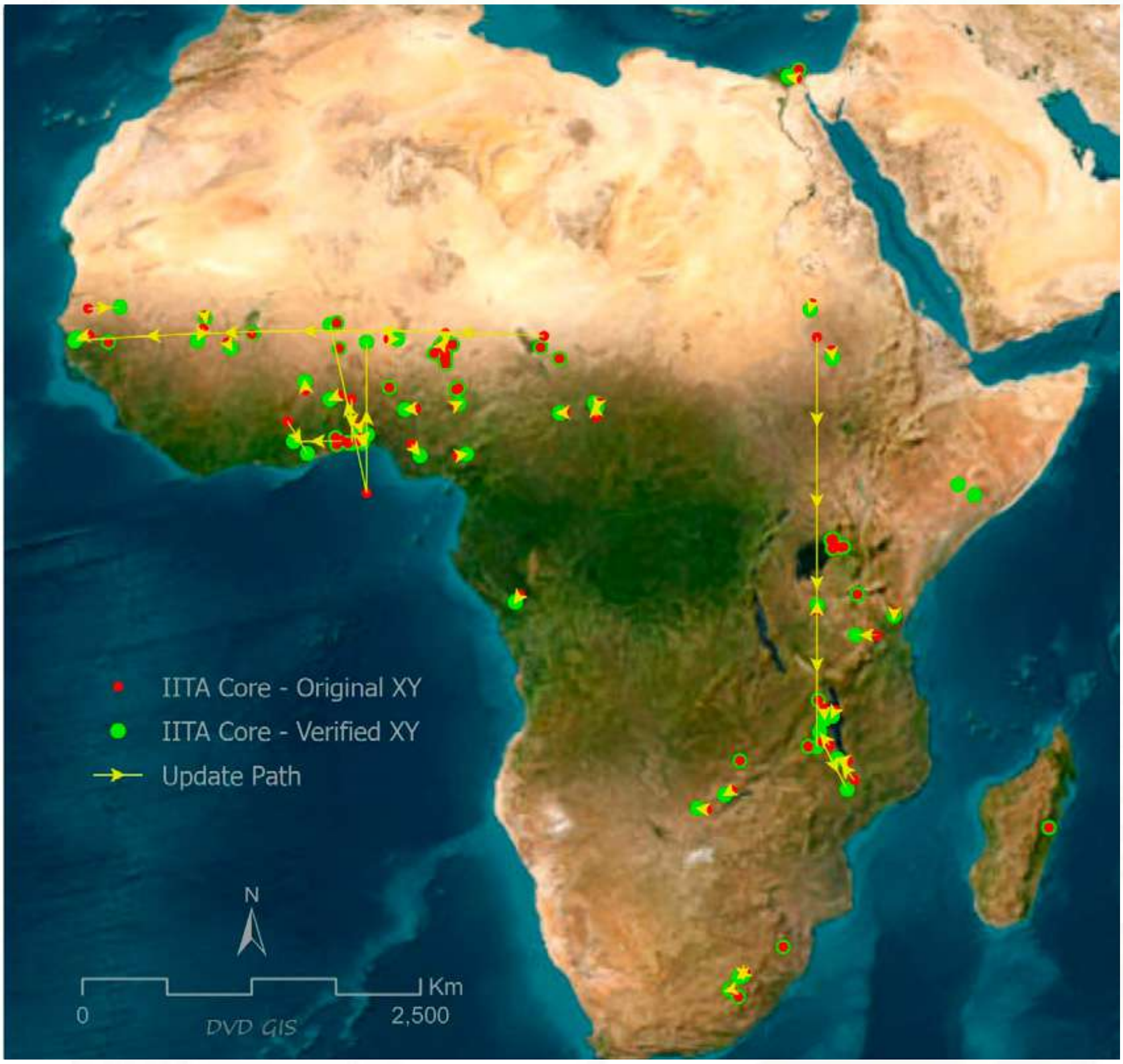

Figure. Location verification results of 94 cowpea accessions in the IITA Core. Red points indicate geographic coordinates available from the original passport file, while green points represent the verified or updated geolocation for the accession. Yellow lines with arrows show paths between the original and updated locations.

Conclusions

The findings of this work highlight the importance of dedicating resources to curating passport data associated with genebank collections. Such an effort is key to obtaining accurate environmental data for envGWAS analyses.

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606/225. GENETIC DIVERSITY OF WORLD-WIDE COWPEA LANDRACES USING SSR MARKERS

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Summary:

Objectives, Description, Main Results & Conclusions

Cowpea (Vigna unguiculata L. Walp.), native of Africa, is a legume crop well adapted to hot and drought-prone environments, and a primary source of protein for millions of people in the developing countries. Knowledge of genetic diversity among cowpea landraces is important for properly preserve and utilize germplasm to obtain improved varieties. The study aimed to amplify SSR markers to evaluate the genetic diversity and structure population of world-wide cowpea landraces. A total of 68 cowpea landraces from 29 countries of four continents were used and eight SSR loci were selected for genetic diversity assessment. A total of 60 polymorphic SSR alleles were identified, varying from 2 to 14 alleles per locus, and with a mean value of polymorphic information content (PIC) of 0.35. This set of SSR markers allowed the identification of two subpopulations related with the geographical origin of the landraces using STRUCTURE. One subpopulation comprises the landraces from southern European, northern African and central American (Cuba) countries, while the other subpopulation includes landraces from countries of central and south African, south American and Asian countries. This distribution was also observed in the PCA analysis. The genetic diversity results from this study agree with results obtained previously with SNP markers (Carvalho et al. 2017), which revealed a clear separation between cowpea landraces. These results provide an in-depth analysis of cowpea genetic diversity that will help on crossing strategies in breeding programs.

Acknowledgments: This work was supported by CITAB project funding from National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

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606/237. AN EXTENSIVE INVESTIGATION OF A GENE BANK POPULATION OF PEA (Pisum Sativum L.) WITH EMPHASIS ON NUTRITIONAL QUALITY, DROUGHT TOLERANCE AND MORPHOLOGICAL DIVERSITY, THROUGH THE COMBINATION OF PHENOTYPI

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Summary:

Objectives

The market for mature pea is currently lacking modern cultivars developed for human consumption. Instead, the industry is relying on a limited number of cultivars mostly developed as fodder peas. Additionally, knowledge about cultivation practices resulting in superior product quality is very sparse. The project "Peas & Love" aims to explore accessions of genebank origin with the goal of identifying genotypes, traits or cultivation practices that might propel the cultivation and quality of peas grown to maturity for human consumption.

Concise description of the work (materials & methods)

The project acquired a unique collection of ~ 300 pea accessions from the NordGen gene bank located in Sweden. In a multi-disciplinary effort, the population is being evaluated through a number of screenings and experiments aimed at identifying traits or accessions that may be of value in the commercial production of mature peas for human consumption. These includes i) various traits related to nutritive quality and agronomic performance as a response to cultivation practices including fertilizer applications and intercropping, ii) extensive characterization through pheno- and genotyping, and iii) Investigations of root architecture and drought tolerance through single-plant studies in transparent PVC rhizotubes, and drought experiments under field conditions. During the season of 2023 comprehensive morphological descriptions are documented on traits like plant architecture, leaf, flower, pod and pea morphology, flowering time and duration as well as ripening time and duration. These traits will be included in a catalogue of information on each accession, easing future uses of the population for research and breeding purposes.

Main Results

Preliminary experiments have revealed variation in traits related to drought tolerance among the accessions, and those results will provide the foundation for future root and drought studies.

During the autumn and winter of 2023, the genetic structure and relatedness of the population will be analyzed using a set of +50.000 DArT markers, and if possible, genetic markers related to protein content and specific amino acid compositions will be identified.

Based on preliminary studies using SSR satellite markers, large variation is present in the population, and valuable information on available pea genetic structure will be published.

Preliminary results include large variation in most traits investigated. The protein content of 72 accessions grown in the field in 2022 varied from 17.8 % to 28.5% and will be compared to that of 211 accessions grown in 2023. Similarly, analysis of stable nitrogen isotope ratios indicated variation in the nitrogen fixing capacity.

Figure 1: Three distinct leaf morphologies of peas, from the left: Tendrill-less, semi-leafless, fully leafless. A visual example of the variation in the population that exists in a variety of traits.

Conclusions

Growing 211 pea accessions of diverse origin allows for the study of variation within a large number of traits, and through an inter-disciplinary approach, the project partners strive to investigate as many relevant traits as possible. Preliminary results will be presented at the conference.

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- *NA as of yet*

606/252. FIELD PHENOTYPING OF LEGUME GERMPLASM COLLECTIONS

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Summary:

Objectives

Legumes are essential for the sustainability of agri-food systems. They require low input to grow and are an important source of vegetable proteins and other nutritional compounds such as vitamins, minerals, and fibres.

Concise description of the work (materials & methods)

Breeding new food legume varieties, more efficient in terms of production and quality, requires access to genetic resources well characterized at genotypic and phenotypic level.

Main Results

The INCREASE project (Bellucci et al., 2021) aims at constituting intelligent collections of food-legume genetic resources, to be exploited in the European agrifood systems. In the frame of the project, collections of three food legume species (chickpea, common bean, and lentil) have been field-phenotyped in central and southern Italy in seasons 2021 and 2022 (Figure). The collections were composed of ~450 single seed descent-purified, domesticated accessions for each species. The experimental design was Randomized Complete Block with repeated checks, in three replicates. Each plot was constituted by 14-15 plants in a single (common bean, lentil) or double (chickpea) row. Several traits, defined by species-specific ontologies, were scored, among which: phenological (time of emergence, time of flowering, pod formation, pod maturation, complete maturation, etc.), morphological (leaf type, flower colour, growth habit, height, etc.) and post-harvest (colour/shape of seeds, number of pods per plant, number of seeds per plant, total plot yield, etc.). Moreover, in pre-flowering phase, leaf samples were harvested on each plot and immediately nitrogen frozen for future metabolomic analysis. Data collected in these trials will be grouped with those coming from twin experiments conducted in Spain and Lebanon for chickpea and lentil, Spain and Poland for common bean, and analysed to study the genotype x environment interactions of the accessions. Another year of field trial will be carried out for lentil in 2023-24, and similar experiments will be set up also with a lupin germplasm collection, in two years and three locations across Europe. All the data obtained from field trials, together with other phenotypic and genotypic information collected in the INCREASE project, will contribute to reveal the structure of the collections and the potential of the single accessions for specific uses (breeding, farming, food industries, etc.).

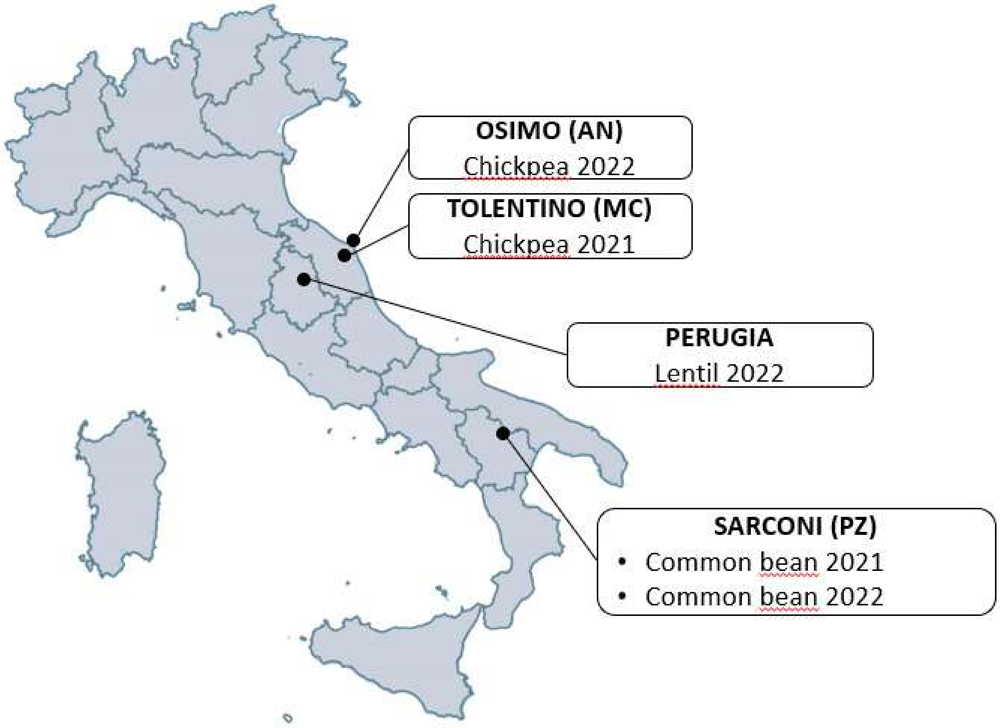

Figure. Location of the field trials carried out in seasons 2021 and 2022.

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606/259. IMPROVING GENETIC DIVERSITY AND BIOTIC STRESS RESISTANCE IN CHICKPEA USING WILD RELATIVES

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Chickpea (Cicer arietinum) is an important pulse crop, cultivated globally and is predominantly used for human consumption, as it is an excellent source of protein and dietary fibre. In the cropping system chickpeas provide a disease break for cereal and oilseed crops and have the unique ability to interact with beneficial rhizobia that fix atmospheric nitrogen. Despite the growing demand for pulses in the human diet, improvement of chickpea varieties is hampered by a lack of genetic diversity within the domesticated gene pool. Wild relatives C. echinospermum and C. reticulatum are cross-compatible with domesticated chickpea and the global collection for these species was significantly expanded between 2013-12016 (von Wettberg et al 2018). This collection has already been demonstrated to harbour sources of biotic stress resistance to Ascochyta blight, root-lesion nematodes and pod-borer (von Wettberg et al 2018; Reen et al 2019, Newman et al 2021). Our team evaluated the wild chickpea collection of 333 accessions for Ascochyta blight resistance and conducted a genome-wide association study (GWAS) for a moderately aggressive and highly aggressive isolate. Furthermore, a subset of 88 wild accessions were evaluated for Sclerotinia stem rot resistance and the performance of Australian varieties were compared. To broaden the genetic diversity and commence incorporation of biotic stress resistance sources into Australian chickpea varieties, 26 wild Cicer accessions were crossed to either cultivar PBA HatTrick or Kyabra. Individual segregants were genotyped and evaluated for several agronomic and postharvest traits. GWAS and quantitative trait locus (QTL) analyses identified multiple loci associated with domestication traits (Figure 1) and candidate genes underlying loci were identified. This research advances our understanding of the genetic and molecular basis of domestication traits and biotic stress resistance in chickpea and may facilitate enhancement of chickpea cultivars through introgression of novel genetic diversity from wild relatives.

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Session 5 Genetics and -omics crop improvement

606/38. A FABA BEAN PAN-GENOME FOR ADVANCING SUSTAINABLE PROTEIN SECURITY

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

The substantial deficit in plant protein production in Europe is met currently by soy meal and beans primarily from South America, leading to reliance both on imported of plant protein in Europe and deforestation in South America. Having an average protein content of 29%, faba bean (Vicia faba L.) is a widely adapted protein crop that could substantially improve European protein sovereignty and security, both for food and for feed. It is well suited to most parts of Europe as well as to regions worldwide where it is a traditional, staple food. Moreover, its range of climatic adaptation is complementary to soy, which is adapted to warm regions and does poorly where faba bean does well. Faba bean like other crops, however, faces unprecedented challenges from the biotic and abiotic stresses made worse by climate change. Breeders need new tools to develop sustainable, secure crops more efficiently, which have the properties processers need [1]. The large size of the faba bean genome (13 Gbp diploid) has until recently hindered a high-quality assembly, but new advances have enabled production of a highly contiguous reference genome [2]. In the PanFaba project, we are now producing a pan-genome spanning the diversity space of the species to deliver the insight and tools needed for rapid improvement of faba bean. We have assembled the genomes of five accessions; these will be complemented by at least 15 others in an international effort. The assemblies are complemented by transcriptomics and gene annotation. The PanFaba project will enable the high-resolution linkage of genotype to phenotype that is needed to improve faba bean as a protein crop and adapt it to likely future climatic conditions in various regions of Europe.

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606/59. DIVERGENCE IN GENOME STRUCTURE AND FUNCTION UNDERPINNING ROOT SYSTEMS IN LEGUMES

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Work centre:

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Summary:

Objectives

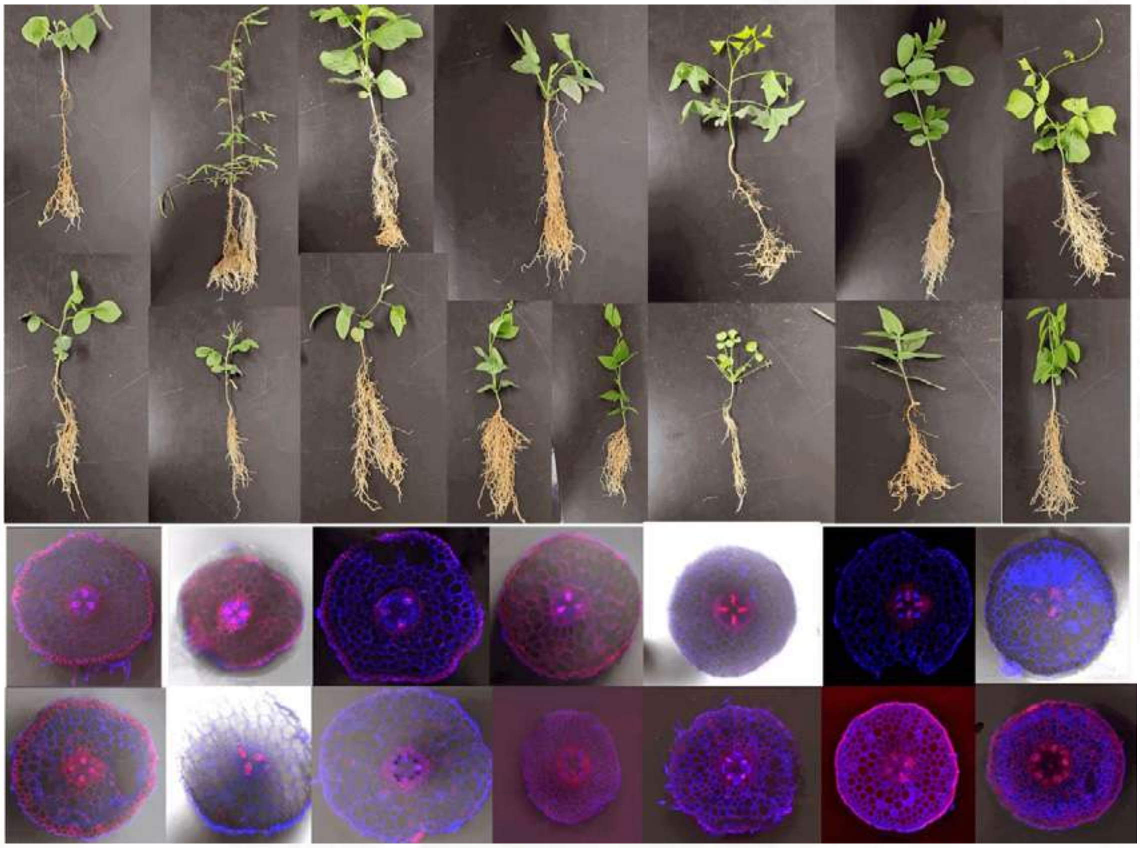
The growing impact of climate change and our need for sustainable food production is driving a shift in our agricultural practices. Conventional approaches primarily focused on maximizing yield have not only failed to address current climate challenges, but have also contributed to a decline in nutrition. Consequently, there is a pressing need to prioritize novel crops with distinct traits that offer opportunities for sustainable food production.

Concise description of the work (materials & methods)

Our research aims to advance the domestication of new legume cultivars by developing the de novo genome assembly and annotation for a diverse range of legume species within the Pallipoinodeae subfamily. We leveraged a combination of Oxford Nanopore Technologies (ONT) and Illumina sequencing, to generate 63 high-quality genome assemblies. Additionally, we generated full-length cDNA (FLcDNA) from a variety of tissue types to improve the accuracy of gene prediction and annotation. These newly added genome assemblies for the Pallipoinodeae subfamily serve as a valuable resource for future advancements in the field.

Main Results

Utilizing these newly assembled genomes, we aim to investigate the molecular mechanisms governing root development in leguminous plants. We aim to establish a comprehensive comparative genomics pipeline specifically tailored for distinct legume clades. In support of our research endeavors, we conducted a detailed characterization of actively developing roots in close proximity to the root meristem. This allowed us to compare differences in anatomical features, which revealed notable differences in root anatomy and lipid distribution, potentially uncovering new pathways underlying root growth in leguminous plants.



Diverse Root Morphologies in Young Roots of Different Legume Species

Conclusions

Our study aims to advance our current understanding of the genetic underpinnings of root development in Pallipoinodeae plants but also contributes to the improvement of denovo domestication strategies of new legume varieties. Ongoing efforts to assemble genomic data from additional legumes hold great potential for enhancing our ability to meet the rising demand for nutritious food, particularly in developing countries.

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606/81. ALTERED FEEDBACK REGULATION OF SHOOT BRANCHING BOOSTS CROP YIELD IN FIELD PEA

Authors:

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Summary:

Objectives

The auxin receptor gene AUXIN-SIGNALLING F-BOX PROTEIN 4/5 (AFB4/5) is known to regulate one or more feedback signal in the strigolactone signalling pathway to control shoot branching[1], however, the nature of these signals and how they interact with AFB4/5 remains unclear[2].

Novel mutant alleles of Psafb4/5 were identified in herbicide tolerant field pea genotypes, which displayed varying effects on plant height and shoot branching. This allelic series provided an opportunity to study the roles of this gene in fundamental processes controlling plant architecture, and the implications for crop production.

Concise description of the work (materials & methods)

Reciprocal grafting and RT-PCR experiments were used to investigate the role of AFB4/5 in regulating expression of strigolactone biosynthesis genes.

Field trials were conducted in South Australia to compare the grain yield of two mutant genotypes and a wildtype control.

Main Results

Grafting studies and gene expression analyses showed that AFB4/5 is required for the transcriptional regulation of strigolactone biosynthesis genes. A hypomorphic allele retained the ability to respond to a branch-derived feedback signal, while a putative null allele maintained exceptionally low transcript levels despite profuse branching, indicating that AFB4/5 is essential for the function of this signal. This functional diversity between alleles likely explains the phenotypic diversity seen in this allelic series.

Field trials in the 2022 growing season compared the performance of two Psafb4/5 mutants to the relevant 'wildtype' variety and found neither showed a significant yield penalty. One genotype with reduced height and increased basal branching, however, produced ~25% higher grain yield than wildtype (Figure 1).

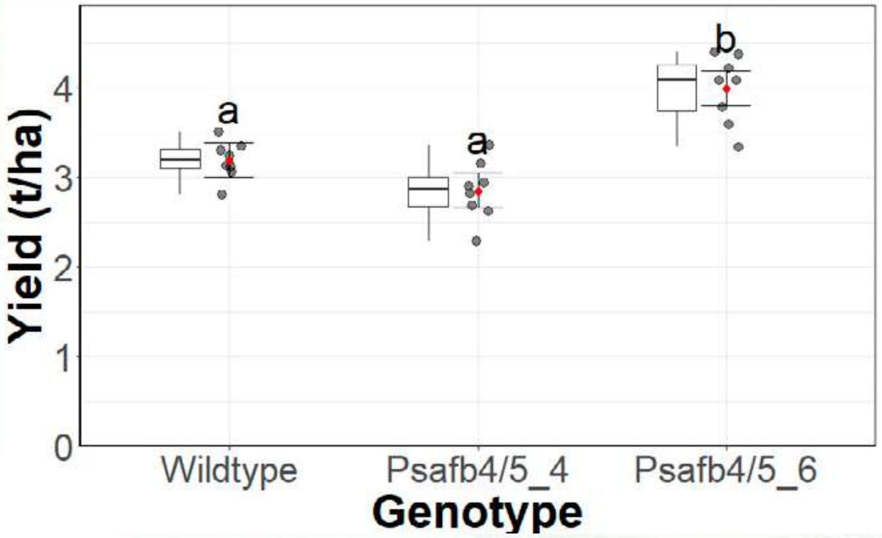

Figure 1: Grain yield of field pea genotypes with altered shoot branching phenotypes. Red dots and error bars represent predicted means ± 95% confidence intervals of 8 replicates. Means not sharing any letters are significantly different (Tukey-test alpha = 0.05). Black dots and box plots represent raw data.

Conclusions

Our increasing understanding of the signalling pathways involved in shoot branching is revealing exciting opportunities to manipulate plant architecture to improve yield potential in grain legume crops.

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606/103. GENETIC BASIS OF MICROBIOME RECRUITMENT IN PEA ROOTS CHALLENGED BY ROOT ROT DISEASE

Authors:

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Work centre:

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Summary:

Objectives

Legumes play a crucial role in the shift towards more sustainable protein production, but root rot complexes can cause massive yield losses in many legume crops such as pea. The pea root rot complex (PRRC) is caused by various soil-borne pathogens that likely act synergistically and influence the composition of the rhizosphere microbiome (Wille et al., 2021). As there is genotypic variation in the abundance of key PRRC taxa and disease susceptibility, we aimed to investigate the genotype effect on the root microbiome composition affecting plant health. This crucial interaction between the plant genotype and its associated microbiome, also known as the holobiont, has the potential to lead to increased resistance to PRRC.

Concise description of the work (materials & methods)

We obtained genetic markers from 253 diverse pea genotypes using genotyping-by-sequencing (GBS). The genotypes were grown under controlled conditions in diseased farm soil (or its sterilized equivalent), showing root rot symptoms caused by a naturally occurring pathogen complex. Several phenotypic traits, such as emergence rate and root rot index, and root samples were obtained at the seedling stage to correlate early root colonizers with disease phenotypes. Root microbial DNA was extracted from the root samples and sequenced for fungi (ITS) and bacteria (16S V3-V4). Operational taxonomic units (OTUs) were assembled based on 97% identity. OTU abundance was used for a genome-wide association study (GWAS) and genomic prediction (GP). Our objectives were to examine if...

- Genomic loci explain a relevant proportion of the variance in the abundance and presence of individual OTUs in pea roots (QTL detection)
- Identified QTLs for differentially-abundant OTUs reveal QTLs additional to the resistance phenotype QTLs
- Microbiome-mediated disease resistance concepts can be implemented into breeding programs via genomic prediction at the holobiont level

Main Results

The GWAS of differentially abundant OTUs revealed 50 highly significant QTLs at 19 independent loci on five different chromosomes (Figure 1). Most QTL can be associated with more than one OTU, while only four loci are associated with both fungi and bacteria. QTLs on chromosome six co-segregated with QTLs for the shoot dry weight, root rotting, and emergence rates. The genomic heritability reached h2 > 0.6 for some of these differentially abundant OTUs. The relative abundance of several OTUs was strongly correlated with plant health. For instance, a strong effect was observed for OTUs from the genus Fusarium (negative) and Dactylonetria (positive), with genomic heritabilities above 0.35. Higher accuracy in predicting resistance to PRRC was obtained using the OTU abundance alone or combined with phenotype QTLs (μ=0.6) in a genomic prediction model. Lower accuracies were obtained using genomic markers for either OTUs or plant phenotypes alone (μ=0.3).

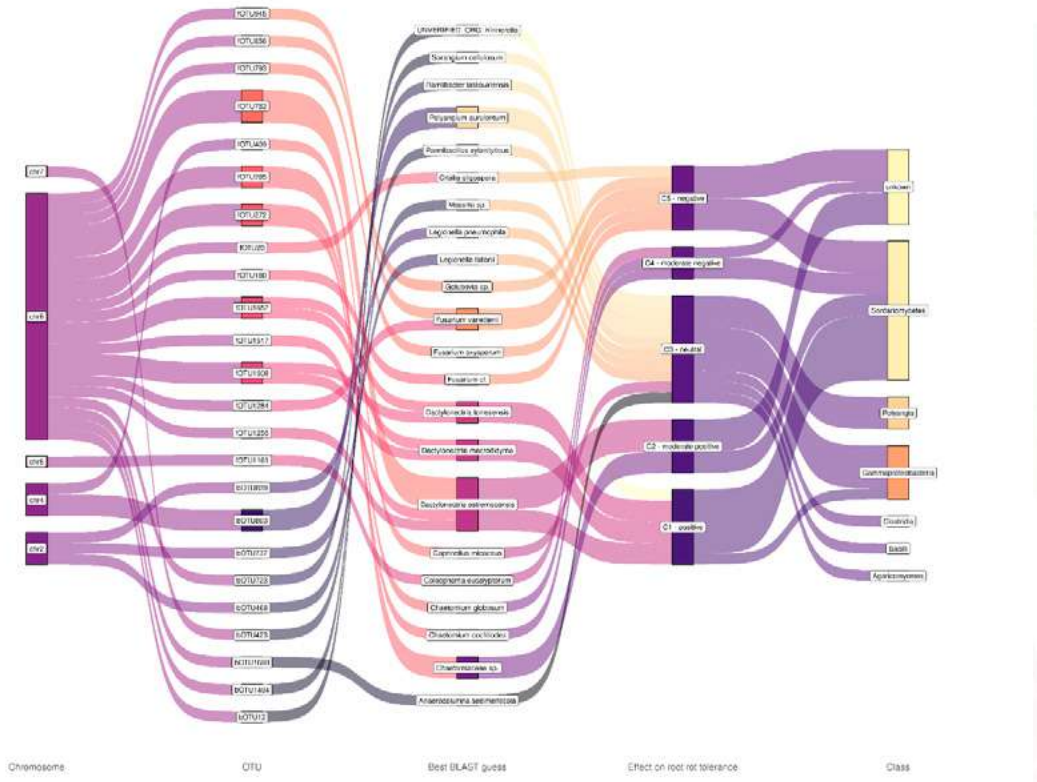

Figure 1. Relationship between pea genetics, root OTU presence and abundance, and resistance to a pea root rot complex. Chromosome refers to the QTL on the pea genome of each OTU. The best BLAST guess is the most similar match against the NCBI genome database. The effect on root rot was categorized into five classes, ranging from negative to positive. The class represents the annotation derived from the curated UNITE database. The most significant QTLs (-log(p) > 9 are presented).

Conclusions

The results obtained indicate that (i) the presence and abundance of microbial taxa are determined (among other factors) by the pea genotype; (ii) the holobiont significantly determines the resistance of the plant to root rot; (iii) the holobiont approach of genomic markers for plant resistance and root microbial recruitment lead to an improved prediction of PRRC resistance compared to predictions based on plant genetics alone.

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606/155. UNRAVELING THE GENOMIC LANDSCAPE OF DELETERIOUS MUTATIONS IN COMMON BEANS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Addressing the pressing global challenge of ensuring food security for a growing population and combating hunger necessitates doubling food production by 2050. However, current yields and projections are insufficient to meet escalating food demands, especially in changing climate conditions. With approximately 30% of producing areas worldwide experiencing stagnating crop yields, crop diversification and innovative breeding approaches are imperative. Legumes play a paramount role in meeting future food and nutrient demands. Leveraging genomic and evolutionary information opens the scope to study

population genetics processes such as inbreeding and its effect on the accumulation of deleterious mutations (DelMut). Using common bean as a self-pollinating legume crop model, we developed a Multiparent Advanced Generation Intercross (MAGIC) population. The whole genome of eight founders was sequenced using long and short reads. Furthermore, intermediate generations were also sequenced at lower coverage. A Practical Haplotype Graph (PHG) using founders' genomes was built (Jensen et al., 2020), and variants were imputed and tracked across generations. To annotate potentially deleterious variants, we incorporated publicly available genomic information from 36 legume species relatives spanning approximately 59 million years of evolution. This information was utilized to train a random forest (RF) model, incorporating 515 parameters derived from protein structure changes based on approximately 7 million coding positions within the common bean genome. This included parameters such as SIFT scores (Vaser et al., 2016) and deep representation learning of fundamental protein features (UniRep; Alley et al., 2019). Using the trained model, putative DelMut variants identified within the MAGIC population were annotated. Our findings revealed distinct genomic regions and genes where DelMut accumulated. Our research not only sheds light on the intricate interplay of DelMut in legumes but also paves the way for designing novel breeding strategies aimed at purging putative DelMut through conventional breeding approaches and genome editing techniques.

606/176. SPATIO-TEMPORAL TRANSCRIPTOME AND STORAGE COMPOUND PROFILES OF DEVELOPING FABA BEAN (VICIA FABA) SEED TISSUES

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Due to its nutritious and protein-rich seeds, the faba bean (Vicia faba), a legume produced in a variety of temperate zones, offers great potential for increased cultivation and use in diverse food applications. Plant breeding targeting specific seed qualities is therefore of high interest. However, basic knowledge regarding the genetic regulation of seed quality and how tissue-specific storage partitioning occurs during seed development in faba bean is needed. In this work, we examined the faba bean seed tissues endosperm, embryo, and pericarp at different developmental stages to pinpoint crucial developmental mechanisms involved in the partitioning of storage compounds. Expression patterns of genes annotated to encode enzymes in the major metabolic pathways and in transcriptional networks were identified using spatiotemporal transcriptome and metabolite profiling analysis, along with chemical analysis of protein, starch, and lipid levels, in the different seed tissues. We found expressed members of the LAFL transcription factor (TF) network (LEC1, ABI3, FUS3, and LEC2) as well as their main repressors VAL1 and ASIL1 (Fatihi et al. 2016). Our findings show that seed storage proteins (SSP), oil, and starch accumulate throughout seed development process, reaching maximum levels of SSPs already early on. Further, the faba bean seems to bear a potential capacity for high lipid biosynthesis and oil storage, as indicated by the early expression of WRI1 transcripts and the late development of oil body proteins. Overall, this work adds fundamental understanding of seed-development processes that may be applied to future development of focused breeding methods for faba bean.

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606/193. NMR METABOLOME-GENOME WIDE ASSOCIATION STUDY REVEALS GENOMIC REGIONS CONTROLLING GRASS PEA METABOLIC QUALITY: IMPLICATIONS FOR PRECISION BREEDING AND IMPROVED DIETS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Growing plant-based eating habits boost legume popularity. Legumes offer rich nutrition and health benefits, having a functional food status. However, nutritional compositional data on some promising legume species is limited, hindering their use. Lathyrus sativus (grass pea) is one such understudied species, being an important source of protein and calories in drier areas, with ability to respond to climate challenges (Gonçalves et al., 2022). In this work, untargeted nuclear magnetic resonance (NMR) spectroscopy profiling was used for a broad and unbiased assessment of seed flour metabolite changes of a collection of 200 grass pea accessions, field trialled over three years. NMR assessment of this collection, for which significant genotype-by-sequence information (5,651 SNPs) exist (Sampaio et al., 2021), allowed also to identify the main genomic regions controlling its metabolome variability, through genome wide association study (GWAS). For that, NMR spectra were consolidated into 514 NMR buckets/metabolome features (NMRprocflow, MetaboAnalyst) and their relative concentrations adjusted means calculated using linear mixed models.

From the metabolomic profiles multivariate analysis, a clear year differentiation was observed. 376 NMR features showed genotypic differences, reinforcing their potential for grass pea improvement, and justifying their GWAS. Using a threshold of –log10(p)=4, 435 SNPs associated with 204 NMR features were identified, prioritizing their annotation. Annotation is ongoing by matching spectral information from 1D and 2D spectra to reference compounds in available databases. Amongst identified compounds are amino acids and derivatives, sugars, sugar-alcohols and organic acids.

The combined genetic analysis highlighted multiple loci impacting compounds abundance through a network of interactions, where individual loci may affect more than one compound and vice versa.

The generated knowledge on metabolite variation and respective genetic basis will support precision breeding efforts to answer consumers' nutritional quality concerns, increasing the use of grass pea in diets.

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606/215. GENOMIC SELECTION FOR ALFALFA: THE CHALLENGE OF THE AUTOTETRAPLOID GENOME

Authors:

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Work centre:

(1) Italy, (2) CREA-ZA

Summary:

Objectives

Alfalfa, the most economically important forage legume worldwide, features modest genetic progress due to long selection cycles and high extent of non-additive genetic variance associated with its autotetraploid genome. Genomic selection (GS) has a special interest for this crop, yielding the promise of substantially shorter half-sib progeny-based selection cycles of parent genotypes for synthetic varieties. This prospect implies GS model construction by a training population whose genotyped parents are phenotyped on the ground of their half-sib progenies. However, the autotetraploid genome complicates also GS predictions because of the substantial sequencing effort and associated costs needed for a reliable allele dosage estimation. Moreover, several strategies do exist for SNP marker calling, filtering, and resulting genome representation, with no clear-cut choice for optimal configurations. This study aims to investigates the effects of such choices and to provide a guideline for both the genotyping phase and the bioinformatics analysis linked to GS in alfalfa. We used Genotyping by Sequencing-generated data and focused on traits of different genetic complexity, i.e., dry biomass yield in moisture-favorable (FE) and drought stress (SE) environments, leaf size, and onset of flowering, assessed on 143 half-sib progenies of a genetically-broad European reference population.

Concise description of the work (materials & methods)

We tested three genome representations: 1) proper tetraploid dosage (AAAA, AAAa, AAaa, Aaaa, aaaa); 2) pooled diploid dosage (with the three heterozygote classes Aaaa, AAaa and AAAa pooled in the single Aa class, together with AA and aa for homozygotes); and 3) allele ratios, where each marker is represented as the observed ratio between A alleles and the total of A+a alleles. These genetic configurations highly depend on other filtering parameters such as the allowed pre-imputation rate of missing marker (mpm) and minimum required number of reads per genetic locus. To compare the various scenarios, we envisaged 10-folds cross-validated predictive ability as the main performance metric. All regressions used ridge regression BLUP as regression model.

Main Results

The number of resulting SNP markers varied from 2387 to 19668 depending on the configuration. Allele ratios maximized the average GS predictive ability (0.206), followed by tetraploid dosage (0.183) and diploid dosage (0.180). The predictive ability of biomass yield decreased markedly in the stress environment (averaging 0.124 in SE vs. 0.349 in FE) along with a reduced broad-sense heritability (0.34 vs. 0.54), in agreement with earlier studies. Predictive abilities averaged 0.335 for leaf size and 0.170 for onset of flowering, the latter possibly hindered by narrow phenotypic variation and genetic control. Filtering on missing rate favoured higher thresholds, while no clear pattern was found for the minimum number of reads. A second result of this study was the release of Legpipe2, a SNP calling pipeline that was used to produce the markers used in the analyses. Legpipe2 is open source, python3-based, modular, easily customizable and supports orthogonal logging and single-file configuration. It is available at https://github.com/ne1s0n/legpipe2.

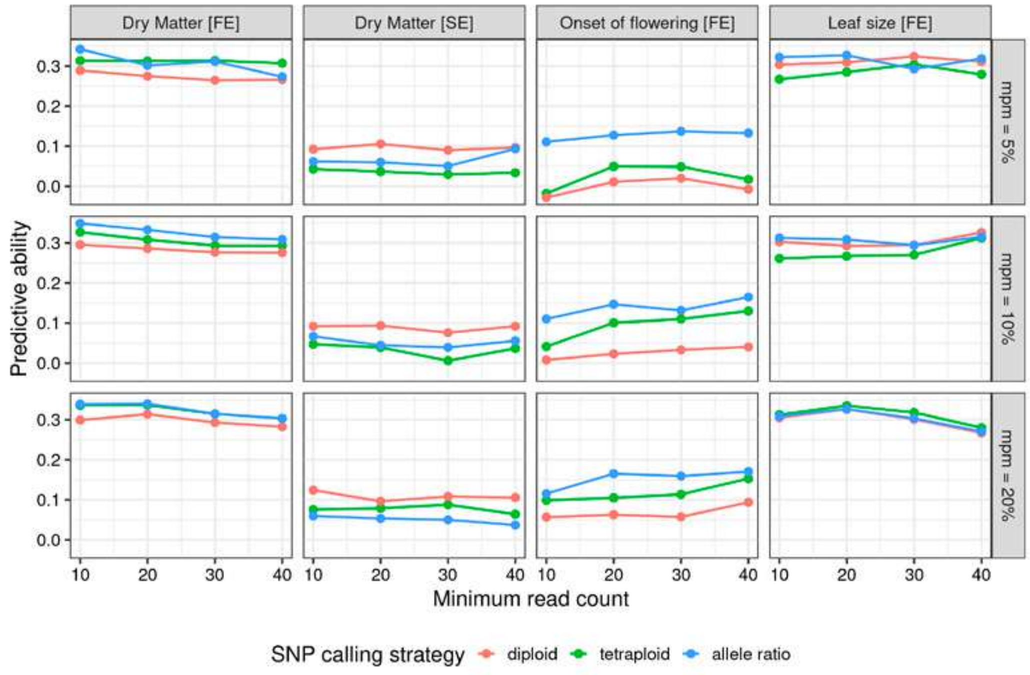

Figure 1. Predictive abilities for three traits in favourable (FE) or drought stress (SE) environment, three levels of filtering on maximum allowed missing rate per marker (mpm), four levels of minimum required number of reads per SNP data point and three SNP representation strategies.

Conclusions

This study shows that SNP ratios is a valid and simple data representation strategy that can improve the GS predictive ability in tetraploid alfalfa by circumventing the difficulties of polyploid dosage calling. It also confirms the greater challenge of predicting biomass yield breeding values in stress environments.

Acknowledgements The research was funded by the project GENLEG granted by the Italian Ministry of Agriculture, Food Sovereignty and Forestry.

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606/234. GENETIC DISSECTION OF SEED PROTEIN CONCENTRATION IN PEA USING MULTIPLE DIVERSE MAPPING POPULATIONS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Improving the seed protein concentration (SPC) of pea is an important breeding objective because of its underlying nutritional value and the demand from the international processing industries. To understand the genetic control of SPC and support the marker-assisted selection (MAS), we explored three recombinant inbred line (RIL) populations and a genome-wide association study panel (GWAS-2) to identify the quantitative trait loci (QTLs) associated with protein content. The RIL populations used, CDC Amarillo x CDC Limerick (PR-25), MP 1918 x P0540-91 (PR-30), and Ballet x Cameor (PR-31), represent moderate SPC x high SPC crosses. The GWAS-2 panel comprised of representative accessions from global pea breeding programs, pea core germplasm, and commercial cultivars released in Canada. One hundred and ten, and 169 RILs of PR-25 and PR-30, and 233 accessions of GWAS panel were genotyped using a Axiom® 90K SNP array. PR-31 was earlier genotyped using the Genopea 13.2K SNP array [1], and the reported linkage map was used in the current study. Individuals of each mapping population were grown in replicated trials at two to three locations in Saskatchewan between 2019 and 2021. All mapping populations were tested in 5 to 7 station-years. Seed samples harvested from each plot were used for the determination of SPC using near-infrared (NIR) spectroscopy. We identified three QTLs each in PR-25 [2] and PR-30, and five QTLs in PR-31 associated with SPC. The LOD value of the identified QTLs ranged from 3.0 – 11.0. The QTLs from the biparental populations will be compared with those identified in the GWAS and with the published literatures. The highly significant QTLs identified in this study are useful for MAS of pea breeding lines for SPC.

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606/238. CONTROL OF THE NUMBER OF FLOWERS AND ARCHITECTURE OF THE INFLORESCENCE OF LEGUMES BY RAX-TYPE MYB TRANSCRIPTION FACTORS

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Summary:

Objectives

Most legumes have compound inflorescences, where flowers/fruits appear not in the in primary inflorescence (I1) stem but in lateral secondary inflorescences (I2; Fig1. A). The number of flowers in the I2 depends on the activity of the I2 meristem and is characteristic of each legume species and variety. Also, in different legumes, mutations have been described whose only apparent effect is to increase the number of flowers/pods produced in the I2 (multipod phenotype; Fig1. B-G). The number of flowers per I2 is a relevant trait, as it contributes to form diversity among legume species and it determines production of flowers/pods, having the potential to influence yield. In spite of the relevance of this trait, little is known about how it is regulated. In collaboration with the labs of T Millán and J Gil (Córdoba University) and J Rubio (IFAPA, Córdoba), we isolated the first gene specifically regulating the number of flowers per I2 (1), the SINGLE FLOWER (SFL) gene, whose mutations lead to the double-pod phenotype in chickpea (Cicer arietinum Ca; Fig 1F-I). SFL codes CaRAX12a, a R2R3-MYB-type transcription factor, similar to Arabidopsis RAX1 and RAX2 (2). In chickpea, CaRAX12a is specifically expressed in the I2 meristem (Fig1J,K), where it acts controlling the time during which this meristem remains active, determining the number of floral meristems produced. Most legumes contain 2-3 homologues of CaRAX12a (Fig1. L). Because different legumes have more than one loci whose mutations lead to multipod phenotypes, our objectives are to understand whether 1) the function of RAX12a controlling t I2 meristem activity is general to other legumes and 2) whether the other RAX12a homologues present in each legume species do also contribute to the control of number of flowers per I2.

Concise description of the work (materials & methods)

With this aim, we are using pea (Pisum sativum, Ps). We perform in situ hybridization with PsRAX12a and PsRAX12b, pea homologues of the chickpea CaRAX12a, to see whether the pea genes have a similar expression pattern than the chickpea RAX12a gene. For function, we silence the expression of the pea homologues using virus induced gene silencing (VIGS).

Main Results

We found that both PsRAX12a and PsRAX12b are expressed at the I2 meristems, PsRAX12a at the same domain as CaRAX12a and PsRAX12b at boundary regions (Fig1. M,N). We also found that PsRAX12a-VIGS plants have I2s that produce more flowers (Fig1 O,P), while PsRAX12b-VIGS plants produce altered I2s that form terminal abnormal flowers (Fig1 O,Q).

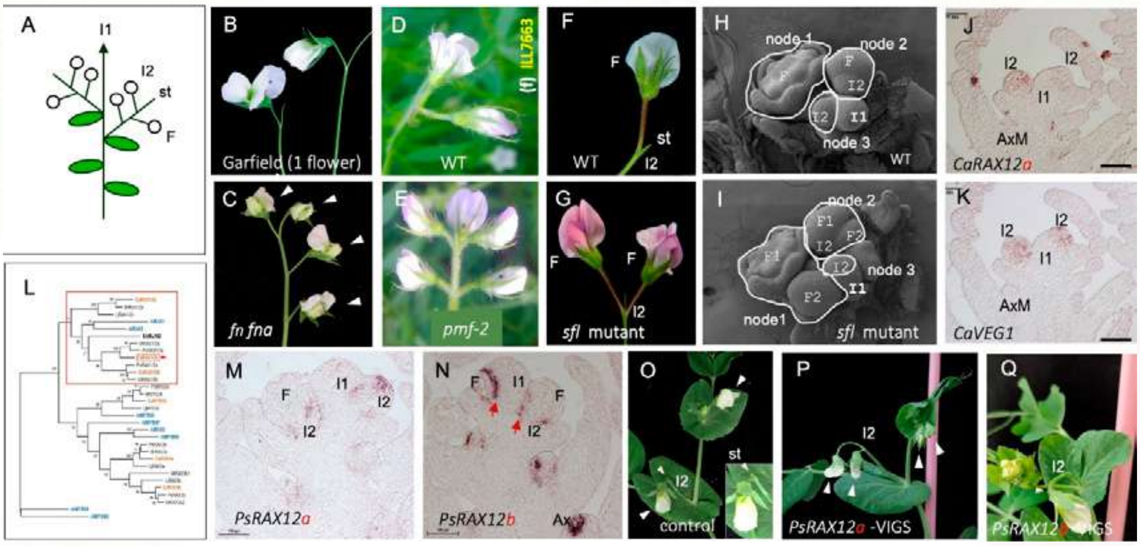

Figure 1. Control of number of flowers in legumes. A. Diagram of the legume compound inflorescence. F, flower; st, stub. B-G. wild-type and multipod mutant I2 inflorescences from pea (B,C), lentil (D,E) and chickpea (F-G). H-I. SEM micrographs of apices from chickpea wild-type (H) and sfl (I) inflorescences. Wild-type I2s make one floral meristem while sfl I2s make two floral meristems. J-K expression of SFL/CaRAX12a in the chickpea inflorescence apex by in situ hybridization. SFL/CaRAX12a expression is observed in the I2 meristem (J), as observed for the I2 meristem marker CaVEG1. Ax, axillary meristem. L. Phylogeny of legume and Arabidopsis RAX-type proteins. M-N. Expression of pea RAX12 homologues in the pea inflorescence apex by in situ hybridization. O-Q. Phenotype of pea plants where PsRAX12a (P) or PsRAX12b (Q) was silenced by VIGS. PsRAX12a-VIGS I2s produce 2 flowers while I2s of control plants only produce 1. Different to control plants the I2s of PsRAX12a-VIGS do not end in a stub (st; arrowheads and close-up in o) but the stub transformed into a terminal flower (orange arrowhead) with abnormal organs. F-K, L images are modified from ref. 1.

Conclusions

Our results indicate that both PSRAX12 genes regulate I2 development, being PsRAX12b also involved in flower development. More general, they suggest that function of RAX12a genes is conserved in legumes, controlling the number of flowers at the I2 inflorescence.

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606/9. GENOME-WIDE ASSOCIATION STUDIES OF NUTRIENTS AND TRACE ELEMENTS IN COMMON BEAN

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Summary:

Objectives, Description, Main Results & Conclusions

As one of the legumes consumed by people all over the world, the common bean (Phaseolus vulgaris L.) has a high nutritional and economic value. Its nutritional value lies in the high concentration of proteins, minerals, vitamins and fiber. In addition, the common bean is an excellent source of calcium, iron, magnesium, phosphorus, potassium, sodium and zinc. Our aim was therefore to search for associations between markers and nutrient content.

A panel of 148 accessions representing Croatian common bean landraces was phenotyped for seed content of 23 nutrients and trace elements. The accessions were previously genotyped, revealing 6,311 polymorphic DArTseq-derived SNP markers with MAF > 0.05. A genome-wide association study (GWAS) was then performed based on the Q + K model in TASSEL 5. Numerous significant SNPs were associated with seed content of six nutrients and trace elements (C, Ca, Cr, Cu, Se and Zn) located on all 11 chromosomes. There were no QTNs associated with the other traits.

The results of this study can be used for future enrichment of common bean seed nutritional traits through genetic biofortification.

606/23. CONSTRUCTION OF AN IMPROVED GENETIC MAP OF A MULTI-TRAIT LENTIL RECOMBINANT INBRED LINE POPULATION: AN IMPORTANT STEP TOWARDS QTL MAPPING AND VALIDATION

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Summary:

Objectives, Description, Main Results & Conclusions

Lentil (Lens culinaris, Medik) is a rich source of protein, zinc, iron, and prebiotic carbohydrates, and is an influential crop in global food and nutritional security. However, lentil yield and productivity are limited by multiple abiotic/biotic stresses (Zeroual et al., 2022). To deal with these constraints, the development of genetic and genomic resources is required to accelerate trait discovery and improvement, especially for polygenic traits that have been difficult to improve using traditional breeding approaches. Recently, cost effective and inexpensive next generation sequencing platforms have allowed efficient assembly of plant genomes and the identification of thousands of single nucleotide polymorphisms (SNPs). SNP markers can be used to develop high-quality genetic linkage maps that are a valuable asset to map, with precision and accuracy, QTL that are associated with important phenotypic traits. In this study, we used 3600 GBS-based SNP markers to construct a high-density linkage map of a lentil recombinant inbred line (RIL) population consisting of 126 F8-derived RILs from the cross ILL6002/ILL5888. The parents contrast for various agro-morphological traits, for resistance to Stemphylium blight and rust, and for drought tolerance, making this RIL population a valuable resource for genetic dissection of multiple traits in lentil. This population has been screened in different drought conditions using both conventional phenotyping methods and an advanced automated high-throughput phenotyping platform. The enhanced genetic map produced in this study has 7 linkage groups with reduced gaps and higher marker density, compared to previous maps. This genetic map will add to the available genomic resources of lentil, and be a valuable tool for genomic research and molecular breeding.

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606/50. RNA-SEQ BASED GENE EXPRESSION ANALYSIS OF SEED PROTEIN AND SULFUR AMINO ACID ACCUMULATION IN DEVELOPING PEA SEEDS

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Summary:

Objectives, Description, Main Results & Conclusions

This research aims at identifying candidate genes associated with the accumulation of pea seed protein and sulfur amino acids by comparing the expression profile of genes in developing seeds of accessions that contrasted in concentration of seed protein and sulfur amino acid concentration. Accessions were selected from PR-25, a recombinant inbred line population derived from a cross between CDC Amarillo and CDC Limerick, based on 7 station-year's data. The selected accessions were high seed protein concentration line PR-25-69, high sulfur amino acid concentration line PR-25-53, and low protein/low sulfur amino acid concentration line PR-25-6. These lines were grown in a phytotron chamber, set at 23ºC for 16 hours (day) and 18ºC for 8 hours (night). Developing seeds of each line were collected at 7, 14, 21, and 28 days after pollination. The harvested seeds collected from three replicates were used for RNA sequencing by AgriGenome Labs (Kochi, Kerala, India). The results were further validated by qPCR assays. On average 73.8 million reads per sample were obtained, with 91.6% alignment to the pea reference genome. By comparison of the gene expression profiles between seed protein/sulfur amino acids contrasting lines, a total of 1781 differentially expressed genes (DEGs), associated with the accumulations of seed protein and sulfur amino acids, were identified in all four sampling times. Of these, 755 DEGs were downregulated and 1026 DEGs were upregulated compared to control (PR-25-6). Their expression level varied from log2 2-fold to log2 10-fold change. Gene ontology enrichment analyses revealed that upregulated DEGs and downregulated DEGs had different impacts on the accumulation of seed protein and sulfur amino acids, as they were involved in disparate biological pathways during seed development processes. The information of identified DEGs and their functional annotations could facilitate gene-base marker development for high protein/good protein quality traits.

606/74. CONSTRUCTION OF A HIGH-DENSITY GENETIC MAP FOR FABA BEAN (VICIA FABA L.) AND FINE MAPPING OF QTLS FOR PEST AND DISEASE RESISTANCE

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicia faba L.) is one of the highest protein crops and the fifth most important legume in production after chickpea, pea and lentil. Ascochyta fabae Speg. and broomrapes (Orobanche spp.) are among the most important pathogens of faba bean and a major constraint to global faba bean production. Broomrapes are particularly important in southern and eastern Europe, Middle East and North Africa. While Orobanche crenata has severely limited faba bean cultivation throughout the Mediterranean area, Orobanche foetida (first detected in Tunisia and later spread to Morocco), represents a future major risk for faba bean cultivation. The spread of this new parasite represents a key threat for legume production not only in Spain, but in all cropping areas of Southern Europe. Several studies on quantitative trait loci (QTL) controlling Orobanche crenata and Ascochyta fabae, resistance have been previously reported. However, few studies are available on the genetic and molecular basis underlying host-pathogen interaction in faba bean. In this work, the resistance to Orobanche foetida was evaluated in two consecutive seasons in Beja (Tunisia), using a recombinant inbred line (RIL) segregating for resistance to broomrape and ascochyta blight derived from the cross of the faba bean parental lines 29H x Vf136. A high-density map has been developed using the Vfaba_v2 Axiom SNP array with 60K SNP (Khazaei et al. 2021) from Affymetrix. QTL analysis was conducted using IciMapping (Meng et al. 2015), together with previous evaluations for Orobanche crenata and Ascochyta fabae performed in this population. The fine-mapping approach proposed here, increases the genetic resolution of relevant QTL regions and paves the way for an efficient deployment of useful alleles for faba bean ascochyta and broomrape resistance through marker-assisted breeding.

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606/75. GENOME-WIDE ASSOCIATION STUDY OF POD DEHISCENCE IN VICIA FABA L

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Summary:

Objectives, Description, Main Results & Conclusions

Yield components are the main factors to improve the production in faba bean (Vicia faba L.) and identifying the genetic determinants for high yield is a major issue to breed varieties. Pod dehiscence (shattering) causes important yield losses in cultivated crops and therefore has been a key trait strongly selected against in crop domestication. In faba bean, yield losses at harvest can be substantial, reaching 30% at maturity. Few studies are available on the genetic basis of this trait in faba bean. The first comprehensive study on faba bean pod dehiscence, identified three QTLs in faba bean chromosomes II, IV and VI, although none of them was stable across years (Aguilar-Benitez et al. 2020). Family-based QTL is limited by the high confidence intervals and the relatively low number of recombination events existing in biparental mapping populations. With the recent development of powerful faba bean SNP array platforms, genome-wide association studies (GWAS) are more time efficient method that increases significantly mapping resolution and make it rather easy for minor effect genes to be detected. The aim of this work, was to detect and validate the genomic regions controlling dehiscence related traits in faba bean by performing a GWAS on a worldwide collection of 352 accessions. Dehiscence scoring was carried in one location and year. Genotyping was performed using the Vfaba_v2 Axiom SNP array with 60K SNP (Khazaei et al., 2021) from Affymetrix. Eight significant single nucleotide polymorphism (SNP) markers associated with shattering were identified in chromosomes I (2), II, III (2), IV (2) and VI and explaining 80.2% of total phenotypic variation. The results have detected new genomic regions associated with this trait and validated a QTL previously reported in chromosome IV. The identification of candidate genes will accelerate molecular marker-assisted breeding for yield related trait in this crop.

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606/84. DEVELOPMENT OF CAPS MARKERS FOR GENOMIC SELECTION OF PEMV RESISTANT PEA LINES AND COMPARING APHID INFESTATIONS IN PEA ACCESSIONS SHOWING HIGH VARIABILITY

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Summary:

Objectives

The aim of this work was to convert the identified SNP markers associated with resistance to PEMV (pea enation mosaic virus) into CAPS (Cleaved Amplified Polymorphic Sequences) markers suitable for selection and breeding. These can be used as an effective tool for the refinement, acceleration and financial efficiency of the selection process and the creation of new competitive pea varieties.

Concise description of the work (materials & methods)

Based on the evaluation of a set of 564 pea genotypes through DArTseq sequencing analysis of DNA libraries with reduced complexity and subsequent association analysis (GWAS), SNP markers associated with resistance to PEMV were identified. 3 of these SNP markers were selected for testing according to their ability to detect the presence of a reference/alternative allele with the use of CAPS method.

Apart from the molecular analysis, 103 field pea accessions showing high variability in morphological and growing characteristics were exposed to natural pea aphid (Acyrthosiphon pisum) infestation under field conditions in 2022. Differences in the aphid abundances (aphids were counted repeatedly from the bud to green pod stage) and the levels of damage induced by PEMV among the compared accessions were assessed. At the same time selected traits of the compared accessions were evaluated and described with intention to find significant relations between them (or their groups) and the levels of aphid / virus infestation levels. Data were analysed with the usage of multivariate statistical methods.

Main Results

The main variability of resistance to PEMV in the analysed pea population is mediated mainly through genes located on chromosome 1 (LGVI linkage group), then on chromosomes 2, 4 and 5. The tested markers were located on chromosomes 5 and 2, corresponding to linkage groups LGIII and LGI. Based on the reference sequence and adjacent sequences, CAPS markers were designed for easy and fast molecular identification of the reference/alternative allele in the sample. Fragments of the expected length were amplified using PCR and during subsequent restrictions variability in the occurrence of alleles was evident for all tested markers.

Field pea accession (as a Factor) had significant impact on variability in the levels of pea aphid infestations (16.5.: F = 2.8410 $p < 0.0001$, 30.5.: F = 2.0028 $p < 0.0001$, 7.6.: F = 2.8410 $p < 0.0001$). There were significant ($p < 0.05$) moderately strong negative correlations (r = - 0.48 to - 0.62) between the level of aphid infestation and crop height, crop density 14 days after emergence even before flowering, and crop biomass at bud formation stage. Aphids were more abundant on greyish than on green and yellow/green accessions. There was tendency for higher aphid counts in accessions showing this combination of traits: lower height, greyish colour of leaves and stipules, earlier flowering and pod formation, green colour of seeds, higher content of N in seeds (after harvest). It was well pronounced especially when the traits were combined with lower density of pea crop at the time between the bud formation and the end of flowering. Surprisingly there was no correlation between the level of aphid infestation and PEMV frequency or incidence.

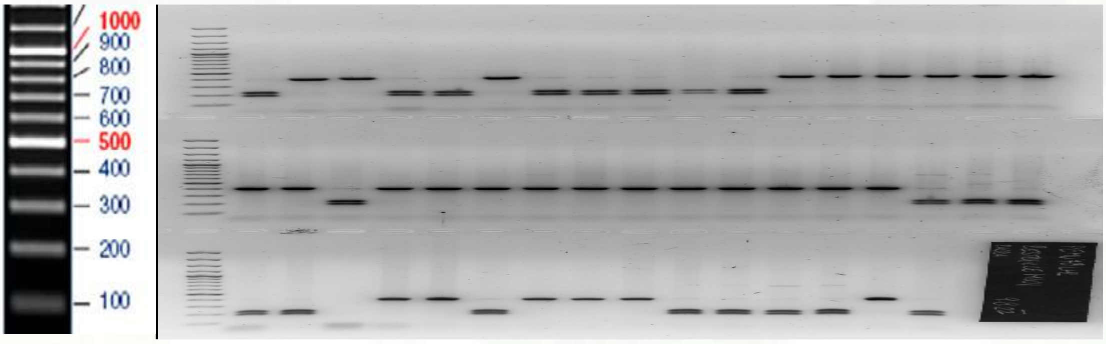

Figure. Result of restriction analysis

Figure. Result of restriction analysis for marker 78: on the left 100 bp Plus DNA Ladder (Thermo Fisher Scientific), 1. – 17. amplified samples after restriction. Samples 1,4,5,7,8,9,10,11 – with resistant allele (206 and 216 bp), samples 2,3,6,12,13,14,15,16,17 with allele sensitive to PEMV (422 bp).

Conclusions

Identified markers can be used for effective pea breeding (marker-assisted selection). The next step of testing will be 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. Significant relation between some field pea plants characteristics (and their combinations) and the level of pea aphid infestation was confirmed.

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606/86. TRANSCRIPTOME ANALYSIS OF VICIA FABA L. FLOWER STYLES

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Summary:

Objectives, Description, Main Results & Conclusions

In angiosperms, the process of pollination depends on a complex communication between the male (pollen) and the female (stigma) reproductive organs. Stigmas can be generally classified into two main groups according to the presence (wet stigmas) or absence (dry stigmas) of a viscous secretion on the stigma surface. In species with wet stigma, the cuticle is disrupted and the presence of exudates is indicative of their receptivity. Most studies are focused on few species, many of them with self-incompatibility systems. However, there is scarce knowledge about the stigma composition in Fabaceae, the third angiosperm family in species richness. Here we report a transcriptome analysis of the Vicia faba L. style and stigma and perform a differential expression analysis between autofertile (flowers able to self-fertilize in absence of manipulation and the exudate is released spontaneously) and autosterile (flowers need to be manipulated to break the cuticle and release the exudates to be receptive) inbred lines. From the 76,269 contigs obtained from the de novo assembly, only a 45,1% of the sequences were annotated with at least one GO term. A total of 115,920, 75,489 and 70,801 annotations were assigned to Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) categories, respectively. Differential expression analyses revealed 5,918 differentially expressed genes (DEGs) between the autofertile and the autosterile lines. Of them, 2,475 genes were downregulated and 3,443 genes were upregulated. The KEGG pathway enrichment analysis showed that the up- and downregulated genes were significantly enriched in 39 functional groups. Upregulated genes were particularly enriched in "Selenocompound metabolism", "One carbon pool by folate", "Monoterpenoid biosynthesis" or "Nitrogen metabolism". Downregulated genes were enriched in "Limonene and pinene degradation", "Phosphatidylinositol signaling system", "Inositolphosphate metabolism", "Phagosome" or "Glycerolipid metabolism". These results suppose a first approach to knowledge the gene expression in faba bean stigmas.

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606/89. HIGH-DENSITY QTL MAPPING OF ARCHITECTURAL, PHENOLOGICAL AND YIELD RELATED TRAITS IN FABA BEAN (VICIA FABA L.)

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicia faba L.) is an important legume grown across the world but in the last decades, genetic improvements were lagging for the lack of a sequenced genome. Nevertheless, the recent publication of the first whole-genome sequencing, assembling and annotation (Jayakodi et al. 2023) will greatly facilitate gene identification and genomic improvement in this crop. In this study, a RIL population of 124 plants from the cross Vf6xVf27, used in previous studies to identify QTLs for yield, plant architecture, flowering time, dehiscence and autofertility (Aguilar-Benitez et al. 2022) was genotyped using an array platform of 60k markers (Khazaei et al. 2021). We obtain a highly saturated genetic map

with 5092 markers joined in six linkage groups and covering 6069.07 cM with an average distance between markers of 0.98cM. Markers in most of the chromosomes, except chr. V, fit the expected 1:1 segregationratio, thus revealing some king of genetic distortion in this genomic region. Using the previous phenotypic data, the new QTL analysis revealed 112 significant QTLs: 31 for autofertility traits, nine for flowering time traits, 52 for plant architecture, 19 for yield and one for dehiscence; with a high accumulation of them in chromosome VI. Several QTLs for the same trait co-localized in closed positions in different years. Markers associated to QTLs could be identified in the genome and their genetic sequence used to determine their biological function and association with the different characters. Many genes were related with cell growing in response to hormones and cell wall development, indicating a complex process of regulation but giving the firsts clues to identify candidate genes for molecular breeding of complex traits in this crop.

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606/98. TOWARDS THE GENETIC BASIS OF CLUSTER ROOTS DEVELOPMENT IN LUPINS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

White lupin (Lupinus albus L.) has the particularity of developing a root structure consisting of densely packed clusters of rootlets called cluster roots in response to low phosphorus levels in the soil. These structures increase the surface area of the roots, allowing for greater phosphorus uptake. Understanding the genetic and molecular mechanisms underlying cluster root development in white lupin could have significant implications for improving the efficiency and sustainability of agriculture. To do so, we first studied mutants in which the development of cluster roots was affected and identified regulators of that development. To go further, we are developing two new projects. On one hand, we will look at the range of cluster root morphologies and functionalities in the species diversity. Plants will be grown in a semi-hydroponics 2D-growth system and their root systems analyzed to extract both basic root architecture traits, as well as cluster root traits such as number, size, or density. Coupled with genomewide genotyping, these traits will be used in a genomewide association study to retrieve the loci controlling them. On the other hand, we will investigate the diversity of the Lupinus genus. Indeed, cluster roots are not conserved in the whole genus and, in particular, seem to be absent in the New World species. We will start by characterizing cluster root development in a large set of species and sequencing the whole genome of species showing the most significant variation in their ability for cluster root development. Sequences at known regulators as well as analysis without a priori will be carried out to better understand what explains those differences within the genus. These two projects aim at the identification of genetic determinants of cluster root formation, as well as providing insights into the evolutionary history of cluster roots within the Lupinus genus.

606/102. ANALYSIS OF THE METABOLIC PROFILES AND GENE EXPRESSION PATTERNS OF CRUCIAL GENETIC ELEMENTS WITHIN THE ANTIOXIDANT METABOLITE BIOSYNTHESIS PATHWAYS IN MUNGBEAN SPROUTS

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Summary:

Objectives, Description, Main Results & Conclusions

Plant secondary metabolites are produced as defense mechanisms against biotic and abiotic stresses. Mungbeans (Vigna radiata. L) are one of the major legume crops and serve as an important nutritional source in many developing countries in Asia. Additionally, mungbeans are known to contain higher contents of flavonoids than other legume crops. The germination of seeds can enhance the functional and nutritional quality of legumes (Kartikeyan et al., 2022). This study aimed to profile secondary metabolites and analyze the expression of key genetic factors associated with the biosynthesis of beneficial antioxidants in mungbean sprouts. Catechin and neo/chlorogenic acid showed the highest and most diverse contents. The reference mungbean elite cultivar, VC1973A, had the highest content of gallic acid, but had lower levels of major legume antioxidants such as catechin, neo/chlorogenic acid, iso/vitexin and isoflavones compared to other genotypes. Wild mungbeans (Genotype no. 36-42) contained higher contents of isoflavones including daidzin, genistin and glycitin, in general. Significant correlations were observed among the contents of isoflavonoids that synthesized by same enzymes. Key genetic factors were identified to be significantly associated with the levels of secondary metabolites through gene expression analysis. The findings suggest that the levels of functional substances are controlled through transcriptional regulation. This knowledge can be utilized in molecular breeding or genetic engineering to enhance the nutritional value of mungbean sprouts. Additionally, wild mungbeans present a valuable resource for improving the quality of mungbean sprouts.

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606/114. GENOME-WIDE IDENTIFICATION OF GENES ASSOCIATED WITH RESISTANCE TO AFLATOXIN B1 Production IN PEANUT (Arachis hypogaea L.)

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Summary:

Objectives, Description, Main Results & Conclusions

Since it was first identified as "Turkey X Disease" in the UK, aflatoxin (AF) has been a significant topic of research. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified AFB1 as having the highest hepatocellular carcinoma-causing toxicity as a member of group I. Utilizing 223 peanut accessions harvested in 2021 and 2022, this study evaluates aflatoxin resistance and identifies resistance genes associated with aflatoxin accumulation. The quantitative analysis of aflatoxin B1 content in peanut accessions inoculated with Aspergillus flavus, which produces aflatoxin B1 and B2 primarily, was conducted using UPLC-PDA in accordance with the protocol of the Korean Food Standards Codex. Using the peanut 58K Axiom_Arachis array chip, a genome-wide association study (GWAS) was conducted to identify the genes associated with aflatoxin B1 content in 223 peanut accessions. Using the FarmCPU model of the GAPIT package, two putative SNPs on chromosomes Araip.B04 and Araip.B09 were identified as being significantly associated with Aflatoxin B1 levels. Within an estimated ±250 kb window, 68 annotated genes flanked by significant SNPs were identified based on the reference genome. Two candidate genes encoding pathogenesis-related thaumatin protein from the PR-5 (Pathogenesis-Related-5) protein family and a leucine-rich repeat receptor-like kinase (LRR-RLK) for disease resistance indicating apoptosis and immune responses may be involved in the peanut response to aflatoxin production. It is anticipated that the outcomes of this research will be useful for evaluating aflatoxin B1 resistance and identifying genomic regions associated with aflatoxin B1 accumulation in order to develop resistant varieties.

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606/119. THE PREVALENCE OF VICINE AND CONVICINE IN GENUS VICIA

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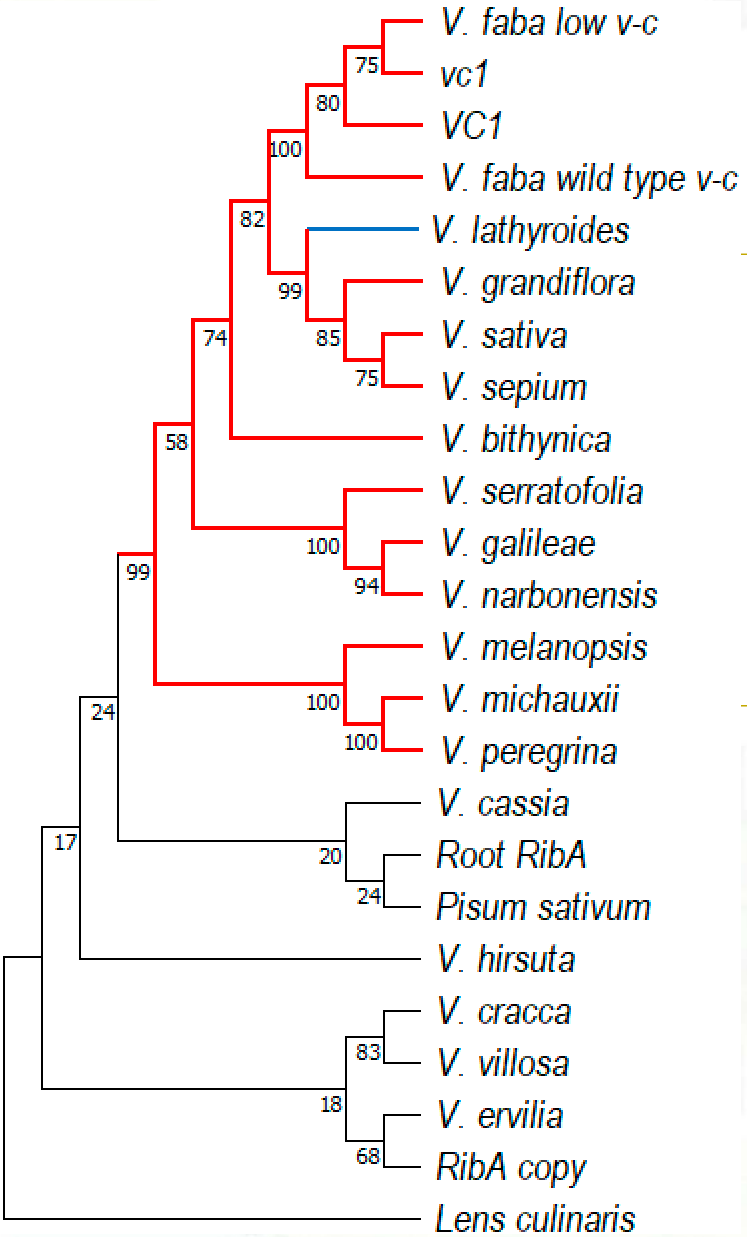
Summary:

Objectives

Faba bean (Vicia faba) contains the anti-nutritional factors vicine and convicine (v-c). These pyrimidine glucosides hydrolyze to divicine and isouramil during digestion. These latter two compounds are powerful oxidants and harmful to humans who have a gene mutation conferring glucose-6-phosphate dehydrogenase (G6PD) deficiency. V-c in chicken feed causes decrease in egg size and quality in laying hens and kills broilers. Other monogastric animals such as pigs are also affected by v-c, but not as strongly as chickens. V-c is the product of an overflow mechanism from the riboflavin synthesis pathway, controlled by RibA gene (Björnsdotter et al., 2021). In faba beans there are several copies of RibA, only one of which is linked to v-c production. This gene copy was named VC1 and its recessive allele vc1 confers lower v-c levels in faba bean. While riboflavin is ubiquitous to plants, v-c is mainly known from some but not all Vicia species. Hence, we set out to investigate the distribution of v-c in the genus, with the aim of gaining insights into the evolution of this peculiar trait and from that, how it might be reduced further in the food chain.



Phylogenetic tree constructed with sequenced DNA. V-c producing species are marked with red. Black have no v-c and blue has the VC1 gene but does not produce v-c.

Concise description of the work (materials & methods)

To achieve this, 17 Vicia species were grown in a greenhouse until maturity in 2020. Seeds were gathered and processed for v-c quantification according to Pulkkinen et. al (2015). Leaf samples were collected for DNA extraction and sequencing. PCR primers based on established VC1 and vc1 sequences were used (Björnsdotter et al., 2021). These primers produced 1000 bp to 1800 bp fragments that were then sequenced commercially by Eurofins genomics. Sequence analysis was done with MEGA 11 program and a phylogenetic tree constructed with maximum likelihood method and Kimura 2-parameter model.

Main Results

V-c measurements from seeds show that amount vicine in species varied widely. Of the 17 species, 6 produced no vicine or convicine. The branching point between the vicine-containing and vicine-free species was high in the phylogenetic tree. In the vicine-producing species, the amount of convicine was unrelated to the amount of vicine. In the phylogenetic tree, the sequences from all the species producing v-c (marked red) are clustered with the VC1 and vc1 sequences. The one exception was V. lathyroides (marked blue), which lacks v-c but sits in the middle of the v-c containing species in the phylogenetic tree, as also shown by morphometric studies (Leht, 2009). This suggests that VC1 has been completely silenced in V. lathyroides. Work is in progress to establish the mechanism of silencing and its potential applicability to faba bean breeding.

Conclusions

We conclude that the v-c production trait emerged early in the evolution of genus Vicia. The trait is present in only one subclade, within which the amounts of v-c varied widely. The evolutionary significance of this trait is still unknown as is the correlation between vicine and convicine .

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606/121. IMPROVING THE UNDERSTANDING OF PISUM SATIVUM RESISTANCE TO ERYSIPHE PISI PROVIDED BY ER2 AND ER3 RESISTANCE GENES, VIA RNA-SEQ ANALYSIS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Powdery mildew (PM) is one of the most concerning diseases affecting pea crop (Pisum sativum), being Erysiphe pisi the major causal agent worldwide. Three major genes conferring resistance to E. pisi has been reported so far, named er1, er2 and Er3. er1 gene has been shown to be a member of the MLO gene family (Humphry et al. 2011), but little is known about the possible function of er2 and Er3 genes and the defense response elicited by these genes (Fondevilla et al. 2011). The objective of this study was to identify candidate genes for er2 and Er3 and to increase our current knowledge about the molecular mechanisms involved in PM resistance mediated by them. For this purpose, RNA-seq was used to compare gene expression profiles, in inoculated and non-inoculated leaf samples, between resistant genotypes JI2480 (er2er2) and cv. Eritreo (Er3Er3), and susceptible cv. Messire (Er2Er2, er3er3). In parallel, the fungal pathogen gene expression was also explored for compatible and non-compatible reactions. Additionally, a Bulk segregant analysis (BSA) for both er2 and Er3 genes was carried out using bulks of homozygous resistant/susceptible individuals derived from the crosses JI2480 × Messire and Eritreo × Messire. Data analysis is in progress. Major outcomes will be shown and discussed in the congress.

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606/125. CANDIDATE GENES ASSOCIATED WITH SEED WEIGHT IN COMMON BEAN IDENTIFIED THROUGH GENOME-WIDE ASSOCIATION AND GENE EXPRESSION ANALYSIS

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Summary:

Objectives

Bean seeds (Phaseolus vulgaris L.) exhibit wide phenotypic diversity, including variations in size, shape, and coat colour. Seed weight is a component of bean yield and a relevant descriptor of market classes. Quantitative inheritance and quan-

titative trait loci (QTLs) associated with seed weight have been reported. The main goal of this work was the identification of candidate genes involved in the genetic control of the seed weight of common beans combining association and differential expression studies.

Concise description of the work (materials & methods)

The Spanish Diversity Panel (SDP) of 308 bean lines described by Campa et al. (2018) was used in this work. The lines were genotyped with the Genotyping-by-Sequencing method. In all, 298 lines were evaluated in a greenhouse at Villaviciosa, Asturias (Spain), during three seasons for the 25-seed weight (average of four sets of 25 seeds per plot). A randomized design with a plot per line was used. A plot included 8-10 plants per line distributed in 1 m. First, a genome-wide association study (GWAS) using the FASTmrEMMA method was led to investigate the genomic regions significantly associated with this trait. Second, an RNA-seq analysis was carried out in the cv Xana to detect the differentially expressed genes (DEG) between three stages of seed development: the beginning of seed development (0.8-1 cm length and green colour), intermediate development stage (1.5-2 cm length and green colour), and final development stage (2-2.5 cm length and green-white appearance). The experimental design had two biological replicates corresponding to two sampling of seeds from different plants and pods. The DEGs were identified with the function NOISeqBIO of the NOISeq package in R, considering a significant q > 0.99.

Main Results

The lines were genotyped with 4,658 SNP (missing values <20%; MAF> 0.05). A total of 6 significant SNP-trait associations were detected, and they were grouped in 6 blocks of linkage disequilibrium located in six chromosomes (Figure 1). The size of these regions ranges between 177,465 and 2,813,327 bp containing a total of 443 annotated genes (https://www.ncbi.nlm.nih.gov/genome/380). Two of those six regions co-located with QTLs previously reported in other studies: SW_Pv08_55.9 and SW_Pv10_39.1. In all, 1,992 DEGs were identified from comparisons among the three stages of seed development. Twenty-two DEGs were located underlying the 6 genomic regions detected by GWAS (Figure 1) and four DEGs were underlying the regions SW_Pv08_55.9 and SW_Pv10_39.1: PHAVU_008G239600g, PHAVU_008G240600g, PHAVU_008G242800g, and PHAVU_010G123100g.

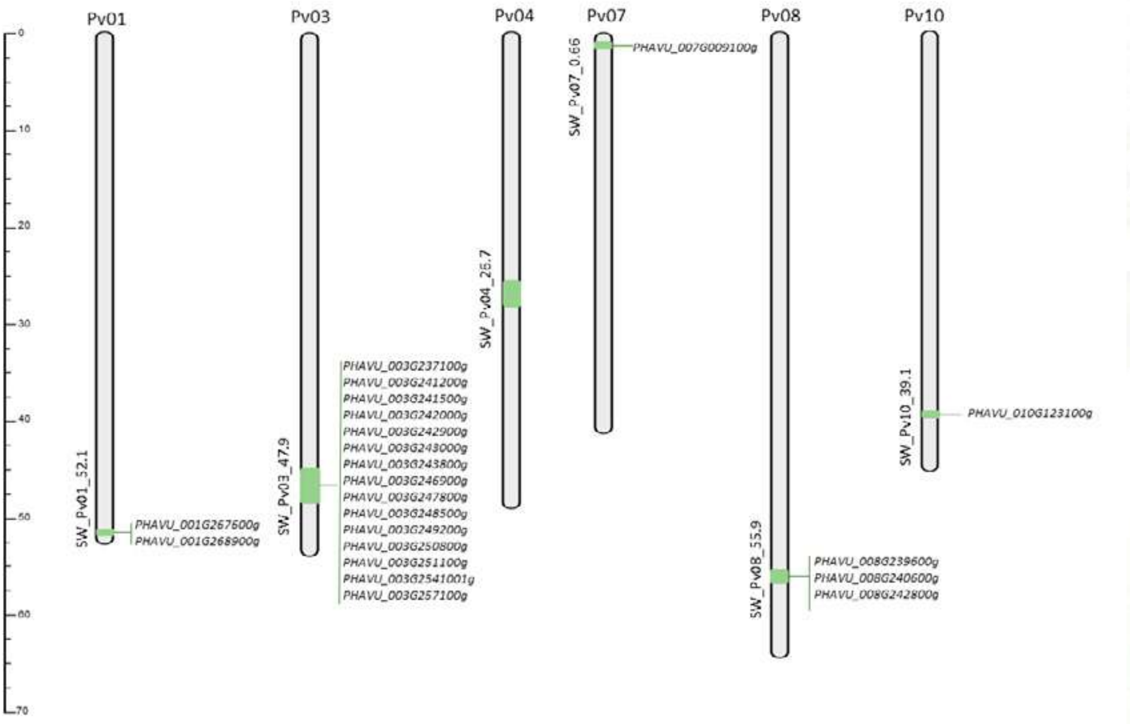

Figure 1. Chromosomal position of genomic regions associated with 25-seed weight identified with GWAS. Genes differentially expressed in seed development underlying those genomic regions revealed by RNA-seq analysis are shown at the right of each chromosome.

Conclusions

Twenty-two DEGs located in regions detected by GWAS can be considered candidate genes to control the seed weight of the common bean. Among them, the four genes underlying the two regions detected by different studies should be considered priorities for further studies.

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606/149. DIGITAL PHENOTYPING FOR BETTER BEANS: PHENOTYPIC AND GENETIC ANALYSIS OF COMMON BEAN GROWTH AND DEVELOPMENT

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Canopy height, area, and volume are informative phenotypic traits used as measurements of crop growth and development. It is advantageous to measure these traits multi-temporally over the growing season to capture plant changes over time. Measuring these traits manually is a laborious, time-consuming, subjective, and impractical method prone to human error when studying large numbers of plots requiring repetitive measurements. High-throughput digital phenotyping using unoccupied aerial vehicles (UAVs) has the potential to overcome the challenges of traditional phenotyping methods and can increase the accuracy and efficiency of phenotyping and selection which ultimately better enables crop improvement and genetic gain. The objective of this work is to evaluate the feasibility of using UAV imagery to measure canopy height, area, and volume in common bean (Phaseolus vulgaris) and use the UAV-derived data to perform phenotypic and genetic analysis. Canopy height was measured manually every two weeks from 6 weeks after planting until maturity. UAV flights were performed weekly beginning in the middle of June and ending at maturity. Canopy height and area data were extracted from the UAV images and volume was calculated from those measurements. A quantitative trait loci analysis was performed to identify genomic regions related to common bean growth and development. It is important to establish the utilization of digital phenotyping methods and improve upon them to facilitate crop improvement.

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606/156. GENETICS OF ASCOCHYTA BLIGHT RESISTANCE IN CHICKPEA

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Summary:

Objectives, Description, Main Results & Conclusions

Ascochyta blight (AB) in chickpea, caused by the fungus Ascochyta rabiei, is a major endemic disease resulting in significant crop loss and management cost for growers. Limited genetic gains have been achieved through conventional breeding. The aim of this work is to identify genetic regions associated with resistance. A diverse chickpea germplasm comprised of domestic x wild introgression material, Vavilov collections, ICARDA FLIP (Food and Legume International Program) lines, and Chickpea Breeding Australia (CBA) material were genotyped using a Multispecies pulse SNP chip and

evaluated for resistance to AB during 2021 and 2022. Two phenotyping environments were utilised i) an outdoor netted pot-based screening facility at the Waite campus, Adelaide, known as SARDI terraces that is inoculated with AB isolates from the northern and the southern chickpea growing regions of Australia, and ii) a field disease nursery at Horsham, Victoria that is inoculated with infested stubble containing a mix of isolates. A total of 1640 genotypes were phenotyped at the SARDI terraces and 1870 genotypes in the Horsham field nursery. There were 300 genotypes in common evaluated across the two environments. Disease Index (%) and stem breakage (%) were used to rate disease severity in the SARDI terraces and in the field nursery. Resistant lines significantly better than the currently released moderately susceptible variety, Genesis™090, were identified. The SARDI terraces and Horsham field assays were highly correlated (r=0.7). GWAS conducted in GAPIT identified significant marker-trait associations for AB resistance associated with percent stem breakage (% stem breakage) in the field on all chromosomes apart from chromosome 3. Genomic regions on chromosomes 2, 4 and 5 were common with loci associated with Disease Index (%) in the SARDI terraces (Figure). Alleles associated with resistance are currently being incorporated into the Australian chickpea breeding program using a genomics-assisted speed breeding approach.

606/178. UNRAVELING GENE EXPRESSION SIGNATURES DURING THE TRANSITION FROM VEGETATIVE TO INFLORESCENCE MERISTEM IN THE COMMON BEAN

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The photoperiodic regulation of flowering in the tropical common bean (Phaseolus vulgaris L.), an obligatory short-day plant, involves the relaxation of photoperiod to induce flowering. As in other crops, photoperiod-induced floral initiation depends on the differentiation and maintenance of meristems. In this study, we investigated global transcript expression profiles in two meristematic tissues: vegetative and inflorescence meristems of two genotypes exhibiting different sensitivities to photoperiods. A total of 3396 differentially expressed genes (DEGs) were identified, with 1271 up-regulated and 1533 down-regulated genes. Notably, 592 genes displayed discordant expression patterns between the genotypes. Comparative analysis with Arabidopsis revealed that many DEGs were novel in Arabidopsis floral transition, indicating evolutionary divergence in the transcriptional regulatory networks governing flowering processes between the two species. However, some genes related to photoperiod and flower development pathways exhibited evolutionarily conserved expression profiles. Additionally, the flower meristem identity genes APETALA1 and LEAFY, along with CONSTANS-LIKE5, were identified as markers distinguishing between vegetative and reproductive stages. Our findings indicate that the down-regulation of photoperiodic genes is directly associated with promoting floral transition under short-day conditions. This study provides valuable insights into the molecular mechanisms underlying meristematic development and contributes to our understanding of photoperiodic adaptation in the common bean.

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- *Keywords: common bean; meristem; flowering; gene regulatory network.*
- *This research was financially supported by PDI2020-114115-RB-100 BEANFLOW (MCIN/AEI). The authors want to thank Xunta de Galicia (www.genecrop.es) and the publication fee by the CSIC Open Access.*

606/183. INSIGHTS FROM TRANSCRIPTOMIC ANALYSIS OF FIBER-RELATED GENES INVOLVED IN POD DEVELOPMENT OF COMMON BEAN

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Summary:

Objectives, Description, Main Results & Conclusions

Pod fiber formation in common bean (Phaseolus vulgaris) involves unique developmental programs. To understand the underlying genetic and molecular architecture of this trait, we carried out a comparative transcriptomic analysis between a wild (PHA1037) and a domesticated accession (PHA0595) with high and reduced fiber deposition, respectively. We analyzed three different pod tissues (dorsal suture, ventral suture, valves) at three developmental stages (10, 20, 30 days post-anthesis). Distinct gene expression patterns were identified in PHA0595 compared to PHA1037. In dorsal suture, Trihelix transcription factors (TFs) and members of Ethylene Response Factor (ERF) family were up- and down-regulated, respectively, at 10 days. By day 30, Lateral Organ Boundaries Domain (LBD) TFs were over- under-expressed, while Arabidopsis Response Regulator B (ARR-B) and Calmodulin Binding Transcription Activator (CAMTA) families were under-expressed. In ventral suture, basic Helix-Loop-Helix (bHLH) and Squamosa Promoter Binding Protein (SBP) families were increased in PHA0595 at day 10, whereas ERF and WRKY TFs were significantly reduced by day 30. In valves, ERF and Heat Shock Factor (HSF) members were over-expressed, and NAC proteins and YABBY TFs were under-expressed at day 10, whereas SBP and Homeodomain-leucine zipper protein (HD-ZIP) families were over- and under-expressed, respectively, at day 30. DEGs also included enzymes involved in metabolic pathways (phenylpropanoid, poly-hydroxy fatty acids and flavonoid biosynthesis), which may be potentially crucial for fiber formation. This study offers insight into the molecular dynamics governing pod fiber development, the role of specific TFs and metabolism enzymes. Our findings pave the way for the genetic improvement of common bean, specifically targeting fiber content reduction.

Bibliography

- *Keywords: Phaseolus vulgaris; transcriptomics; fiber development; gene expression; transcription factors; enzymes; bean improvement; metabolic pathways*
- *PDI2020-114115-RB-100 BEANFLOW (MCIN/AEI) supported this research. The authors thank to Xunta de Galicia (www.genecrop.es) and the CSIC Open Access. RL thanks to Junta de Andalucía (DOC_01129).*

606/202. INCREASING THE CULTIVABILITY OF LUPINUS LUTEUS IN GERMANY BY COMBINATION OF CLASSICAL AND INNOVATIVE BREEDING APPROACHES - ANTHRACNOSE RESISTANCE AND YIELD POTENTIAL

Authors:

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Work centre:

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Summary:

Objectives

Lupins have a tradition as a protein plant in Germany. Beside narrow leafed lupin (L.angustifolius) and white lupin (L. albus) found in Germanys agricultural landscape, the yellow lupin (Lupinus luteus L.) is a promising legume that is well adapted to poor soils, has good drought tolerance and the highest seed protein content of all old world lupins. Nevertheless, due to its high susceptibility to the fungal disease anthracnose and its low yield capacity, the yellow lupin has almost completely disappeared from the fields in Germany and breeding activities have discontinued.

Concise description of the work (materials & methods)

Our research activities have been focused on improving these essential traits in order to initiate the return of the yellow lupin. Examining the diverse plant genetic resources of yellow lupin for anthracnose resistance led to a Polish variety with a substantial degree of anthracnose resistance under our field conditions. In order to investigate genetic inheritance, we

have created three independent mapping populations. The F2 and F3 progenies were subjected to an established greenhouse resistance test and were genotyped by using different approaches.

Main Results

The results of phenotyping revealed a 3:1 split ratio between resistant and susceptible plants, which indicated a monogenic dominant inheritance of the novel resistance. F3 progeny tests confirmed the result and allowed the differentiation of homo- and heterozygous resistant genotypes. The resistance locus was designated Llur and originates from a different genetic resource than the previously postulated Llur1 (Lichtin et al. 2020) or Lanr1 and LanrBo (Fischer et al. 2015) known from NLL. Genome data, differential transcriptome analysis as well as public databases were used for map construction of Llur. Differentially expressed gene analysis (DEG) by RNA-Seq allowed first insights into the functional regulation of anthracnose resistance in Lupinus luteus. Genome data from L. angustifolius enabled an in silico mapping of Llur on LG 18 of L. luteus, which is not homologous to Lanr1 and LanrBo resistance loci. A marker bracket with moderately linked markers is available for marker assisted breeding (MAS) of Llur. In a second step, a forward genetic approach based on Llur resistant plants resulted in genotypes showing novel growth types with high yield potential (Fig.1). After several generations of selfing, field tests were conducted at two distinct locations. As a result, we have identified M5 and M6 lines with significantly higher yields compared to the resistance donor and former cultivars from L. luteus. Astonishingly, the yield level of novel M- lines is comparable to that of the current cultivars of L. angustifolius. Considering the high protein content of the seeds, L. luteus promises to become an additional valuable protein resource for food and feed in Europe.

Figure. Identification of novel growth types after EMS-mutagenesis of non-branched cv. 'Taper'. Seed treatment of 5000 seeds of non-branched determined cv. Taper leads to novel branching types in M2 generation. Propagation of stable new growth types resulted in promising high-yielding lines that provide high protein yields with consistently early maturing.

Conclusions

Within the scope of this research work, an effective resistance to anthracnose in yellow lupin was genetic and molecular characterized. Development of molecular markers based on genomic and transcriptomic sequence data. Combining resistance with novel high-yielding growth types set the basis for the return of yellow lupin.

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606/211. MAPPING OF DROUGHT TOLERANCE RELATED QTLs AND GENE EXPRESSION ANALYSIS IN LENTIL (Lens culinaris MEDIK.)

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Drought stress is one of the main abiotic stresses capable of negative affecting growth and yield of the crops, including lentil. The main objective of this study has been to find and analyze different genes associated with drought stress responses in lentil, in special those candidates related to tolerance to lack of water at germination and seedling state. Seeds of Lens culinaris cv. Alpo, Lens odemensis ILWL235 and 85 RILs F7 obtained from the cross of these two species were subjected to drought stress conditions, simulated by using a 15 % polyethylene glycol 6000 solution. The QTLs related with the trait "relative percentage of seed germination" in comparison with the controls were located in the reference map constructed from the same RIL population by Polanco et al. (2019). The results showed two significant QTLs mapping in the linkage group related with the lentil chromosome 4, presenting an epistatic interaction, and explaining 40 % of the total variance. Additionally, we analyzed the two parental genotypes for the expression changes of several genes in roots and leaves described as partially responsible for tolerance by Singh et al. (2017), to assess if they could be used as biomarkers to predict the level of resistance to this stress. The data obtained have shown differences with those expected according to the available bibliographic data. These results could be due to the existence of different responses depending on the genotypes and the plasticity of the changes in the transcriptome in different tissues in the face of to cope with water deficit.

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606/212. TRANSLATIONAL RESEARCH OPPORTUNITIES IN GRAIN LEGUMES: WHERE ARE WE?

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Summary:

Objectives, Description, Main Results & Conclusions

Researchers are under constant pressure to provide solutions to meet the challenges of a growing world population and advance One Health. Translational biology can play an important role in achieving these goals by transforming basic research discoveries into practice quickly and efficiently. In plants, basic science can indeed be translated into methods to develop and improve crop varieties and thus achieve food security in a sustainable and safe way. Grain legumes are an important source of protein for animal and human nutrition. They are part of a large plant family with several, closely-related, agronomically-important crops. As the demand for plant-based protein continues to grow, more land is being devoted to these crops, but many environmental influences still constrain their productivity. Improving the response of grain legumes to limiting factors is key to ensuring the stability of yield and grain quality. Here, we report the development of OrthoLegKB, a knowledge graph database for grain legumes. The framework relies heavily on orthology and synteny relationships to build bridges between species, and transfer and/or compare information between them. Users can integrate published and unpublished data related to their species of interest including genetic, genomic and transcriptomic information. Heterogeneous datasets from multiple species can be accessed and explored with quasi-instantaneous query times, allowing relevant biological questions to be addressed, hypotheses to be generated, and information from a single or group of species to be transferred to others. OrthoLegKB is a significant step towards translational approaches in grain legumes and a great tool for research and breeding. The number of legume species will be constantly increased. Future developments to accommodate pangenomic datasets as well as proteomic and metabolomic information will further enrich the query capabilities.

606/217. IMPROVING LEGUME PERFORMANCE FOR MORE SUSTAINABLE AGRICULTURAL PRACTICES: FUL AND AP2 GENES EFFECT ON POD YIELD AND ROOT DEVELOPMENT

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes are valued crops with highly interesting sustainability traits.The ability to fix nitrogen in the soil through nodulation is useful to reduce the use of nitrogen-based fertilizers that have a tremendous negative impact on the environment. Legumes are also a major source of high-quality protein, reducing the need for animal-derived protein for human consumption, as well as for animal feed. In addition, legumes are a basic component of the mediterranean diet, intrinsically embedded in our cultural heritage, and a wealth of landraces and local varieties are available, which constitute a huge reservoir of biodiversity still to be fully exploited and developed. However, nowadays the legumes are not the crop of choice for most farmers because of reasons such as their low and unstable yield. Some factors that limit legume yield are biotic and abiotic stresses, the little improvement of farming practices and a low effort in plant breeding. Therefore, it is necessary to generate knowledge that could support the development of more resilient varieties. We are studying the function and the biotechnological potential of pea (Pisum sativum) PsFUL genes, key controllers of the length of the reproductive phase*, and their putative role in root development. Loss-of-function mutants in PsFUL genes show an extended reproductive phase, with many more flowering nodes produced before inflorescence growth gets arrested, meaning a significant increase in pod/seed production per plant, which leads to up to 60-80% more yield, with no observable changes in seed composition and quality. Additionally, psful mutants show a dramatic increase in root development, a trait that might have a potential positive effect in water usage and drought resistance.

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606/218. TRANSCRIPTOMICS OF WILD LENTILS IN ASCOCHYTA BLIGHT EARLY RESPONSE

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Summary:

Objectives

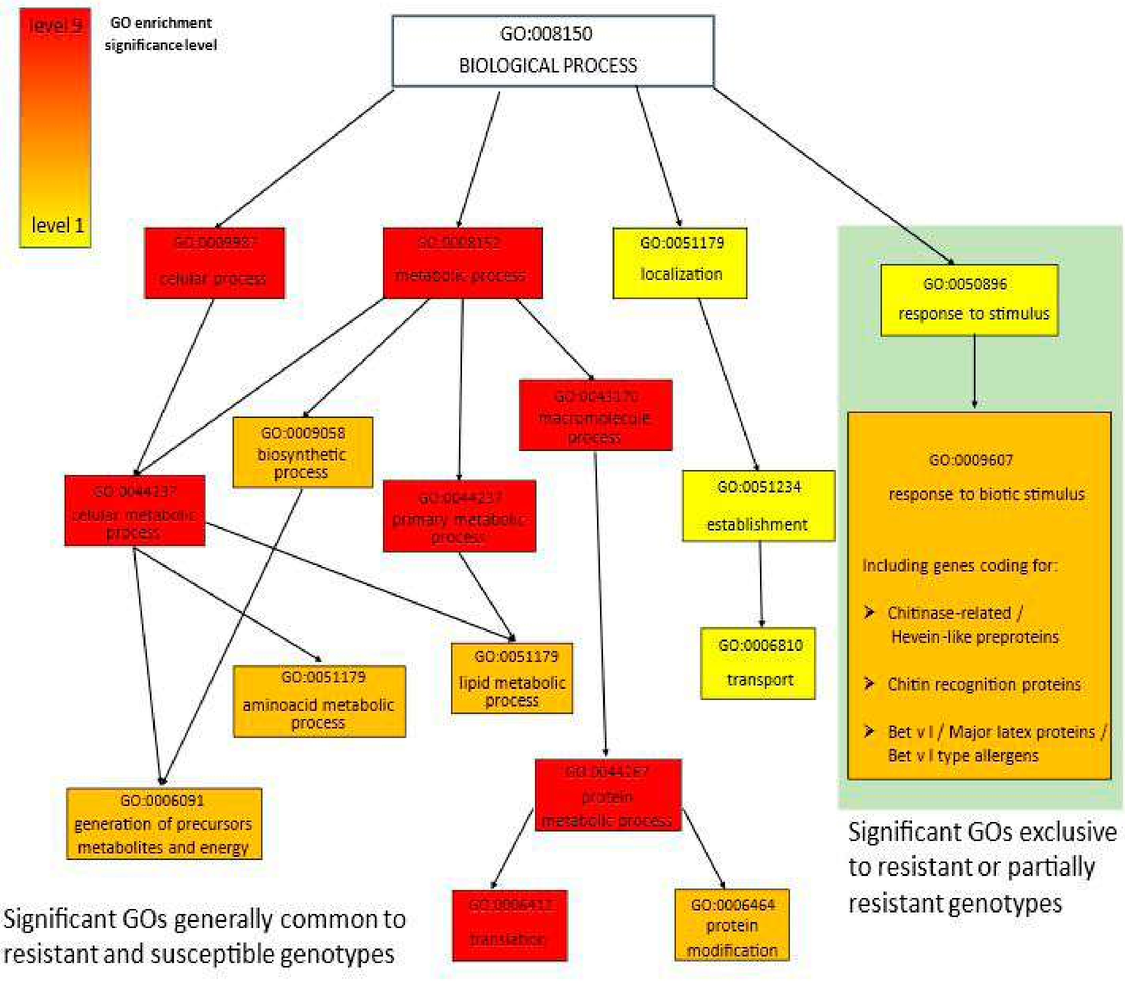
The preferred cultivation of certain elite varieties by main producers has led to a decrease in lentil genetic diversity available to growers. Consequently, one of the approaches to obtain new resistant varieties is to exploit other gene pools. The lentil's wild relatives L. orientalis, L. tomentosus, L. odemensis, L. lamottei, L. ervoides, and L. nigricans could be reservoirs of many desirable traits including Ascochyta resistance that are worth assessing.

Concise description of the work (materials & methods)

We have recently published the first draft of the lentil pan-transcriptome evaluating a commercial cultivar of the cultivated species and accessions of the six wild species close to it (Gutierrez-Gonzalez et al., 2022), and we have ranked the resistance to Ascochyta of these accessions by infecting 15-day-old seedlings. From the material extracted 24 hours after treatment in three replicates of infected plants and control plants, the RNAseq methodology has been employed to carry out both a study of differential expression analysis in the seven species and the corresponding functional analysis of gene ontology.

Main Results

In all species, genes showing differential expression (both up and down regulation) after infection were detected. A pairwise comparison of genes exhibiting a similar expression pattern showed genotype-dependent responses to infection. The functional analysis of differentially-expressed genes indicated that both resistant and susceptible genotypes have a significant enrichment in certain biological processes including protein modifications or kinase activity. However, only up-regulated genes in the resistant genotypes had a significant enrichment in categories related to biotic stimuli and defense response to fungi (Figure). Among them, genes related to the recognition of fungal elicitors such as chitin and proteins related to pathogenesis are candidate genes in the resistance response to Ascochyta and will be studied in greater detail.



Significant GO term enrichment in "biological process" category.

Conclusions

This information can be useful in the identification of promising genotypes for lentil breeding programs looking for Ascochyta resistance.

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606/233. EXPLOITATION OF PLANT GENETIC RESOURCES FOR SUSTAINABLE SWEETNESS OF LUPINUS ANGUSTIFOLIUS

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Summary:

Objectives, Description, Main Results & Conclusions

Narrow-leaved lupin (NLL) (L. angustifolius) is a protein crop with a wide range of applications in the feed and food industry, although its use is limited by certain thresholds for alkaloid content (0.05 % for feed, 0.02 % for food) in the seeds. Due to the genetic bottleneck that has occurred during the selection of sweet NLL varieties in the 1930s, it is necessary to increase the genetic diversity in current breeding-material. In order to overcome this bottleneck, a wide range of sweet and bitter varieties, gene-bank accessions, mutant-lines and landraces of different or-

igins have been compiled. Potentially interesting results are expected from the mutant-lines, which showed low alkaloid content in several environments. In addition, they did not test positive for the widespread iucundus locus.. The main objective of this project is to develop environmentally stable NLL lines for high yield and low alkaloid content. To achieve this goal, GWAS with alkaloid rich and poor varieties, development of markers for the performance of marker-assisted selection and linkage analysis, genetic and functional characterization of alkaloid biosynthesis by transcription analysis, genetic analysis with segregating populations and pyramidization of gene variants for low alkaloid content will be conducted. Furthermore, the quantity and quality of alkaloids in leaves and pods will be measured by GC-MS and FID. With potentially new sources of genes and mutations for low alkaloid content in actual unused plant genetic resources, we hope to improve future breeding programs and secure the application of NLL in the feed and food industry.

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606/256. ELUCIDATION OF GENETIC REGULATION OF VICINE AND CONVICINE IN FABA BEAN

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean is a valuable grain legume widely used for human and animal consumption. However, the presence of vicine and convicine (v-c) in the seeds reduces the nutritional value of faba bean protein. To enhance its efficient use for food and feed, it is crucial to reduce or eliminate these compounds. Recently, it was reported that the gene VC1 encodes a bi-functional RIBA 1 protein catalyzing the initial step in the vicine-convicine biosynthetic pathway, and a 2 bp inactivating insertion in this gene leads to loss of function in low vicine-convicine cultivars. However, since this mutation did not eliminate vicine-convicine completely, involvement of other genes or gene copies remains unclear. The aim of this study was to dissect the genetic regulation of vicine-convicine contents and to identify active RIBA 1 genes and functional polymorphisms underlying vicine-convicine variations in faba bean. Comparative analysis showed that at least 3 genes in the reference genome assemblies of the cultivars Hedin (high v-c) and Tiffany (low v-c) shared over 99% identity to VC1. Two variants, VC1a and VC1b, were differentiated by a major structural variant with the absence of intron 3 in VC1b. Additionally, we identified another locus with 60% identity to VC1, here referred to 'vc2'. Expression analysis revealed the expression of only VC1a and vc2, suggesting that the deletion in VC1b is necessary for transcription. Cumulative expression of VC1 and vc2 in seeds was six-fold higher in cultivars with high v-c content. Furthermore, VC1 copy number was found to correlate negatively (R =-0.36) with gene expression and phenotype. As described previously, cDNA sequencing confirmed the presence of a 2bp inactivating insertion in VC1a, resulting in a truncated protein found in all low vicine-convicine cultivars. These results conclusively demonstrate that all VC1 copies are inactive in low v-c cultivars, where vc2 appears to be the only functional RIBA 1 gene. Based on these results, we developed efficient KASP markers for VC1 as a basis for improved selection of low vicine-convicine faba bean.

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606/260. PHENOTYPIC AND GENOMIC-BASED GENETIC DIVERSITY ANALYSES IDENTIFY USEFUL ACCESSIONS FOR THE IMPROVEMENT OF PROTEIN CONTENT IN NORWEGIAN CULTIVATED PEA (Pisum sativum L)

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Summary:

Objectives: Concise description of the work (materials & methods)

Enhancing yield and protein content, together with standing ability are the main breeding goals for pea (Pisum sativum L) in Norway to improve its production and quality for feed and food industries. The identification of pea varieties with broad adaptation is likely the best breeding strategy for cultivation under the Norwegian climate. The aim of this work was to characterize 43 different pea varieties and breeding lines of European origin in 3 field trials located in the South-East of Norway, and to identify those which performed best across environments. The plant material underwent genotyping using the GenoPea 13.2 K SNP Array chip. To integrate the information on genetic diversity based on phenotypic traits with that at the genomic level pattern analyses (PA) of phenotypic traits (yield, YLD; thousand kernel weight, TKW and percent protein content on DM, PRT) was compared with clustering based on discriminant analyses of principal components (DAPC) of SNPs col. PA combines cluster analysis of an entry by multiple traits matrix and principal component analyses of the same matrix. Here best linear unbiased predictors (BLUPs) for the phenotypic traits were calculated to summarize the performance of accessions across multiple environments, and this BLUPs matrix was used as input in PA. BLUPs were estimated with the following equation: Y_ijm=M+g_i+l_j+ (gl)□_ij+b_jm+ε_ijm; where Yijm is the value of a trait measured in population i in replicate m at location j; M is the overall mean; g_i is the random effect of population i, N(0, σ_g^2); lj is the fixed effect of location j; bjm is the random effect of replicate m within location j N(0, σ_b^2); (gl)ij is the random effect of the interaction between population i and location j, N(0,σ_gl^2); and ε_ijm is the residual effect for population i in replicate m at location j, N(0, σ_ε^2). The range of BLUPs for YLD, TKW and PRT were 427.5-630.6 kg/da, 222.5-350.4 g and 20.83-24.55%, respectively. PA and DAPC identified four and three different variety groups based on phenotypic traits and allele frequencies, respectively. Clustering based on PA assigned accessions with the best performance across locations in terms of YLD and TKW to the same group (2, figure A), however, the three accessions with the highest PRT belong to a different group (3, figure A). These same 3 accessions were found to be highly genetically diverse from the best performing accessions and were clustered to a different group by DAPC (figure B). These accessions were all bred in eastern Europe and belong to a different gene pool than the best performing ones which have Nordic European origin. These results suggest that accessions with eastern European genetic background can be utilized to further improve the protein content of pea accessions adapted to the Norwegian climate. The inclusion of a wider genetic diversity within a breeding program for the selection of accessions with broad adaptation is to be suggested, especially for minor crops with limited economic resources.

Main Results

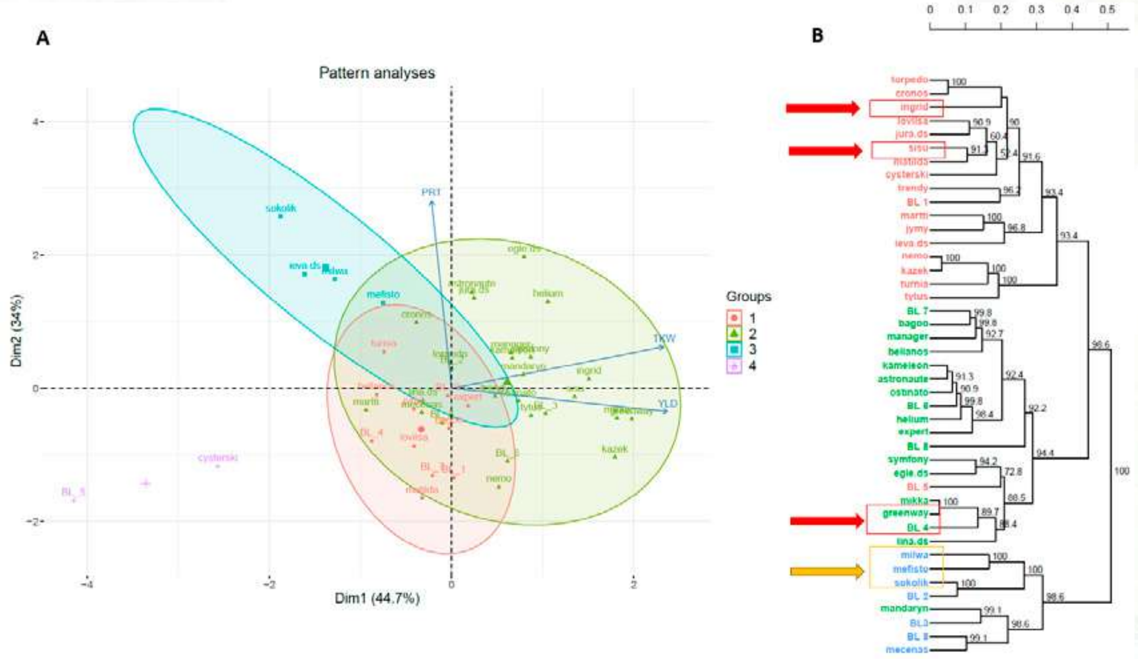

Figure. Results of pattern analysis (PA) of phenotypic traits and of discriminant analyses of principal components (DAPC) of SNPs col. (A) PA was conducted on BLUPs from entry by phenotypic traits (yield, YLD; thousand kernel weight, TKW and protein content, PRT). Accessions were assigned to four different groups highlighted by different colors. (B) Cluster analyses of DAPC scores based on SNP separated the 43 accessions in three different groups. The yellow and red boxes highlight accessions with high PRT belonging to the eastern European gene pool and the best performing varieties across environments in terms of YLD and TKW, respectively.

606/39. ACCELERATING LENTIL BREEDING: THE EFFICIENCY OF A MODIFIED SINGLE SEED DESCENT BREEDING METHOD UNDER AN EXTENDED PHOTOPERIOD-BASED SPEED BREEDING

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Summary:

Objectives

Lentil productivity is affected by several biotic and abiotic stresses becoming more severe due to climate change in recent decades. In arid and semi-arid regions drought and heat stress are the major constraints that reduce lentil yield and productivity, which require innovative and sustainable management strategies (Zeroual et al., 2022). Plant breeding for desirable traits is a long and tedious process, which often requires several years of generation advancement after crossing before obtaining homozygote fixed lines and starting field evaluation. Speed breeding, a technique for accelerated plant selection, can help speed up this process by reducing generation time and increasing the number of generations per year thus increasing genetic gain (Idrissi, 2020; Mitache et al., 2023).

Concise description of the work (materials & methods)

In this study, we evaluated the use of extended photoperiod in speed breeding to accelerate the generation turnover in lentil segregating populations from different crosses between selected parents with desirable traits. We compared the results obtained using this technique to those obtained with the traditional plant selection technique. A modified single-seed descent method was used to advance generations.

Main Results

The use of extended photoperiod significantly reduced the generation time for the tested lentil populations. We found that the generation time (F2-F3; F3-F4, F4-F5, and F5-F6) was more than 150 days after sowing in the greenhouse while it was only around 63 days in the speed breeding growth chamber (Figure 1). Furthermore, it should be pointed out that this method allowed the production of several F6 populations that were introduced to field observation nurseries as new genetic material with internal crosses designed and generation advancement as an alternative to the systematic introduction of international nurseries from international research centres. Thus enhancing the efficiency of breeding for specific objectives.

Figure 1: Speed breeding growth chamber

Conclusions

In conclusion, this study showed that the use of a modified single seed descent method under extended photoperiod is an efficient speed breeding method to accelerate lentil generations, select desirable traits and efficiently enhance genetic variability among the genetic material under a selection of the breeding program. By reducing the generation time and increasing the number of generations per year, this technique can help to speed up the plant selection process significantly. This approach can be useful for plant breeding programs to improve productivity, disease resistance, or crop quality.

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606/45. BULK SELECTION AND EVOLUTIONARY POPULATIONS AS LOW-COST BREEDING STRATEGIES TO COPE WITH INCREASING CLIMATE VARIATION: A FORMAL ASSESSMENT FOR PEA IN DIFFERENT TARGET REGIONS

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Summary:

Objectives

Grain legume breeders are challenged by increasing year-to-year climate variation and low budgets due to limited cultivar market value. Preliminary bulk selection, which exploits natural or mass selection of segregating material in a target environment across several generations, may represent a low-cost alternative to single-seed descent (SSD) for generation of inbred lines. Bulk selection could also be used to select evolutionary populations (EPs), which can be marketed for EU organic systems and may display wider adaptability than ordinary pure line cultivars. This study aimed to compare selection of SSD-derived lines vs. selection of bulk-derived lines vs. selection of EPs generated from the same genetic base (3 connected crosses among elite cultivars).

Concise description of the work (materials & methods)

The comparison was based on actual yield gains achieved under autumn sowing in each of 3 climatically different regions, i.e., inland Morocco (mild winter; severe terminal drought), Algeria (mild winter; moderate drought) and northern Italy (cold winter; moisture-favourable). Three cycles of stratified mass selection applied onto F2 to F4 plants of each cross under managed severe drought (in a rain-out sheltered environment) produced bulk-derived lines and the initial selection of EP material for Morocco and Algeria; bulk-derived and SSD-derived lines and one EP pooling bulked seed from the 3 crosses underwent further selection in each country (using 3 selection environments per country). For northern Italy, bulk-derived lines were obtained from 3 years of natural selection and a fourth year of mass selection, whereas EP material was obtained from 4 years of natural selection, followed by selection of bulk-derived and SSD-derived lines and natural selection of one EP pooling bulked seed from the 3 crosses (using 3 selection environments). We assessed the grain yield advantage over the higher-yielding parent of the 20% top-yielding bulk-derived lines issued from drought-prone or cold-prone environments (testing 30 lines per cross for each target region) and SSD-derived lines (testing 60 lines per cross) in 5 environments encompassing the overall relevant climate variability, i.e., the managed severe drought environment used for bulk selection, inland Morocco, coastal Algeria, northern Italy, and an additional environment of central Italy (moderately cold- and drought-prone).

Main Results

The best bulk-derived lines for each target environment displayed 4% to 14% greater mean yield, and at least 5.5% greater genetic gain, than the best SSD-derived lines, with relatively greater advantage in the environments featuring greater levels of the stress targeted by bulk selection. The impact of agroecological adaptation was confirmed by nil or negative gains exhibited by best bulk-derived lines tested in their non-target environment (severe drought for cold tolerance-selected lines; northern Italy for drought-tolerance selected lines). Following the region-specific, multi-environment selection of bulk-derived and SSD-derived lines and EPs, we compared the top-yielding bulk-derived line and SSD-derived line and the EP selected for Morocco and Algeria in these regions and in non-target environments represented by central Italy or intercropping with barley. The three materials did not show marked yield differences in their respective target regions; however, EPs displayed wider adaptability, as they tended to outyield bulk-derived or SSD-derived lines in any non-target environment (Morocco, for material selected in Algeria; Algeria,

for material selected in Morocco; Perugia; intercropping; Table 1). The comparison of material selected in northern Italy across 6 environments (organic or conventional, in pure stand or intercropping) revealed comparable mean yield of the top-yielding BS-derived line and the EP (1.775 vs. 1.765 t/ha) and somewhat lower yield of the top-yielding SSD-derived line (1.580 t/ha).

		Pure stand				Intercropping	
Material	Selection	Marchouch	Alger	Perugia	Mean	Marchouch	Alger
Top line from BS	Morocco	0.438	1.876	1.028	1.114	0.108	
Top line from SSD	Marocco	0.627	1.597	1.326	1.183	0.156	
Evolutionary population	Marocco	0.422	2.485	2.083	1.664	0.196	
Top line from BS	Algeria	0.365	1.773	1.511	1.216		0.552
Top line from SSD	Algeria	0.324	1.734	1.794	1.284		0.950
Evolutionary population	Algeria	0.471	1.735	1.970	1.392		1.236
LSD (P<0.05)		0.224	0.682	0.641	0.355	0.058	0.504

Table 1. Grain yield (t/ha) in pure stand or intercropping with barley in Marchouch (inland Morocco), Alger and Perugia (central Italy) of the top-yielding line issued from bulk selection (BS) or single-seed descent (SSD) and the evolutionary population selected in Morocco or Algeria

Conclusions

Selecting EPs has special interest for small breeding programs targeting organic or highly variable environments, or for cultivar introduction in informal seed systems of developing countries. The exploitation of bulk-derived lines has large interest, and could integrate genomic selection.

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606/48. CLIMATE-FRIENDLY PEA AND SOYBEAN BREEDING FOR WESTERN CANADA

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Summary:

Objectives, Description, Main Results & Conclusions

For pea to remain a key component of western Canadian crop rotations, continuous research efforts must be made in improving the crop, i.e., improving biological nitrogen fixation ability, root health, biotic and abiotic stress resistance, end-use quality, and ultimately grain yield. We have developed pea lines with improved nitrogen fixation traits based on crosses with nodulation mutants. We are exploring marker-trait associations for nitrogen acquisition traits in a Genome Wide Association study (GWAS) mapping panel. Our goal is to deploy these markers in pea breeding. Successful nitrogen fixation relies on a healthy root system, thus we are developing pea varieties with improved root rot resistance. Previously reported alleles and new alleles related to resistance to Aphanomyces euteiches, Fusarium avenaceum, and Fusarium solani will be pyramided. We have identified markers for traits associated with heat tolerance and seed protein concentration in pea and are deploying these in breeding. We are also exploring the GWAS panel to identify markers for water use efficiency. All of these efforts are aimed at improving the resilience of the pea crop to the warming climate. As nitrogen fixing crop alternatives are of major importance globally, soybean is an interesting option for Saskatchewan and Alberta where it is not grown to a large extent yet. We are developing varieties well adapted to this frontier production region, with emphasis on early maturity, high grain yield, and high seed protein concentration using a combination of conventional and molecular breeding approaches.

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606/66. LEGUME CROP DEVELOPMENT CONSIDERATIONS FOR PLANT-BASED MEAT

Authors:

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Work centre:

(1) The Good Food Institute, Washington, DC, USA

Summary:

Objectives, Description, Main Results & Conclusions

Concerns about sustainability, food security, and the public health impacts of industrial animal agriculture have spurred a sense of urgency to develop plant-based meats that appeal to mainstream consumers rather than niche markets. This involves crafting products that mimic or improve upon the taste, texture, nutrition, and overall experience of animal-based meat.

Because plant proteins serve as the primary raw material for plant-based meat, optimising their crop sources results in less costly and time-consuming downstream processing. The more closely tailored a raw plant protein is for plant-based meat, the less effort is needed to achieve the desired functional and sensory aspects through subsequent steps in the process, such as flavouring, formulation, and mechanical production. However, conventional plant protein sources such as soy and wheat have been optimised for oil and starch production and are used simply because they exist in abundance as side streams of other processes.

Given their rich genetic diversity and widespread domestication, legumes are critical for the breeding of new crops with excellent protein quality and functionality that can provide a healthy and sustainable protein source tailored for plant-based meat. This talk will present an overview of the challenges and opportunities this presents, highlighting key technical hurdles which need to be addressed for plant-based meat to reach widespread consumer acceptance.

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606/110. MODELLING DEPLOYMENT OF 21st CENTURY BREEDING TECHNOLOGIES TO MAXIMISE GENETIC GAIN

Authors:

Garry Rosewarne[1]

Work centre:

(1) Agriculture Victoria. Horsham Vic. Australia

Summary:

Objectives, Description, Main Results & Conclusions

Breeding programs are undergoing a revolution as modern technologies such as genomic selection, high throughput phenotyping and speed-breeding become routine. These technologies offer a plethora of opportunities to re-structure breeding programs to optimise genetic gain. Improved gain should be considered in light of the breeders equation, where key components are selection intensity, accuracy and time taken to select new parents from a previous cross. Genomic selection impacts on all of these parameters. For example, culls on new lines can be based on genome estimated breeding values for traits including responses to multiple diseases, edaphic constraints, phenology, quality aspects and yield, whereas a nongenomic breeding program only select on easily observed phenotypes such as a disease or physiological traits such as phenology and architecture. Genomics can therefore offer large increases in selection intensity. This technology also has a profound effect on the time taken to select new parents from a previous cross. Conventionally, this takes anywhere between 7-10 years, but can be reduced to as little as 6 months with genomic information. Speed breeding is based on a shorted life cycle of a plant and our experience using increased day length and warm temperatures has resulted in a lifecycle of only 2 months. This reduces the time taken to develop fixed lines by 75%. We have also developed a continuous crossing strategy by combining speed breeding with genomics to undertake 3 crossing cycles per year. Finally, high throughput phenotyping improves the accuracy and magnitude of trait observations, as well as assessing additional traits such as biomass accumulation and canopy height. This paper models the impact of these technologies and outlines their optimal application to improve genetic gain on a cost basis.

606/123. INFRARED THERMAL IMAGING TO EVALUATE LENTIL WILD GENOTYPES FOR DROUGHT TOLERANCE

Authors:

Navya Beera[1], Dorin Gupta[1], Rebecca Ford[3], Garry Rosewarne[1], Prabhakaran Thanjavur Sambasivam[1]

Work centre:

(1) Australia

Summary:

Objectives, Description, Main Results & Conclusions

Water limitation is a critical factor during the reproductive phase, that often-affecting pulse crop sustainability.Lentil is one of the important pulse crops in Australia and consistently encounters terminal moisture stress that leads to lesser yields.Lentil wild germplasm is a vital source of variability and novel alleles, as most are native to areas that experience frequent droughts. Thus, evaluating drought tolerance mechanisms in the available wild germplasm is vital to identify genotypes that sustain frequent drought intervals during crop growth.The present study was designed to evaluate 35 genotypes from Lens ervoides species for drought tolerance at the reproductive phase in a controlled glasshouse facility. All the genotypes were evaluated using Infrared thermal imaging (IRTI) along with physiological and agro-morphological traits for a reliable selection of drought-tolerant genotypes. We identified 43% of the total screened genotypes showing better adaptation to water-limiting environments based on their overall performance during the drought stress treatment. Interestingly, the better-performing genotypes maintained higher relative water content (60%-20%) at the end of drought stress treatment. Also, these genotypes indicated a lower canopy temperature (Tc:17ºC-20ºC) and crop water stress index (CWSI:0.2-0.6) after drought stress treatment. All these genotypes show positive canopy temperature depression (CTD). Similarly, these results resemble better transpiration efficiency under drought stress and are consistent with Linda& Albert(2017), who also reported that Lens ervoides has a superior performance under drought stress with good transpiration efficiency.Even though few genotypes in germplasm have exhibited drought-escaping mechanisms by early flowering, drought tolerance has been a significant observation with most of the genotypes in Lens ervoides.The current screening experiment also signifies that drought tolerance mechanisms vary within the species, and it depends on the centre of origin that it comes from as well as the genetic constitution of the plant.

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606/203. TOWARDS RELIABLE, INFORMATIVE, COST-EFFECTIVE GENOTYPING TOOLS FOR THE TOOLBOX OF GRAIN LEGUME BREEDERS

Authors:

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Work centre:

(1) Agroécologie, INRAE, Institut Agro, University of Bourgogne, University of Bourgogne Franche-Comté. Dijon. France, (2) Biogemma. Chappes. France

Summary:

Objectives, Description, Main Results & Conclusions

Single nucleotide polymorphisms (SNPs) are the most frequent source of genetic variation in eukaryotic species. Having access to well-distributed SNPs across the genome, segregating between and within the diversity groups of each species, is key to genetic studies and applications. Recently, we took advantage of the advances in second-generation sequencing technologies and the development of bioinformatics tools to characterise genetic diversity panels in pea and faba bean, two grain legume species with large genomes (4.45Gb and 13Gb, respectively). We chose a target capture technology focusing on coding regions only because whole-genome resequencing is relatively expensive for species with large genomes and because polymorphisms capture in repetitive non-coding regions is difficult to achieve or to interpret. We generated exome-enriched genomic libraries for 240 and 248 pea and faba bean accessions, respectively. After paired-end Illumina sequencing, the reads were mapped to the latest pea and faba bean genome assemblies, allowing the discovery of approximately 2.2 and 1.75 million robust SNPs in the two panels, respectively. The development of these SNP resources paves the way for a wide range of applications requiring low, medium or high marker density. These data can be used directly to set up genome-wide association studies using the sequenced panels in both species, as has already been demonstrated for aphid resistance in pea. They can also be used for the exploitation of genetic resources and for marker- and genomic-assisted selection. Here, we show how the pea and faba bean research and breeding communities can benefit from recent developments, and we highlight the importance of common tailored genotyping tools that can be developed based on the available SNP sets. We also discuss the importance of identifying and incorporating functional markers related to traits of interest including biotic and abiotic stress resistance and seed and protein quality.

606/243. GRAIN LEGUME RE-DIVERSIFICATION THROUGH TRANSDISCIPLINARY BREEDING APPROACHES

Authors:

Sebastian Kussmann[1]

Work centre:

(1) Switzerland

Summary:

Objectives

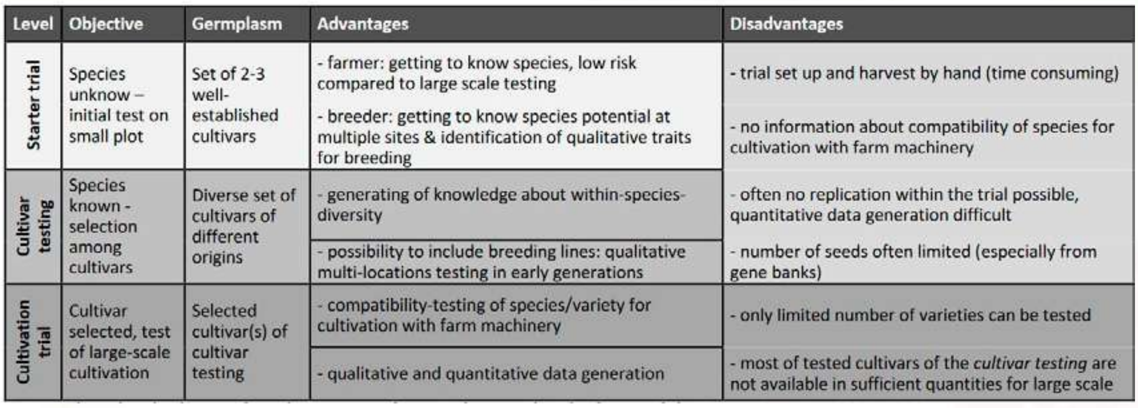
For the agroecological transition of agriculture, re-diversification of field crops is necessary (Kahane et al. 2013). In Switzerland, this especially concerns grain legumes. Locally adapted cultivars and knowledge about their cultivation in the diverse Swiss agroecosystems are often lacking, preventing farmers from cultivating them. Aim of this project is to test transdisciplinary and action-research based cultivar testing and plant breeding approaches for the re-diversification of cultivation and breeding of grain legumes in Switzerland, bringing together farmers, processors, and plant breeders. Basis of the project are methods for transdisciplinary action research and decentralized plant breeding (Ceccarelli et al. 2007, Chevalier et al. 2019).

Concise description of the work (materials & methods)

The project was started by grain legume breeders of gzpk, an independent plant breeding organisation based in Switzerland. First, a network of ten farms across Switzerland has been established in 2021. In 2022, small-scale on-farm trials were performed at each farm to assess the potential of nine grain legume species for cultivation, processing, and marketing. Each species was grown on minimum two square meters. In 2023, farm-specific trials with species pre-selected by the farmers based on the results of the trials of the previous year were conducted, with an increased number of tested cultivars per species to qualitatively evaluate the within-species diversity and compare cultivar performance. Responsibilities of the breeder were cultivar selection, organisation of seeds and preparation of templates for trial assessments, while farmers made assessments in the trial during the season and after harvest and participated in the exchange of and discussion about observations and results. A three-level scheme was developed for the realisation of the trials within three years (see figure).

Main Results

The collection of mainly qualitative data and the exchange between breeders and farmers increased the knowledge about the crop species and varieties. Farmers experienced the diversity of species and varieties, while breeders learnt about farmers priorities regarding agronomic and qualitative traits. Successfully tested varieties which were brought to Switzerland for the trials can be used by farmers for cultivation from the next season on, while at the same time the breeder characterised basic material of species in several environments as a basis for crop improvement. However, logistical, social, and scientific limitations of the approach were also recognised. Not all farms participated in the second year for various reasons and the generation of quantitative data (e.g. yield) from small-scale trials turned out to be challenging.



Level	Objective	Germplasm	Advantages	Disadvantages
Starter trial	Species unknow – initial test on small plot	Set of 2-3 well-established cultivars	- farmer: getting to know species, low risk compared to large scale testing	- trial set up and harvest by hand (time consuming)
			- breeder: getting to know species potential at multiple sites & identification of qualitative traits for breeding	- no information about compatibility of species for cultivation with farm machinery
Cultivar testing	Species known - selection among cultivars	Diverse set of cultivars of different origins	- generating of knowledge about within-species-diversity	- often no replication within the trial possible, quantitative data generation difficult
			- possibility to include breeding lines: qualitative multi-locations testing in early generations	- number of seeds often limited (especially from gene banks)
Cultivation trial	Cultivar selected, test of large-scale cultivation	Selected cultivar(s) of cultivar testing	- compatibility-testing of species/variety for cultivation with farm machinery	- only limited number of varieties can be tested
			- qualitative and quantitative data generation	- most of tested cultivars of the *cultivar testing* are not available in sufficient quantities for large scale

Figure: Three level-scheme of the participatory on-farm trial approach, tested in the frame of the integraL project 2021-2023. The figure summarizes objectives, used germplasm, and advantages and disadvantages from farmers' and breeders' perspective of the three levels of the approach. The objective of the respective level impacts the size of the trial, number of tested varieties and species, and the infrastructure used. When working with an unknown species, all three levels should be followed successively, while for known species with cultivation and/or breeding experience, each level can be chosen depending on the goal of the trial.

Conclusions

The tested approach showed after two of the three project years its potential for qualitative evaluation of cultivars on-farm and transdisciplinary identification of breeding targets. Taking its limitations into account, the method seems to be promising for testing and breeding of grain legumes as part of re-diversification processes in Switzerland. Further optimization of the communication, and the collection and analysis of data is required.

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606/245. BREEDING BY EDITING-USING GENOME EDITING TO CREATE DETERMINATE COWPEA VARIETIES

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Tal Sherman[1], Mor Friedman[2], Ester Yeshayahou[2], Mariana Aroch[2], Shira Corem[2]

Work centre:

(1) Israel, (2) BetterSeeds

Summary:

Objectives

Labor scarcity in agriculture poses significant challenges to crop production. Cowpea (Vigna unguiculata) is a vital legume crop, and its mechanical harvesting is hindered by its indeterminate growth habit. Drawing inspiration from the industrial tomato industry, where the natural mutation causing determinate growth, SELF PRUNING (SP), has revolutionized cultivation, this study aimed to utilize genome editing, specifically the CRISPR/Cas9 system, to edit the Cowpea homolog of SP and develop determinate Cowpea varieties.

Concise description of the work (materials & methods)

In this study, the CRISPR/Cas9 system was employed to target and modify Cowpea homologs of SELF PRUNING (SP). Constructs containing the CRISPR/Cas9 machinery and specific guide RNA (gRNA) targeting the Cowpea SP homologs were designed and introduced into Cowpea tissue using Agrobacterium-mediated transformation. The edited plants were screened for desired mutations using molecular techniques such as PCR and DNA sequencing. Phenotypic analysis was performed to evaluate plant height, flowering synchronization, and pod formation.

Main Results

The results of this study demonstrated successful genome editing of two homologs of Cowpea SP, resulting in the development of determinate Cowpea varieties. Several editing events were identified, resulting in small deletions of a few base pairs and up to 150 base pairs. All editing events led to the same indeterminate phenotype. The edited plants exhibited a distinct short stature and bushy growth habit, which is in contrast to the unedited control indeterminate Cowpea plants. Furthermore, flowering and pod formation were observed to be synchronized and uniform across the edited plants.

Phenotypic comparison of edited and wild-type Cowpea plants. The edited plants (right) display a shorter stature and bushy growth habit, in contrast to the indeterminate phenotype of the wild-type control plant (left).

Conclusions

Genome editing, specifically utilizing the CRISPR/Cas9 system, enabled successful modification of the Cowpea homolog of SELF PRUNING (SP), resulting in the development of determinate Cowpea plants. The edited plants exhibited a compact growth habit, which facilitates mechanical harvesting, overcoming the labor limitations faced in traditional indeterminate Cowpea cultivation. Additionally, the synchronized and uniform flowering and pod formation in the edited plants offer advantages in terms of crop management and yield prediction. The findings of this study demonstrate the potential of genome editing technologies to enhance crop productivity and address challenges associated with labor scarcity in agriculture.

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606/54. DESIGNING CLIMATE SMART LEGUMES FOR SUSTAINABLE LIVELIHOOD SYSTEMS FOR DROUGHT PRONE ECOLOGIES

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Field crops and legume cultivation are the main activities in terms of practising Agriculture in India. Germplasm, genotyping and phenotyping, combined with a clear definition of product targets, are the foundation of a successful crop breeding programme on long term basis. Breeding crop genotypes with superior yield for the climatically challenging regions of the region involves integration of multiple drought-specific technologies together with all of the other technology components that comprise a successful crop breeding programme. Characterization of genetic diversity for drought resistance and identification of novel germplasm is the first step for conventional breeding, genomics-assisted plant breeding and functional analysis of the genes involved in various pathways associated drought stress responses. Although final yield returns under drought stress is the primary trait for measurement of drought resistance in many legumes, secondary traits may be particularly suited to improving selection response to stress conditions. Desirable screening methods based on secondary traits should be established to effectively trace out target phenomes in quickest possible time. Selection for improved performance under drought based on per se yield alone has often been considered inefficient, but the use of secondary traits of adaptive value whose genetic variability increases under drought can increase selection efficiency. Root screening has proved to be effective tool in culling out poor genotypes with respect to drought. Highest positive correlation was observed between root biomass and yield of common bean lines in artificially controlled conditions. The similar trend was also observed in field trials indicating relevance of root architecture with yield performance under drought stress.

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606/65. BREEDING RESEARCH NEWS FROM THE FAMOUS GÖTTINGEN WINTER FABA BEAN

Authors:

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Work centre:

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Summary:

Objectives

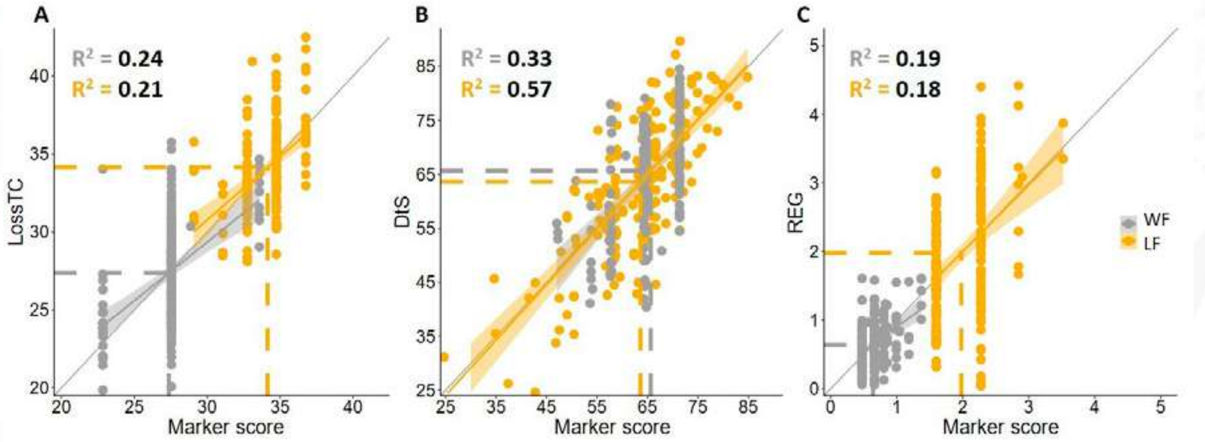
Facing climate change, an urgent breeding objective for winter crops is their adaptation to both milder winters with less predictable, strong frost spells and late-frost spells. German winter faba beans still need marked genetic improvement in overall winter hardiness and late-frost tolerance. The aim of this study was to identify QTL for winter-frost tolerance (WFT) and late-frost tolerance (LFT) in winter faba bean, and further genetically investigate these traits.

Concise description of the work (materials & methods)

We phenotyped a panel of 188 inbred lines (Association-set; A-set) as juvenile plants in two series of controlled experiments (Ali et al., 2016; Windhorst et al., 2023). Two treatments, namely winter-frost (WF) and late-frost (LF), were applied. We assessed 14 traits in both treatments, including the main freezing tolerance related sub-traits (FTRS-traits): loss of leaf turgidity and color (LossTC), dis-position to survive (DtS), and regrowth after freezing (REG). We performed genome-wide associa-tion studies (GWAS) on all traits, utilizing 60K SNP chip data. The A-set showed broad significant phenotypic and treatments specific variation.

Main Results

Correlations among the main FTRS-traits between treatments were 0.46 ≤ r ≤ 0.72. FTRS-traits re-peatability ranged 0.63 ≤ h2 ≤ 0.91. GWAS analysis for WFT revealed two, four, and three unique marker-trait associations for LossTC, DtS, and REG, respectively. For LFT, we identified three, nine, and two unique marker-trait associations for those FTRS-traits. After extensively analyzing local linkage disequilibrium structure of the respective markers, all markers were considered as repre-senting independent QTL (23 in total). Accordingly, we calculated specific marker scores for LossTC, DtS, and REG per treatment for each inbred line. The coefficient of determination (R2) between the marker scores and the phenotypic values ranged from R^2=0.194 (REG) to 0.334 (DtS) for WFT, and from R^2=0.179 (REG) to 0.569 (DtS) for LFT (Figure 1). Interestingly, none of the reported markers showed significant associations with other FTRS-traits within or across treatments. Within treat-ments, however, several markers for different FTRS-traits were located in close physically proximity on the respective chromosome. These findings suggest QTL with pleiotropic effects on multiple FTRS-traits, such as DtS and REG in the LF treatment.



Correlation between inbred line-specific marker scores and their phenotypic values per freezing-related sub-trait (FTRS-trait) and treatment. Marker scores were derived from the GWAS-estimated marker effects and the inbred line-specific marker genotypes. The coefficient of determination (R2) is given per each FTRS-trait and treatment. Vertical and horizontal dashed lines indicated means of x- and y-axis, respectively. Treatments: winter-frost, WF; late-frost, LF. FTRS-traits: loss of turgor and color (scale 8-72), LossTC; disposition to survive (0-90°), DtS; regrowth after freezing, REG (g).

Conclusions

In conclusion, the identification of 23 marker-trait associations for the FTRS-traits represents significant advancement towards marker-assisted selection (MAS) for WFT and LFT in winter faba bean breeding.

Brand new: The estimated marker effects were employed to predict the phenotypic values of a separately phenotyped winter faba bean inbred line panel. The prediction abilities, reaching up to r=0.53, substantiate the potential of our findings for marker-assisted selection (MAS); analyses are ongoing.

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606/73. POLLEN PRODUCTION, POLLEN VIABILITY AND AUTOFERTILITY IN FABA BEAN (VICIA FABA L.) AND THEIR RELATIONSHIP WITH PATERNAL SUCCESS OF FABA BEAN GENOTYPES

Authors:

Lisa Brünjes[1]

Work centre:

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Summary:

Objectives

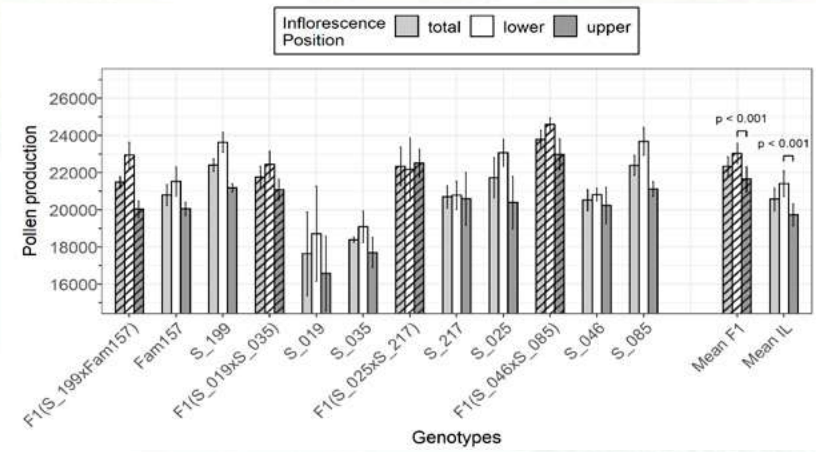
The paternal success describes the success rate of a genotype in its role as pollen donor to cross-fertilized seeds. Different paternal success between genotypes means different efficiencies to distribute the own pollen for the cross-fertilization of surrounding individuals; thereby, the paternal success of a genotype influences its genetic contribution to the next generation. When breeding synthetic cultivars of faba bean, equal contributions of genotypes to the next generation are desired to reduce inbreeding and to keep the genetic composition stable. Direct assessments of paternity are elaborate and costly, yet, were conducted in our prior experiments (Brünjes and Link 2021). Here, we studied whether components of plant fitness such as pollen production or pollen viability can be used as a proxy for paternal success. Hence, in a field and a caged outdoor pot experiment, eighteen winter faba bean genotypes (14 inbred lines, 4 F1 hybrids) were evaluated for pollen production, pollen viability and autofertility.

Concise description of the work (materials & methods)

In the field experiment, two F1 hybrids and six inbred lines were grown with twelve replicates (one individual per replicate, full randomization). In the pot experiment, plants were grown in 18 x 18 cm pots in bee-proof outdoor cabins, with four replicates (pollen production) or two replicates (all other traits) and two plants combined as one entry. To eliminate potential differences caused by a different number of flowers per plant, plants were standardized to bear two tillers and a restricted number of inflorescences and flowers. Four flower buds per tiller were harvested at one day prior to presumed anther dehiscing to ensure that all pollen grains of a flower were collected. Pollen production and pollen viability were estimated using an impedance flow cytometer (type Ampha Z30; Amphasys, Lucerne, Switzerland).

Main Results

Pollen production was between 17700 and 24900 pollen grains per flower in the pot experiment and higher at the lower, i.e. earlier inflorescences than at the upper, i.e. later inflorescences. Inbred lines differed significantly in pollen production and we found significant heterosis for this trait (Figure). Pollen production was lower in the field; the rank correlation to pot data was low and not significant. Relative pollen viability was overall high (93% to 97% in the pot experiment, 88-95% in the field experiment) and only in the field experiment, pollen of F1 hybrids was significantly more viable than pollen of inbred lines. Autofertility ranged from 0% to 98% and heterosis for this trait was pronounced and highly significant (mean of F1 hybrids: 72.9%, parental mean: 11.2%). We found no evidence for a clear relationship between autofertility and pollen production, pollen viability or paternal success. However, pollen production and pollen viability were highly correlated with paternal outcrossing success in the six inbred lines which were tested in the field experiment.



Pollen production (pollen grains per flower) of the four F1 hybrids (striped) alongside their parental inbred lines (not striped) at the total plant, at the lower inflorescences and at the upper inflorescences. Note that the four F1 hybrids are sorted by pollen production of the total plant, which is the mean of the values for pollen production at the lower and upper inflorescences. Error bars show standard error of the mean. The differences of mean values between lower and upper inflorescence position were tested with Welch's t-test.

Conclusions

When selecting inbred lines as components of a synthetic cultivar, data on pollen production and viability might be useful to choose components with small differences in their paternal successes, thereby reducing inbreeding and better exploiting heterosis. As pollen production seems to be environment-specific, genotypes should be selected in the environment where seed production with the thus-selected genotypes shall take place.

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606/93. DEVELOPMENT OF MULTIPLE STRESS-RESISTANT KABULI CHICKPEA GERMPLASM FOR DIVERSE GROWING REGIONS

Authors:

Aladdin Hamwieh[1], Tawffiq Istanbuli[1], Seid-Ahmed Kemal[1]

Work centre:

(1) Egypt

Summary:

Objectives, Description, Main Results & Conclusions

Chickpea (Cicer arietinum) is an important crop in dry areas of the world. The major production challenges of chickpea production are Ascochyta blight (AB), wilt/root rot, cold, and drought. ICARDA is mandated to develop elite germplasm resistant to key production challenges of spring and winter-planted chickpea. The wilt/root rot was assessed by recording the percentage of plants showing symptoms of wilting, while the AB was evaluated using a 1-9 disease rating scale where 1 is highly resistant, while a rating of 9 is highly susceptible. ICARDA chickpea breeding program follows the pedigree breeding method to develop multiple stress resistant/tolerant germplasm targeting demands of National Partners in South Asia, Sub-Saharan Africa, and the Mediterranean regions. In this abstract, we present the outcomes of crosses made in 2014 to develop wilt/root rot, and AB resistant chickpea genotypes. A total of 106 crosses were derived from 212 parents and F1 seeds were harvested and planted as F2. A total of 613 selected plants (F3) were evaluated in the wilt/root rot sick pilot at Terbol station, Lebanon. About 62% of progenies with < 15% mortality were retained and screened for AB. A total of 383 lines were selected AB resistant. Only 144 F5 lines were selected from the second round of wilt/root rot screening with a mortality of < 15%. Finally, 48 genotypes with multiple resistance were shared with the national partners.

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- *https://doi.org/10.1007/s10681-019-2389-2(0123456789().,-volV()0123456789().,-volV)*
- *https://doi.org/10.3390/agronomy12040850*

606/112. ASSESMENT OF PEA FLOWERING BY USING HIGH THROUGHPUT FIELD PHENOTYPING

Authors:

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Summary:

Objectives

High Throughput Field Phenotyping (HTFP) is a promising approach to support breeding by gathering a vast amount of standardized data on relevant traits in an objective manner. The Crop Science group of ETH Zürich is using their Field Phenotyping Platform (FIP) 1 since many years by conducting field experiments e.g. with wheat, soybean and since 2022 with pea. The FIP is a rope-suspended carrier system which allows to collect RGB-images of individual experimental plots (9 m2) throughout the whole vegetation period.

Flowering is one of the most important processes in a plant's life. Before flowering, the pea plant uses most of its energy to accumulate nutrients from the soil, while after flowering the focus is on generative growth and grain filling. Therefore, this switch in its phenological stage is of great interest also in breeding and variety testing. The start and duration of

flowering are traits that indicate how well adapted a variety is to a certain location2. Moreover, there are indications that flowering time might be an early trait for high protein content3, at least in soybean. However, flowering time is today visually rated by human evaluators. Our HTFP-research seeks to provide a more reliable assessment of flowering time.

Concise description of the work (materials & methods)

We will investigate the time of flowering as an early trait for protein content in pea by testing the hypothesis: "The later the flowering, the higher the protein content in the seed". To test this hypothesis, a workflow will be established to detect the timepoint of flowering on RGB images using HTFP and deep learning approaches. RGB-Images of 12 pea varieties in 36 plots were acquired with the FIP twice per week throughout flowering in 2022. These images were then used to train a deep learning model (YOLOv8), allowing to detect flowers on images (Figure 1B). In 2023, a further field experiment was conducted with a set of 50 genetically diverse pea genotypes. Additional ground truth data, by manually counting flowers in the field within a region of interest (50 x 50 cm) is being collected.

Main Results

The results from manual counting are now compared to the results from the deep learning model to validate it. In these preliminary results, more open flowers were counted manually than by the object detection model with an accuracy of R2 = 0.796 (Figure 1A).

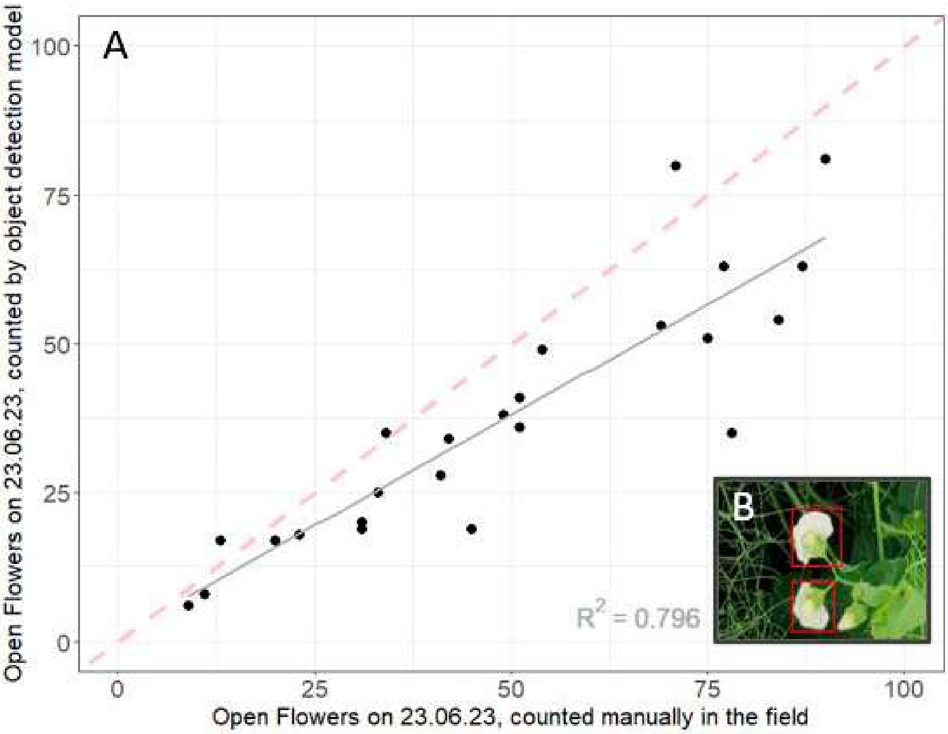

Figure 1A: Open flowers manually counted and open flowers that are recognized by the object detection model. Dashed pink line indicates 1:1. Preliminary Result. Figure 1B: Example of "open flowers" in a FIP-Image.

Conclusions

To analyze the correlation between flowering time and seed protein content, nine additional year-sites were analyzed, for which flowering time was assessed visually in 2022 and 2021. Together with the expected results from 2023, the above-mentioned hypothesis will be tested, and preliminary results will be shown in the conference. This work will help to establish a workflow to precisely detect flowering time via HTFP-images with the final aim to improve legume breeding.

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- *3. Roth, L., Barendregt, C., Bétrix, C.-A., Hund, A. & Walter, A. High-throughput field phenotyping of soybean: Spotting an ideotype. Remote Sens. Environ. 269, 112797 (2022).*

606/129. LEGUME INNOVATION: TRANSCRIPTOMIC PROFILING BASED ON RNA-SEQ ANALYSIS OF RESISTANT AND SUSCEPTIBLE VICIA ERVILIA GERMPLASM REVEALS MOLECULAR MECHANISMS INVOLVED IN THE RESPONSE TO OROBANCHE CRENATA

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

In this work we developed an RNA-Seq strategy in the grain legume Vicia ervilia Willd infected by the root parasitic plant Orobanche crenata Forsk. Taking into consideration that O. crenata is one of the most limiting factors for this crop, lines showing resistance to this parasite are of most interest as well as the knowledge of the genes involved in this resistance. To carry out the experiments, it was considered the previously selected V. ervilia material Ve125 (resistant line) and Ve123 (susceptible line) for their contrasting response against the infection of O. crenata (González-Verdejo et al., 2020). Firstly, we set up two time points of interest in the infection process for this pathosystem: t1 (20 dai) when the parasite radicle has reached the host root and has started the invasion to the host tissues and t2 (25 dai) at initial stages of the parasite tubercle development. Preliminary results showed that the total number of transcripts obtained was 93.463. Among the differentially expressed genes and in the specific comparison between the resistant and susceptible lines at t1, genes encoding for specific surface receptor proteins involved in the recognition of other parasitic plants by resistant hosts were found. These results will contribute to obtaining a deeper knowledge of molecular events underlying resistance in the V. ervilia – O. crenata pathosystem, which is the key to develop molecular markers that assist breeding programs in the species.

Dai: days after inoculation.

Acknowledgements: This work was supported by European Regional Development Fund (FEDER)-Andalusia Operational Program 2021-2027 (TRA.TRA23.007). MFA thanks the Spanish Ministry of Science and Innovation for the postdoctoral support with a Ramón y Cajal fellowship (RYC-2015-18961)

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606/131. PRECISION BREEDING FOR RED CLOVER: OPTIMIZING PHYTOESTROGENS LEVELS WITH ASSOCIATED MARKERS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Red clover, a key forage crop in temperate regions, is recognized for its phytoestrogen content, which impacts the utilization of its biomass. Phytoestrogens are plant compounds that can exhibit estrogen-like activity with physiological response not only in livestock. They are reported to possess several health benefits, including cardio protective, antidiabetic, antioxidant, anti-osteoporotic properties and mitigating postmenopausal symptoms in women (Cornara et al. 2016). However, intensive consumption of oestrogenic pasture has been shown to have negative effects on reproductive performance of livestock. Major phytoestrogens in red clover include formononetin and biochanin A. Assessing phytoestrogen content is essential for evaluating forage quality. The production of varieties with varying phytoestrogen levels is desirable, as lower levels benefit

animal hormonal rhythm, while higher levels enhance animal growth for meat production (Moorby et al. 2004). Our research emphasizes the importance of molecular tools in red clover breeding. We have identified SNP associated with phytoestrogen levels using GWAS and prepared a selection set consisting of 9 CAPs markers. These SNP markers enable breeders to selectively target DNA polymorphisms associated with specific phytoestrogen levels. The tool includes optimized PCR primers and the appropriate restriction endonuclease for detecting SNP markers related to formononetin and biochanin A content. Its implementation in a marker-assisted breeding program facilitates molecular breeding for conventional breeders. The presented markers enhance the efficiency of developing new varieties and enable the targeted selection of beneficial traits that are challenging to achieve through conventional breeding methods. Red clover breeding is complex due to its outcrossing nature, which hampers the fixation of desired alleles. Therefore, the utilization of molecular selection tools is crucial for breeders to make substantial progress in fine-tuning the content of these important red clover chemical compounds.

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606/171. ASSOCIATION ANALYSIS (GWAS) OF PEA (PISUM SATIVUM L.) AND IDENTIFICATION OF SNP MARKERS FOR GENOMIC SELECTION OF ECONOMICALLY IMPORTANT TRAITS

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Summary:

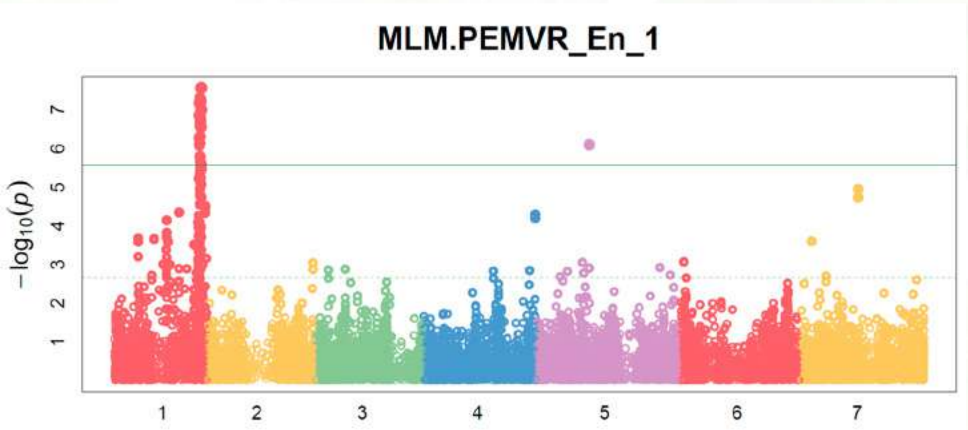
Objectives

The aim of the work was to use new breeding techniques and accelerate the development of new varieties of pea.

Concise description of the work (materials & methods)

Genome-Wide-Association Study (GWAS) was performed to identify DNA markers associated with agronomic (plant height, number of branches, leaf type, seed shape and colour, seed yield and seed weight, TSW, resistance to powdery mildew and PEMV) and seed quality (protein and starch content) traits in pea (Pisum sativum L.). Pea enation mosaic virus (PEMV) causes an important disease of peas, resulting in significant yield loss worldwide. A set of 564 accessions of field and garden pea was evaluated in 2019-2022 on three locations (Šumperk, Olomouc, Smržice). DArTseq sequencing of DNA libraries with reduced complexity was used for identification of SNP variants.

Main Results



376 SNP markers were obtained, whose position on individual chromosomes (Fig. 1) was determined based on pea reference sequence. For SNP detection, a 69 bp sequence attached to associated SNPs was provided, which can be used for any available approaches of SNP markers detection (hybridization, enzymatic, sequenation and physical SNP detection methods).

Figure 1. Manhattan plot of PEMV resistance association using MLM model. The solid green line indicates the FDR-adjusted P value of association level of 0.01, the dashed green line indicates the FDR-adjusted P value of association level of 0.05. The seven chromosomes of pea are distinguished by colour. The association level of each SNP in the form of a P value is transformed by the decadic logarithm and plotted against its position in the sowpea genome.

Conclusions

The identified SNP loci will be used to design PCR markers for marker-assisted selection to streamline and accelerate the breeding process and to generate qualitatively new, competitive field and garden pea varieties.

Acknowledgments: This work was supported by the Ministry of Agriculture of the Czech Republic via long-term development concept of research organization (RO1023).

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606/179. UNDER THE LIGHT OF NIRS: EXPLOITING MULTIVARIATE STATISTICAL METHODS TO PREDICT PROTEIN AND OIL CONTENT IN FABA BEAN SEEDS

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicia faba L.) is a promising protein crop for its high protein content and yield potential. The growing interest of the food industry in this crop is driving increased breeding investments on enhancing the quality of its seeds. Breeding for quality requires the use of high-throughput phenotyping for large-scale screening, such as Near-Infrared Spectroscopy (NIRS). Chemometric models are required to extract information from the spectrum resulting from the interaction between the Near-Infrared light and the samples [1]. This study aimed to develop and assess statistical approaches for the prediction of protein and oil content of a breeding population phenotyped trough NIRS. The predictive ability of four statistical models - Partial Least Square (PLS), Elastic Net, Memory-based Learning, and BayesB – was tested. The models were trained using a 125-sample calibration set and then evaluated on an independent 70-sample validation set. The samples were selected by the K-means and a modified Kennard-Stone algorithm, respectively. Before modeling, the raw spectra were pre-processed with Savitzky-Golay and 1st derivative filter. Due to the high costs of large calibration sets for chemical analysis, this study explored cost reduction by simulating smaller calibration sets for additional NIR calibrations. The chemical analysis performed on the calibration and validation set for protein (DUMAS method) and oil content (a hexane-based extraction) revealed a range of variation from 18.39% to 35%, and from 1.17% to 2.17%, respectively. Overall, the root mean square error (RMSE) indicated that protein content was a more predictable trait than oil content. The choice of the best statistical approach was trait specific. PLS was best method for predicting protein, while BayesB for oil content. Moreover, reducing the training set size resulted in reduced prediction accuracy as general trend. The NIR models we developed are robust and valuable tools for faba bean breeding.

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606/180. DEVELOPMENT AND CHARACTERIZATION OF AN EMS-MUTAGENIZED COMMON BEAN POPULATION AND IDENTIFICATION OF MUTANTS ALTERED IN REPRODUCTIVE DEVELOPMENT

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Universidad de Almería. Almería. Spain, (4) Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almería. Spain, (5) Genética del Desarrollo de Plantas, Misión Biológica de Galicia-CSIC, Pontevedra. Spain

Summary:

Objectives, Description, Main Results & Conclusions

Common bean (Phaseolus vulgaris) is the major food legume worldwide, making it an important target for approaches of genetic analysis. In order to provide genetic diversity for identifying important genes, an ethyl methane sulfonate (EMS) mutagenized population was generated (≈3000 M2 families). Several phenotypes with altered morphological and agronomic traits were observed from a preliminary characterization of the M2 population. This work reports the phenotypic and molecular characterization of an EMS-induced mutation that results in the complete absence of flowering under any environmental conditions. PHAM2020 mutants exhibit a perpetually vegetative state, indicating that the mutated gene is essential for the maintenance of inflorescence meristem identity. The vegetative phenotype was inherited as a monogenic recessive trait in the M2 progeny, as inferred from the 3:1 (WT: mut) segregation ratio observed. To gain insight into the molecular basis of the vegetative phenotype, we employed a mapping-by-sequencing cloning strategy to identify the mutated gene. Firstly, we obtained F2 families by self-pollinating the F1, involving plants heterozygous for the mutation. Subsequently, we classified the F2 plants into two distinct pools (WT or mut), which were Illumina sequenced and mapped against the reference genome sequence. Based on the allele frequencies, a tight candidate interval on chromosome 2 was defined, in which searched for mutations that could explain the observed phenotype. Finally, five candidate genes were chosen based on their location, function and type of mutation. To pinpoint the specific mutation, we devised tailored markers for each candidate gene and analyzed their segregation patterns. The results indicate that this EMS population represents a useful resource for the common bean research community, which will provide novel allelic diversity for improvement and functional genomics.

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- *Keywords: common bean; EMS; mutagenesis; mapping-by-sequencing.*

606/189. IDENTIFICATION OF A PRIORI INBRED INDIVIDUALS IN FABA BEAN (VICIA FABA L.) POPULATIONS AND PREDICTION OF THEIR GENETIC VALUE FOR BREEDING

Authors:

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Work centre:

(1) Georg-August-Universität Göttingen

Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicia faba L.) is a partially allogamous grain legume, standing out due to its relatively high seed protein content, its high symbiotic performance and its climatic adaptation. However, its acreage in Germany is relatively low, partly because of yield instability and high seed cost.

Faba bean breeding research recently experienced great progress thanks to the availability of SNP chips, the sequencing of its genome and analyses on the global diversity of the species, allowing the introduction of new breeding approaches.

In this context, the project 'Abo-Direkt' aims to invent, develop, test and implement methodological improvements in faba bean breeding. It especially focuses on the development of a cost-efficient and fast method for the identification of a priori existing inbred individuals in faba bean populations, and the estimation of their genetic value through genomic prediction, to ultimately optimize breeding.

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606/197. MODERNIZING FABA BEAN FOR MAXIMUM GLOBAL BENEFITS – A PROGRESS REPORT FROM CANADA

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Objectives

We redesigned faba bean nutritional, environmental and economic attributes for economic/ecological adaptation to the Canadian prairie environment to improve crop rotations and global food production systems. Main objectives were (1) reduce seed size (2) eliminate vicine-convicine , (3) reduce tannins and (4) days to maturity and (5) increase economic value of the crop.

Materials and Methods

The F2-derived family method was used to reducing seed size, improve seed shape, reduce vicine-convicine, tannins and and maturity, and increase yield.

Main Results

(1) Seed size reduced from 650 mg per seed to less than 300 mg per seed

in adapted germplasm to reduce seed volume and increase value.

(2) Seed shape altered from the traditional flat shape to almost round.

(3) Molecular marker for the low vicine/convicine (vc1) was discovered and implemented in the entire breeding program.

(4) The white flower (low tannin gene zt1) trait was introduced into the entire

breeding system.

(5) Crop maturity was reduced through genetic, physical and environmental breeding strategies.

(6) Yield potential was maintained or increased depending on the production area, while crop production risks were reduced.

(7) A much greater range of phenotypic and genotypic diversity with economic potential was established for faba bean.

Conclusions (Outcomes)

(1) Effective breeding objectives and methods were established to improve the economic, nutritional, environmental, ecological, agronomic, and culinary attributes of faba bean.

(2) New genetic baseline accelerated production of faba bean

(3) Faba bean protein is now in high demand for expanding plant protein industry in western Canada.

(4) Breeding resulted in greater genetic diversity and production and product diversification.

(5) The economic potential for faba bean in western Canadian crop rotations was increased.

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606/201. GENOMIC SELECTION FOR PEA GRAIN YIELD AND PROTEIN CONTENT: PREDICTIVE ABILITY FOR INDEPENDENT ITALIAN ENVIRONMENTS AND FOR TARGET AND NON-TARGET GENETIC BASES

Authors:

Margherita Crosta[1], Nelson Nazzicari[1], Barbara Ferrari[1], Luciano Pecetti[1], Tommaso Notario[1], Giovanni Cabassi[1], Paolo Annicchiarico[1]

Work centre:

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Summary:

Objectives

Genomic selection (GS) has special interest for complex polygenic traits, but its actual value for cool-season grain legumes is poorly known. Since GS involves substantial initial phenotyping for model construction, information is needed not only on GS inter-environment predictive ability (as challenged by genotype × environment interactions) but also on the predictive ability penalty suffered by a GS model built for a genetic base (GB) when predicting breeding values for another GB. Pioneer studies were encouraging for pea GS targeting Italian environments for a GB represented by three

recombinant inbred line (RIL) populations issued by connected crosses among three elite cultivars, with average values of inter-environment predictive ability for top-performing GS models of 0.40 for grain yield, 0.53 for protein content, and 0.41 for protein yield (Annicchiarico et al., 2019; Crosta et al., 2022). These values were about halved for inter-population predictions using one RIL population for model construction to predict data of the other populations. This study aimed to provide a thorough assessment of the predictive ability of GS models defined from data of three environments, when applied to data from independent environments and inbred lines from both the target GB (the same three RIL populations) and a non-target GB (three other RIL populations, each including one parent cultivar different from those of the target GB).

Concise description of the work (materials & methods)

While GS model construction was based on data of 276 lines grown in three environments of Northern or Central Italy in the cropping years 2013-14 or 2014-15, GS model evaluation was based on data of 131 independent inbred lines (64 for the target genetic base, and 67 for the non-target base) grown in Northern Italy in the years 2018-19 and 2019-20. All the experiments were autumn-sown and had three replicates. GS predictions relied on 5,537 polymorphic SNP markers made available by ApeKI-based genotyping-by-sequencing.

Main Results

Genotype × year interaction across validation environments was substantial, as indicated by only moderate phenotypic correlation for line values across environments (<0.55 for all focus traits). Grain yield and protein content in each validation environment were poorly correlated (r = 0.10-0.21). On average, GS displayed moderate predictive ability (0.28-0.40) for grain yield, protein content and protein yield of genotypes within individual RIL populations of the target GB or protein content of genotypes of the non-target GB, with minor test year differences (Table 1). Somewhat greater predictive ability was obtained for all focus traits when GS was applied to all genotypes of the RIL populations representing the target GB (Table 1).

		Within-RIL prediction		Across-RIL prediction
Target trait	Cropping year	Target GB	Non-target GB	Target GB
Grain yield per ha	2018-19	0.256	0.113	0.355
Grain yield per ha	2019-20	0.258	0.011	0.444
Grain yield per ha	Average of 2 years	0.292	0.079	0.399
Grain protein content	2018-19	0.313	0.372	0.390
Grain protein content	2019-20	0.425	0.314	0.449
Grain protein content	Average of 2 years	0.403	0.360	0.419
Protein yield per ha	2018-19	0.245	0.085	0.308
Protein yield per ha	2019-20	0.267	-0.089	0.447
Protein yield per ha	Average of 2 years	0.279	0.003	0.378

Table 1. Predictive ability of top-performing Ridge regression BLUP genomic selection models for three pea traits constructed for a genetic base (GB) represented by three connected crosses, when tested on 126 independent inbred lines belonging to the target and a non-target GB grown in two independent environments. Predictions within RIL populations (reporting results averaged across three populations) or across RIL populations

Conclusions

On the whole, these findings confirm and reinforce the usefulness of GS for pea grain yield and protein content of the GB used for model training, while restricting to protein content its interest for a non-target GB.

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606/214. GENETIC BASIS OF GRASS PEA AGRONOMIC TRAITS FOR PRECISION BREEDING TOWARD CLIMATE CHANGE MORE ADAPTED VARIETIES

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Grass pea (Lathyrus sativus L.) is an annual cool-season grain legume recognized as a stress-resilient crop. In the Mediterranean region, climate change has increased environmental instability, creating a fundamental need for more adaptable plant varieties. To assist the development of such varieties, we studied the genetic basis of 13 agronomic traits in a worldwide collection of 182 grass pea accessions, making use of the genotypic screening of the same accessions with genotyping-by-sequence SNP-markers. For the agronomic evaluation, the grass pea accessions were field trailed, over 4 growing seasons, in a Mediterranean region, and the agronomic traits (3 plant growth-, 2 inflorescence-, 4 seed-, and 4 yield-related) were scored according to the “Descriptors for Lathyrus spp.” (IPGRI, 2000). The genetic basis of these important agronomic traits was studied through a genome-wide association. A panel of 5,651 high-quality SNP markers was used to test for SNP-trait associations, based on linear mixed models, accounting for population structure. Using a -log10P-value≥4, and a grass pea reference genome (Rajarammohan et al. 2023), we detected a total of 33 SNP markers significantly associated with the traits. Fourteen SNP markers were associated with plant growth-, 6 with yield-, 6 with seed-, and 7 with inflorescence-related traits, explaining from 2.75 to 62.96 % of the observed variability. Two SNP markers, S3424 and S3097, were common to one plant growth- and one yield-related traits, the S3803 was common to one yield- and one seed-related traits, the S3891 was common to two growth-related traits, and the S1194 was common to two seed-related traits. The candidate genes and metabolic pathways underlying the associations are currently being explored. The detected associated SNP markers constitute promising genomic targets for the development of molecular tools to assist stress-resilience precision breeding.

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606/223. INVESTIGATING INTERSPECIFIC LENTIL GERMPLASM: NEAR-INFRARED SPECTROSCOPY FOR PROTEIN AND AMINO ACID PROFILE AND QUANTITATIVE TRAIT LOCUS ANALYSIS

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Cultivated lentil (Lens culinaris Medik.) has a relatively narrow genetic base which poses many challenges to the improvement of the crop. Introgressing traits like biotic and abiotic stress resistance from wild relatives is often hindered by linkage drag of undesirable traits including shattering and low palatability. The objective of this study was to investigate the effects of crossing with wild lentils on protein content and amino acid profile. An interspecific recombinant inbred line population, LR-68 (L. culinaris ‘CDC Greenstar’ and L. orientalis ‘IG 72643’) was grown in four site-years and seed collected. Protein and amino acid concentrations of LR-68 samples for all site-years were estimated using a previously developed NIR model (Hang et al., 2022). CDC Greenstar is a large-seeded lentil with green seed coat and yellow cotyledons and relatively low mean protein content (24.8%). IG 72643 is small seeded with a marbled brown seed coat and red cotyledons with a relatively high

mean protein content (30.0%). The RILs exhibited significant variation for predicted protein content and amino acid profile, with protein content ranging from 24.0-32.8%. Quantitative trait locus (QTL) analysis was conducted to identify regions of the genome associated with protein content and amino acid profile. QTL analyses were performed on an individual site-year basis due to significant effects of environment on protein and amino acid profiles. There were multiple significant QTL for protein, histidine, arginine, isoleucine, and phenylalanine at all site-years. Breeders can use this to make informed decisions about maintaining or perhaps enhancing lentil quality when using L. orientalis as a source of diverse traits.

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606/229. FEASIBILITY STUDY OF CHICKPEA CULTIVATION IN FLANDERS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

In Flanders (Belgium), consumers and the food industry show increasing interest in locally produced grain legumes, and particularly in chickpea (Cicer arietinum). Farmers show interest in this new crop due to increased market opportunities and advantages in their rotation. In three consecutive years (2019-2022), variety trials with 7 varieties were sown and evaluated at two locations in Flanders. Varieties with germination at lower temperatures and rapid juvenile growth are needed for our climate conditions, with 'Elmo' and 'Flamenco' suited to early sowing (end of March). Yield varied between 2.5 - 3 t/ha (2919 and 2020) and 3 - 4 t/ha (2022) with the high yielding Flamenco as the most promising cultivar. However, in 2021 yield was lost totally (cold spells). Chickpea is a promising crop for Flanders but with the risk of complete failure in case of unfavorable weather conditions. A chickpea breeding program was set up at ILVO to create varieties with improved adaptation, improved yield stability, sufficient grain quality and Phoma rabiei (Ascochyta rabiei) disease resistance using artificial inoculation. As chickpea is a new crop in Belgium inoculation with Rhizobium bacteria is necessary to ensure adequate nodulation. In addition, the feasibility of local cultivation on different soil types is being investigated through citizen science and a network of 15 start-up chickpea growers. As well commercial chickpea innoculants as locally trapped Rhizobia bacteria from the citizens experiment are currently assessed for nodulation efficiency. In 2022, 18% of assessed soils showed nicely formed nodules and bacterial strains are now being isolated from these soils for further characterization. Other concerns are the harvest losses due to the indeterminate growth of the crop with possible regrowth before the end of the growing season.Results in breeding, efforts, yield and innoculants will be presented.

606/232. INTRODUCING LEGUME GENERATION

Authors:

Donal Murphy-Bokern[1], Sanu Arora[2], Fred Eickmeyer[3], Charlotte Jones[4], Tania Gioia, Jasmin Karer[5], Lars-Gernot Otto[6], Elizabeth Ninou[7], Nick Nick Vangheluwe[8], Johann Vollmann[9]

Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

This presentation will introduce Legume Generation to the legume research community in Europe. Funded by the European Union as an Innovation Action, Legume Generation (Boosting innovation in breeding for the next generation of legume

crops for Europe) will boost the breeding of legumes in Europe by combining the entrepreneurial focus of breeders with the broad inventiveness of the supporting research base. Six innovation communities will link practical breeding with the research-base. They lead the innovation work and each is focused on the breeding of a single species or species type: soya bean (Glycine max); lupins (Lupinus spp); pea (Pisum sativum); lentil (Lens culinaris); phaseolus bean (Phaseolus spp. e.g., 'common' bean); and white and red clover (Trifolium repens and T. pratense). These are supported by intelligence on ideotype concepts, beneficial traits, a catalogue of legume species and cultivars, and breeding methods assembled in the Legume Generation Knowledge Centre; the production and validation of novel resources; screening, demonstration and testing of germplasm and new cultivars; training to support breeding gains; governance and financial models. All this will be supported by the extension of the European Legume Hub as a platform for sharing of knowledge. We currently run 44 breeding and pre-breeding programmes. We provide access to resources that accelerates the production of novel germplasm, innovating up to the point where newly bred germplasm and cultivars are proven on farm. An important point with respect to the ILS conference is that our innovation communities will be open to all relevant actors and provide a direct route for the dissemination of results to other users and interested stakeholders. Their sustainability beyond the life of the project will be supported by business plans.

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606/251. PIGEONPEA BREEDING NETWORK TARGETING PRIORITIZED MARKET SEGMENTS IN EASTERN AND SOUTHERN AFRICA: ACHIVEMENTS & OPPORTUNITIES

Authors:

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Summary:

Objectives

Pigeonpea is the crop of about six million smallholder subsistence farmers in Eastern and Southern Africa (ESA), in providing food and nutritional security, feed, fodder and fuel wood supply and income generation. Its production is steadily increasing over the last 15 years and attained about 883,099 t (FAO Stat 2023) with Malawi, Tanzania, Mozambique, and Kenya being the major pigeonpea producers. Pigeonpea has huge regional and international export potential and India alone imports about 600,000 t annually. At present, ESA countries export about 470,000 t of grain/year worth of $ 230 million. Pigeonpea provides multiple benefits to cropping and farming systems through biological nitrogen fixation, nutrient recycling, climatic resilience, and sustainable intensification options.

Concise description of the work (materials & methods)

ESA is the secondary centre of diversity with large amount of native and useful genetic diversity and recent germplasm collections in Kenya, Mozambique, Uganda and Tanzania assisted in capturing this. NARES and ICRISAT in the region holding about 1200 unique germplasm accessions of the ESA region. Pigeonpea improvement in ESA started by mostly relying on native germplasm and through these 41 high yielding varieties that are belonging to short (8), medium (21) and long (12) maturity group were released.

Main Results

A strong region-specific network-based breeding program is in operational in close collaboration with NARES and other stakeholders with prioritized market segments and target product profiles emphasizing on high grain yield, inter-cropping compatibility, photo-period insensitivity, grain quality, resistance and/or tolerance to Fusarium wilt and resilience to climate change. Most of the cultivated varieties are susceptible to insects but regional germplasm contributed to develop insect-cum-drought tolerant, high yielding and big seeded genotypes with > 28 g. Pigeonpea being a drought tolerant crop, leaves remain green longer compared to other legumes grown in these areas such as cowpeas and beans, hence,

it offers an alternative source of fodder to the livestock with >25 t of biomass yield for animal feed coupled with > 1.6 t of grain yield. Sustainable intensification options through P-micro dosing, intercropping with cereals and double up legumes had increased yields of both pigeonpea and associated crops. This led to productivity gains in the region up to 1021 kg/ha and in Malawi it has reached about 1674 kg/ha. However, a huge gap exists between realizable and actual yields with present management options.

Conclusions

To further develop strong breeding pipeline, genetic enhancement using diverse gene pools and trait specific/multiple trait donors will be used in pre-breeding program. More emphasis will be given to develop varieties based on prioritized market segments at regional/country level along with streamlined advancement procedures, on-farm testing, sustainable seed systems, and effectively utilizing genomic and genetic resources.

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606/254. FOOD LEGUMES EXPLOITATION: VARIETAL COMPARISON AND AGRONOMIC INNOVATIVE TOOLS TO IMPROVE THE BREEDING AND THE PERFOMANCE

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Lentil, chickpea and common bean are among the most consumed pulses in Europe, with a crucial role in Mediterranean basin. Legumes are staple crops in rotation systems and from a nutritional point of view providing a crucial source of vegetable proteins. The lack of significant investments in plant breeding activities compared to other species and the poor knowledge on cultivation techniques and varieties pose the challenge of identifying strategies for boosting legumes cultivation and consumption. In this context, the RESO -REsilienza and "SOstenibilità- project aims to provide knowledge and strategies to ameliorate the supply chain, for several relevant crops of the Mediterranean basin, including legumes, in order to create novel market opportunities in the South Italy, to be adopted elsewhere too. RESO by investigating the molecular and phenotypic diversities in plant genetic resources of lentil, chickpea and common bean, aims to develop new varieties for adaptation, sustainable cultivation practices and key quality traits. Several experiments in open field and under controlled conditions allow to test the agronomic values of selected genotypes, also considering the intercropping system with cereal, and by conducting metabolomics and metagenomics studies. RESO is also characterizing the nutritional quality of lentils and chickpea grains and investigates the phenotypic variability of root architectural traits in chickpea by implementing high-throughput phenotyping platforms. By combining this information with genomic data, RESO will identify genomic regions and candidate genes underlying useful root traits for plant breeding. Data collected during the first year of the project highlighted the variability for agronomic performances among different selected varieties of lentil across environment conditions. The intercropping field trial system proved a significant difference in terms of weeds and pests resistance, probably for the allelopathic effect of the cereal-legume system.

Data obtained from field trials, together with phenotypic and genotypic information collected from sister projects (e.g., INCREASE project; Bellucci et al., 2021) will contribute to reveal the potential of the single accessions for the projects aims.

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606/258. SELECTION FOR HIGH AND STABLE YIELD IN SOYBEAN BREEDING LINES USING DIGITAL PHENOTYPING METHODS

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Summary:

Objectives, Description, Main Results & Conclusions

Soybeans have become a valuable alternative in crop rotation for sustainable protein production. To achieve high yields, plants must efficiently absorb sunlight energy, convert it into photochemical energy and finally into biomass. To ensure stable yields, soybean has been successfully adapted to the European climate and must be robust to a range of environmental conditions.

In several field trials in Switzerland, the conversion efficiency of sunlight into photochemical energy in elite soybean lines was determined by photosynthesis measurements using chlorophyll fluorescence. Energy conversion into biomass was approximated by measuring canopy cover and plant height using segmented RGB images and 3D laser scans. This allowed growth dynamics to be monitored under different conditions during the growing season. Linear modeling was used to assess the influence of environmental variables such as precipitation, light intensity or temperature on growth rate. In this way, high-throughput phenotyping will allow the selection of lines that are tolerant to specific environmental conditions and efficient in biomass production. This will facilitate the adaption of soybean lines to the future climate and ensure high yield and yield stability.

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Session 7 Physiology, biochemistry and systems biology

606/14. NUCLEOTIDE METABOLISM IN COMMON BEAN FRUITS DURING SEED FILLING PHASE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Common bean is among the most cultivated legumes in the world. During seed formation, the pods that surround the seeds would have an important and dual role protecting the developing seed while contributing to its nutrition. In this phase, the seed needs to incorporate nitrogen in order to synthesize the storage proteins. An important reservoir of nitrogen that has been poorly studied are nucleic acids. These are quite abundant molecules in plant tissues that, in addition to nitrogen, can function as a reservoir of carbon and phosphorus (Stasolla et al., 2003). For this reason, nucleic acids can play a crucial role in processes that involve mobilization of nutrients. The enzymes involved in the breaking down of nucleic acids are nucleases and ribonucleases, nucleotidases and nucleosidases, enzymes that release nucleotides, nucleosides and the nucleobase and the sugar moiety, respectively.

This research aims to carry out a comprehensive study of nucleic acid metabolism in the fruit filling phase, since a better knowledge could be crucial to improve both crop yield and nutritional properties, processes with great agronomic impact that can contribute to developing fruits with better nutritional properties (Bennet et al, 2011). To do that, gene expression and enzymatic activity of S1 nucleases, T2-S-Like ribonucleases, HAD superfamily phosphatases and cytosolic nucleosidases have been analysed during fruit development. Overall, the data presented allow to conclude that there is an intense nucleotide metabolism in pods during seed filling stage with an especial involvement of seed coats.

Aknowledgement:

Universidad de Córdoba (pre-doctoral contract: M D-B), Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía (Grant P20_00440), Ministerio de Ciencia e Innovación (Grant PID2020-117966RB-I00).

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606/22. DOMESTICATION HAS ALTERED GENE EXPRESSION AND SECONDARY METABOLITES IN PEA SEED COAT

Authors:

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Summary:

Objectives

The seed represents a characteristic feature of seed plants that has strongly enhanced the reproductive and dispersal potential of taxa. It is mainly the seed coat (SC) that provides protection for the seed by physical and chemical barriers to prevent the entry of pathogens or the impact of an adverse environment. Wild and crop plant seeds differ in their SC

properties as a result of the domestication process. Domestication led to the formation of crops with favorable traits for humankind. Wild ancestors had both advantageous features comprising protection mechanisms but at the same time also not desirable traits such as seed dormancy. This has been lost or modified during domestication. Legumes due to their protein-rich seeds provide world food security. Mature pea seeds consist of the SC and embryo (E) where SC is crucial for seed protection, dormancy, dispersion, and reproduction success.

Concise description of the work (materials & methods)

To understand the mechanism of pea seed domestication, we conducted an anatomic, transcriptomic, proteomic, and metabolomic analysis of pea seeds comparatively between 3 wild and 3 cultivated genotypes sampled at 5 developmental stages (Balarynová et al. 2022, Krejčí et al. 2022).

Main Results

Anatomical analysis showed differences in SC anatomy between wild and cultivated genotypes. Comparison of SC to E has shown a constant number of shared genes expressed in both tissues, while in the E a comparably lower number (1,000-2,000) of tissue-specific genes than in SC (3,000-4,000) was expressed. This highlights the importance of SC and shows the complexity of gene expression and biosynthetic pathways particularly related to secondary metabolites. 2,651 up-regulated and 1,809 down-regulated genes were identified between Cameor and wild pea genotypes. Most of DEGs belonged to GO category with oxidoreductase activity. Gene co-expression analysis of 26,951 SC-expressed genes identified 20 gene expression clusters (Fig.1). Four clusters showed a pattern of developmentally regulated genes, while two were positively associated with seed dormancy or domestication with genes differing in the expression level between wild and domesticated genotypes. Concomitantly, proteomic analysis detected a higher abundance of proteins involved in secondary metabolite biosynthesis, including caffeic acid 3-O-methyl transferase enzyme active in lignin biosynthesis in wild pea seeds. Metabolites involved in the monolignol biosynthetic pathway were also identified in the SC during development. Cinnamate and p-coumaraldehyde were present in a higher content at the early developmental stages compared to more matured ones. On the other hand, sinapyl alcohol and coniferyl alcohol contents rose during maturation of seeds of wild genotypes.

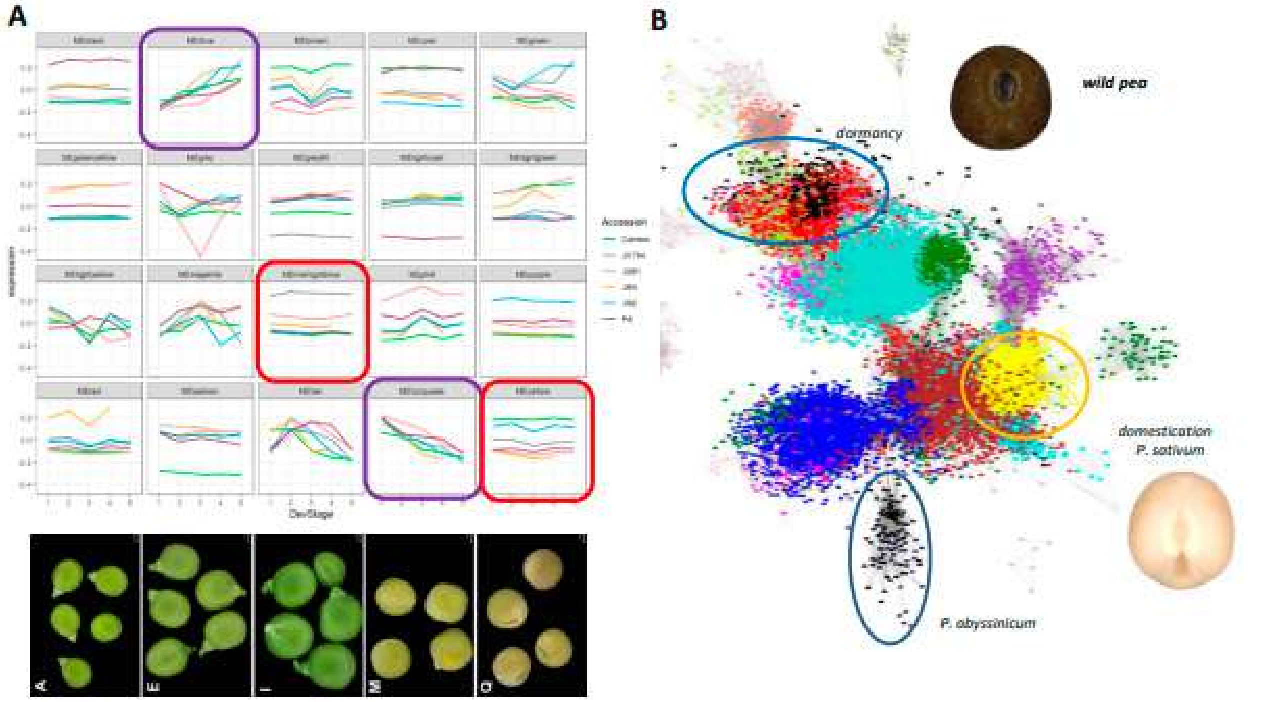

Figure 1: Gene expression during the seed coat development. A) Gene expression of different modules during pea development. B) Spatial resolution of modules according to their relationship to particular traits.

Conclusions

This work provides the first comprehensive gene expression, proteomic and metabolomic profiling of pea SC development. The comparison of wild and cultivated pea genotypes allows analysis of the gene expression in relation to seed development, dormancy, and also domestication. The impact of the domestication process on plant traits is well-known (Smýkal et al. 2018) while less is known about the alternation of metabolic pathways. Understanding seed development is important for biology but has also an impact on breeding, agronomy, and food processing.

Acknowledgment: This work was supported by Grant Agency of Czech Republic (19-07155S) project.

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606/28. PEXOPHAGY IN SUGAR-STARVED EMBRYONIC AXES OF GERMINATING LUPIN (LUPINUS SPP.) SEEDS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Plant autophagy is a process by which cells degrade and recycle their own components such as organelles and protein complexes. Under adverse conditions, including nutrient deficiency, autophagy is enhanced to increase the pool of respiratory substrates and hence increase the potential of cell survival. We found that advanced autophagy occurs under sugar starvation conditions in isolated embryonic axes of white lupin (Lupinus albus L.; up to 14% total lipid content in seed dry matter) and Andean lupin (Lupinus mutabilis Sweet; about 20% total lipid content in seed dry matter) cultured in vitro for 96 h. Simultaneously, we observed disruption of storage lipid breakdown, which was reflected in higher lipid content in the sugar-starved (-S) than in sucrose-fed (+S) axes. Our results indicate that the disturbance in storage lipid breakdown is caused by the pexophagy, i.e. a selective autophagic degradation of peroxisomes - the key organelles in storage lipid degradation. Evidence for such conclusion are: i) peroxisome localization in the autophagic bodies, ii) higher content of total lipid, iii) higher transcript levels of genes coding for proteins of the pexophagy machinery, and iv) lower content of the peroxisome marker Pex14p and its increase caused by an autophagy inhibitor (concanamycin A) in -S axes than +S axes. We did not find significant differences in pexophagy and storage lipid breakdown between axes of two investigated lupin species despite the different lipid content in seeds. Importantly, our results show that autophagy is an adaptive response to adverse conditions also in very young plant organs like embryonic axes. The research was financed by the National Science Centre, Poland, grant no. 2016/23/B/NZ3/00735.

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606/40. b-CONGLUTINS' MOBILE ARM IS CRUCIAL IN THE NUTRACEUTICAL PROPERTIES OFBLUE LUPIN

Authors:

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Summary:

Objectives

Sweet lupin species, particularly narrow-leafed lupin (NLL; Lupinus angustifolius L.), have gained significant attention in recent years for their nutraceutical properties, specifically their anti-inflammatory and anti-diabetic effects. β-conglutin proteins, particularly the NLL β1, β3, and β6, have been extensively studied for their health benefits [1], with some studies reporting their cytotoxic effect on breast cancer cells and prevention of malignant transformation of healthy cells, reducing metastasis and recurrence in breast cancer cell lines [2]. These nutraceutical properties may be attributed to the unique structural domain of β-conglutin proteins, characterized by an N-terminal mobile arm [3] absent in other vicilin proteins of legume species. This study focused on the anti-inflammatory activity and oxidative regulatory capacity of the NLL β5 and β7 conglutins.

Concise description of the work (materials & methods)

Biochemical and molecular biology techniques were employed using in vitro and ex vivo systems: LPS-stimulated in vitro (HepG2) model culture cells and ex vivo (isolate PBMC from blood samples of T2D-diagnosed patients and healthy control subjects). We purified recombinant forms of complete (β5 and β7) and truncated (tβ5 and tβ7, missing the N-terminal mobile-arm domain) conglutin proteins using affinity chromatography.

Main Results

The results revealed that β5 and β7 proteins reduced the levels of pro-inflammatory mediators and mRNA expression levels of iNOS, TNFα, and IL-1β (Figure 1). In addition, the complete forms of β5 and β7 decreased the protein levels of pro-inflammatory cytokines such as TNF-α, interleukins (IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, IL-27), and other pro-inflammatory mediators (INFγ, MOP, S-TNF-R1/-R2, and TWEAK), while exerting a regulatory effect on cellular oxidative balance through glutathione levels, catalase, and superoxide dismutase enzymatic activities. Interestingly, the truncated forms (tβ5 and tβ7) did not exhibit these molecular effects [3].

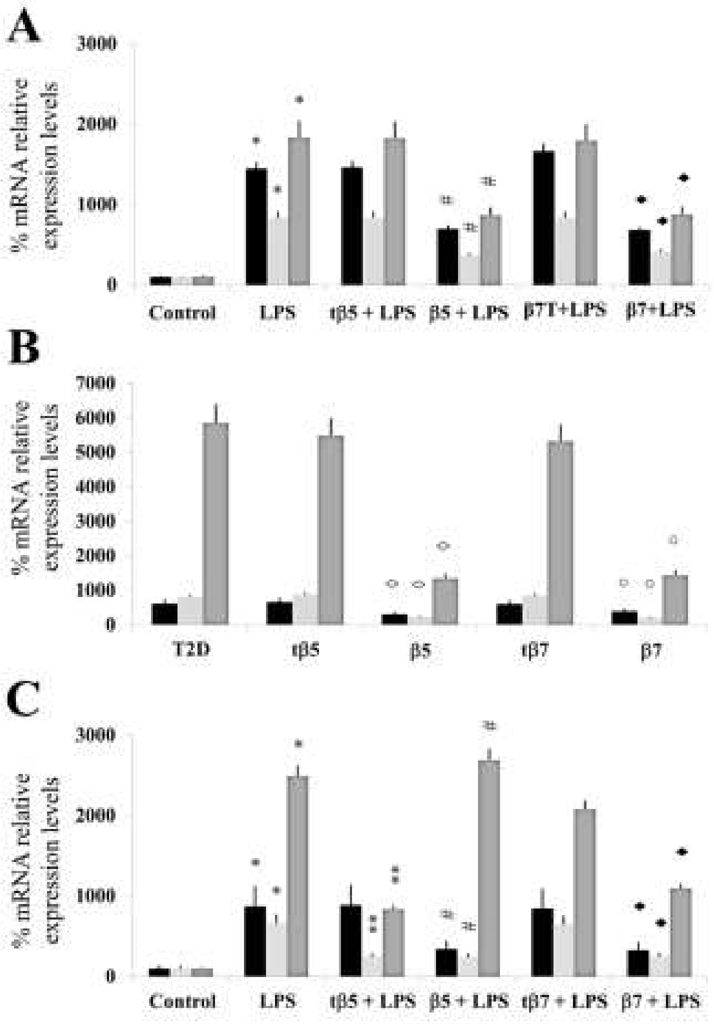

Figure 1. Example of the effect of β5 and β7 compared to truncated forms in all experimental groups: Assessment of mRNA expression levels of TNF-α, IL-1β, and iNOS genes. Culture cells of HepG2 (A), T2D (B), and healthy control subjects (C). Each group was incubated for 24 h in LPS, LPS+tβ5, LPS+β5, LPS+tβ7, or LPS+β7. Bars show TNF-α (black), IL-1β (light gray), and iNOS (gray) color. *p< 0.05 LPS vs. control; **p< 0.05 LPS+tβ5 or tβ7 vs. LPS; #p< 0.05 LPS+β5 vs. LPS; ⍰p< 0.05 LPS+β7 vs. LPS. ◊p< 0.05 LPS+β5 vs. T2D, and ○p< 0.05 LPS+β7 vs. T2D in T2D cell cultures. Data represent the mean ± SD of three independent experiments. [3]

Conclusions

Our findings highlight the anti-inflammatory and oxidative cell state regulatory properties of β5 and β7 conglutins. Furthermore, we identified the N-terminal mobile-arm domain as a critical element of β-conglutin proteins contributing to these nutraceutical properties.

Consequently, these newly discovered anti-inflammatory proteins (NLL β5 and β7) might play a key role as potential functional food components for preventing and treating inflammation-related diseases and cancer. Furthermore, our study highlighted the importance of the unique β-conglutin' mobile arm as a key structure in mediating these beneficial effects.

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Acknowledgments

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606/128. IDENTIFICATION OF MENDEL'S POD COLOUR CHARACTER IN PEA: CHARACTERISATION OF THE ALLELE CONDITIONING YELLOW POD COLOUR

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The inheritance of yellow-podded versus green-podded peas was studied by Mendel more than 150 years ago, but, until now, the allelic variation underlying this difference remained unknown. A report of a novel, spontaneous gp mutant (Pellew and Sverdrup, 1923) suggested that at least two independent gp alleles existed. We undertook genetic complementation crosses with 19 yellow-podded lines present in the John Innes Pisum germplasm collection, one of which is described as "Pellew's gp", and we found that all were allelic to gp carried by the type-line, JI0128. This showed that Mendel's yellow-podded character is conditioned by variation at a single locus. We used genetic mapping of Axiom markers in a large F2 population to define the position of gp within a 4.4 cM interval, encompassing approximately 8 Mb. We used exome capture to compare genome structures within this interval, in 227 Gp accessions and the 19 gp accessions. We found that all 19 gp lines carry the same large 100 kb deletion, while all 227 Gp accessions do not, indicating that only one gp haplotype exists in the germplasm we studied, and that gp must have been crossed into different backgrounds by breeders and researchers in the past. Three candidate genes associated with the deletion were assessed. After crossing a null TiLLING mutant for one of these candidates with a gp line, yellow-podded F1 progeny were obtained. This genetic complementation test showed that we have identified a new allele of the gp gene. We used backcross lines to gain a better understanding of the mutant phenotype. It was already known that thylakoid membranes are underdeveloped in gp lines (Price et al., 1988). Additional effects will be described.

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606/132. PEA RESILIENCE TO WATER DEFICITS: TOLERANCE, ACCLIMATION, AND STRESS MEMORY OF THE NODULATED ROOT SYSTEM

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Peas have the potential to play a significant role in ensuring future food security. However, the yields of peas, both in terms of quantity and quality (such as grain content), are vulnerable to the impacts of climate change, specifically water deficits. Nowadays, water deficits occur more frequently during crop growth cycles and persist for longer durations. The effects of soil water stress on plant development and growth are complex, as they depend on soil water availability, the modulation of available soil elements, and the plant's ability to acclimate to continuous stress or remember previous stress events. To understand the strategies underlying plant responses to water stress events, we conducted controlled experiments with pea plants subjected to optimal water conditions, and different types of water stresses: transient stress during vegetative or reproductive periods, recurrent stress, and continuous stress throughout the entire growth cycle. An integrative approach, including a structure-function ecophysiological framework characterizing plant hydromineral nutrition, enriched with root and nodule transcriptomic analyses (RNA-seq), revealed the mechanisms underlying specific and common responses to the different types of water stress. In addition, a complementary field experiment was carried out in order to validate the observations made under controlled conditions and to assess the level of expression of the candidate genes identified. This study introduces a fresh perspective by highlighting the importance of plant memory and acclimation in designing ideotypes that are more resilient to longer stresses, or multiple stress events in the context of climate change. This innovative approach has the potential to enhance crop productivity and ensure food security. This work was supported by Plant2Pro as part as the ARECOVER project, by FUI, BPIFrance, the Regional Council of Burgundy, Dijon Metropole, and FEDER, as part as the EAUPTIC project and by ANR-PIA as part as the PHENOME-EMPHASIS project.

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606/172. ELUCIDATING THE ROLE OF NF-YB TRANSCRIPTION FACTORS IN PEA SEED DEVELOPMENT

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes such as pea (Pisum sativum L.) can accumulate large amounts of protein in their seeds even without nitrogen fertilizer thanks to their symbiosis with N2-fixing soil bacteria. Pea cultivation offers undeniable economic and environmental benefits in the context of a growing demand for plant proteins. The aim of this project is to elucidate the molecular processes underlying the accumulation of seed storage proteins (SSPs) in pea, focusing on the NUCLEAR FACTOR YB (NF-YB) transcription factors, including LEAFY COTYLEDON 1 (LEC1) and LEC1-like (L1L), which have been shown to be key regulators of seed development and storage compound accumulation in Arabidopsis thaliana. Phylogenetic and transcriptomic analyses identified four seed-specific NF-YB candidates for the regulation of SSP synthesis in pea, two of which are orthologs of the AtLEC1 and AtL1L genes. Pea mutants for these genes were obtained by TILLING (Targeting Induced Local Lesions IN Genomes), and preliminary phenotyping showed that loss of function l1l mutants are affected in seed size and seed protein composition, suggesting that L1L may regulate SSP accumulation. A transient expression system in moss (Physcomitrella patens) protoplasts (thevenin et al.,2016), was used to test the ability of these regulators to activate the promoter of genes related to storage activities.

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606/227. PROSPECTS FOR THE USE OF MAGNETIC RESONANCE IMAGING IN LEGUME BIOLOGY AND BREEDING

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Summary:

Objectives, Description, Main Results & Conclusions

Scientific research begins with observation. We focus on magnetic resonance imaging (MRI), an in vivo technology that has not only revolutionized medical and clinical investigations but in recent years has become a versatile platform for plant imaging (Borisjuk et al., 2023). We provide examples of how MRI simultaneously captures anatomy and metabolism and can track processes. New approaches will reinforce the relevance of MRI for legume applications. At the same time, we will address the specificity of experimental design and share our experience from non-invasive research. Our presentation will include animated 3D models showing the interior of intact, living seeds (pea, soybean, Vicia sp.), highlighting, among other things, the structural arrangement of the internal embryonic vascular network and the deposition of storage products that are normally hidden from the human eye. Using legume seeds as an example, we demonstrate a new in vivo approach that allows visualization of the most abundant metabolites in plants, such as amino acids and sugars. MRI can be used in combination with -omics technology to elucidate the mechanisms of metabolic and hormonal control of seed filling (Meitzel et al., 2021). In this way, modern plant MRI can help to better understand the inner workings of a plant, its development, and its response to a dynamic environment. With our presentation we want to show the potential of MRI and to inspire future developments in legume research and breeding.

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606/246. CHARACTERIZATION OF FACTORS CONTROLLING THE END OF FLOWERING IN PEA

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Summary:

Objectives, Description, Main Results & Conclusions

In monocarpic plants, after the production of a certain number of fruits/seeds, the activity of the shoot apical meristem (SAM) and of the meristems of the secondary axes stop in a coordinated way, leading to the cessation of flower production. This phenomenon has been named Global Proliferative Arrest (GPA), likely a general phenomenon in monocarpic plants, as it has been described in different species including major crops. Much effort has been devoted to studying flower initiation in the last years, but there is not much known yet about the regulation of the end of flowering. Studies in the model species Arabidopsis thaliana have identified factors controlling

GPA timing. One of them is the production of fruits and seeds: sterile mutants produce more flowers than fertile ones and pruning fruits can delay GPA or induce reactivation of the meristem. In addition, we have recently proposed a genetic pathway that controls GPA: FRUITFULL and miR172 regulate the levels of APETALA2 (AP2) and AP2-LIKE genes in the SAM, which finally control SAM arrest. Recent studies in Pisum sativum (pea) indicate that the mechanisms that control GPA could be very similar to those of Arabidopsis. We are currently working to characterize the differences and similarities that exist in this process between both species. For that aim, we have carried out different types of physiological studies to assess the impact of fruit/seed production in pea. Also, we have investigated the transcriptomic response of the pea inflorescence meristems in different stages related to GPA and compare it to those already characterized in Arabidopsis. Finally, through VIGs and VIGe techniques, and with the use of TILLING mutants, we are looking to new modulators of the GPA, to better understand the genetic pathway that controls this process in pea.

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606/12. USING FABA BEAN TO ADVANCE A PHYSIOLOGICAL THEORY OF YIELD FORMATION

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Summary:

Objectives, Description, Main Results & Conclusions

Research at low levels of biological organisation (gene, molecule, cell) must be scaled up for agronomic relevance, and research at higher levels (field, farm) needs reduction for explanation. Crop physiology facilitates both moves because it explains yield at the level at which it occurs – a population of interacting plants. A comprehensive physiological theory of grain yield formation should: 1) describe the acquisition of resources for growth, 2) predict the proportion of growth allocated to grain, 3) characterise the limits and disruptions imposed on yield formation by the target environments, 4) link grain production (seed number and size) to fitness, 5) account for plant-plant interactions in crop stands, 6) be diachronic, 7) incorporate the way past meristem allocation affects future growth options, and 8) account for how plants forecast the environment from past and present experience. To put this in propositional form, plants within crops grow and produce seeds through time with decreasing degrees of freedom while accounting for future uncertainty. Criteria 1 through 5 are addressed in the literature, 6 is gaining prominence, and 7 and 8 are at the hypothesis stage. In this paper, I will a) present new data from faba bean experiments that address 5-8, and b) reflect on their implications for a physiological theory of grain yield formation of pulses and other annual grain crops.

606/15. PHOSPHATASE HAD GENE SUPERFAMILY DURING GERMINATION IN COMMON BEAN

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Nucleotides are important molecules in plant physiology. Nucleotides are elementary units of the genetic material, are involved in bio-energetic processes, play a role as cofactors, and are also components of secondary metabolites and the hormone cytokinin. Purine nucleotide metabolism has an additional role in ureidic legumes, such as common bean, as the precursor of ureides, compounds used as nitrogen transport from nodules to the rest of the plant. In addition, nucleotides are important during germination and early seedling development since the developing seedlings require high amount of these compounds (Delgado-García et al., 2021).

In this work, we have performed an expression analysis of the 11 genes recently identified as belonging to the HAD superfamily (Gálvez-Valdivieso et al., 2021) in common bean seeds during germination. The analysis was performed both at 1 and 3 days after start of imbibition (DAI) as not germinated and germinated seeds, respectively. Results show that expression of genes PvNTD9 and PvNTD10 are highly induced at 3 DAI, being these two genes the most expressed in germinated com-

mon bean seeds. Phosphatase activity with nucleotides as substrates was determined as well in germinated seeds. The expression pattern of PvNTD9 prompted us to further analyse gene expression in other nutrient mobilization process. The expression remains unchanged in both cotyledon and leave senescence, whereas the data suggest involvement in seed development during fruit filling phase.

Aknowledgement:

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606/51. FREQUENCY OF OUTCROSSING AND ISOLATION DISTANCE IN FABA BEAN

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Summary:

Objectives

Faba bean (Vicia faba L.) is a partially outcrossing species requiring an isolation distance to maintain genetic purity when more than one variety is grown in field conditions. This information is crucial for seed growers and faba bean breeders.

Concise description of the work (materials & methods)

A study was conducted at the University of Sydney's Plant Breeding Institute, Narrabri, Australia over two years to examine the extent of natural outcrossing using a creamy white flower characteristic as a morphological marker, which is controlled by a single recessive gene. The white flowered genotype (IX225c) was grown in paired rows of 150 m length in four directions from a central 480 m2 plot of the normal flowered genotype PBA Warda. This resulted in four contiguous set of plots sown at right angles to each other, such that the resulting array formed cartesian axis (X, Y). Each axis radiated from a central plot of PBA Warda, a genotype with normal flower colour. A beehive was placed in the central plot at flowering time and natural pollination was allowed. At maturity, seed samples were taken from the white flower genotype at designated intervals along each axis and 100 seeds from each sample were grown in the glasshouse/birdcage to the 4-5 leaf stage in the following season. The proportion of plants displaying stipule spot pigmentation (normal flower colour and spotted stipule are linked) was used to determine the percentage of outcrossing.

Main Results

Maximum outcrossing of 2.28% occurred where both genotypes were grown side by side (0 m) and the degree of outcrossing decreased as the distance along each axis from the central plot increased. At 6 m distance the outcrossing was less than 1%, however on occasion it increased to 1% beyond a distance of 100 m, indicating the volatile and unpredictable nature of bee flights. Distance had the major effect on outcrossing, but direction (00, 900, 1800, 2700) and its interaction had no effect. The results suggest that to limit outcrossing to below 0.5%, a distance of more than 120 m between plots of different faba beans cultivars would be required.

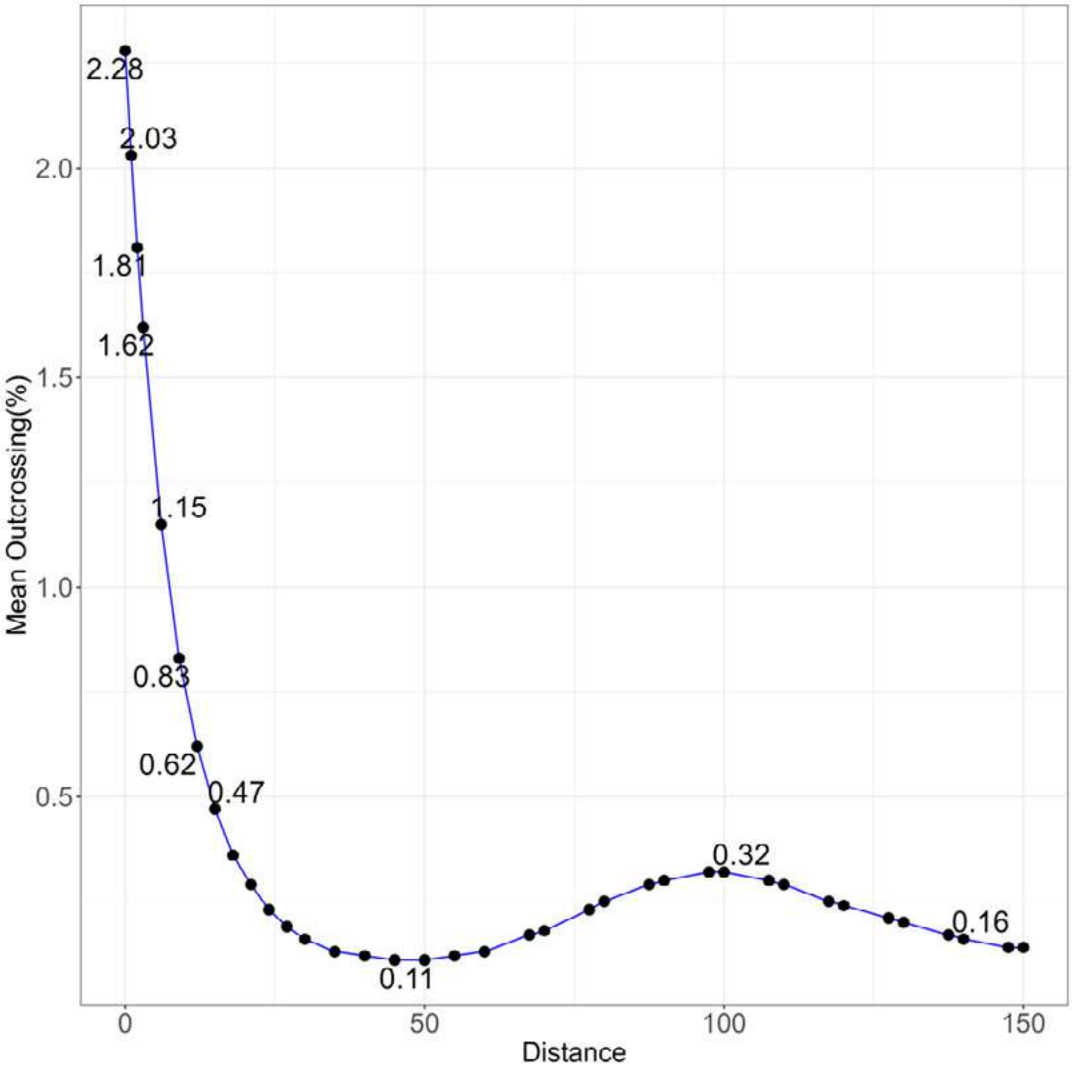

Figure. Mean outcrossing % after combining all directions within a distance (the data were combined because the direction was not significant).

Conclusions

Maximum outcrossing of less than 3% suggested that outcrossing in faba bean in Australia is limited and honeybees do not take long flights while foraging among faba bean plants. Thus, Australian faba bean genotypes are mostly self-fertile and a relatively narrow isolation distance will ensure self-fertilization in seed production and breeding programs.

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606/148. SHOULD WE MEASURE FORAGE LEGUME TOTAL CONDENSED TANNINS OR PROTEIN-PRECIPITATING POLYPHENOLICS?

Authors:

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Summary:

Objectives

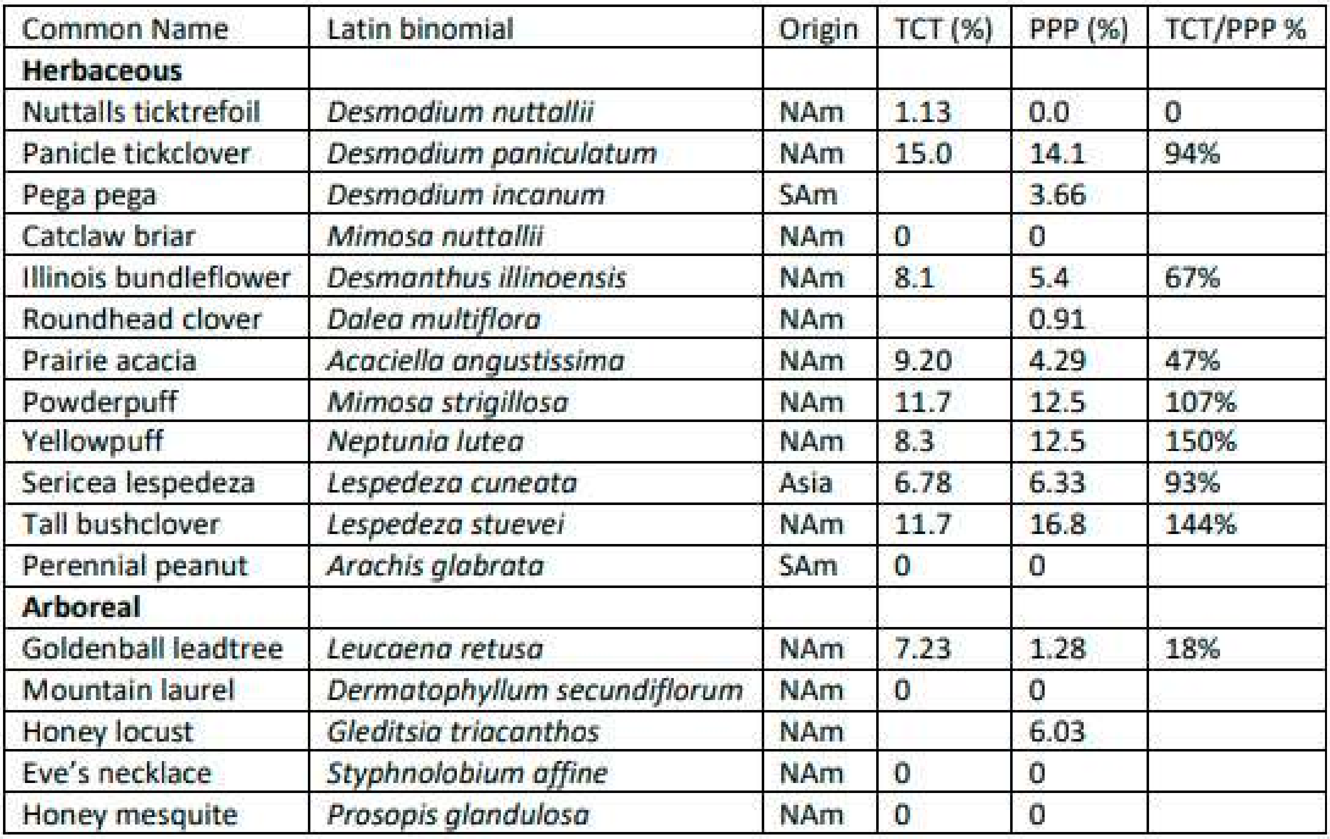
Legume research often reports forage total condensed tannin (TCT) content (percentage of dry matter) rather than biologically active fractions, defined here as protein-precipitating polyphenolics (PPP). As a result, dietary CT effects on animal nutrition are often inconsistent and misleading. For example, the widely reported ideal 5% TCT (dry matter basis) maximum diet inclusion in ruminant diets becomes nonsensical when comparing CT that are biologically active with those that are not. We suggest that research and resulting animal nutrition recommendations should more efficaciously focus on biologically active CT when recommending ideal minimum or maximum CT for dietary effects such as rumen-bypass protein, health benefits such as gastro-intestinal parasite control or environmental mitigation such as green-house gas emission reductions.

Concise description of the work (materials & methods)

We hypothesized that TCT content in a range of forage legume herbage differs from PPP content. We compared TCT (Terrill et al., 1993) versus PPP (Naumann et al., 2014) content in 26 forage legumes of various origins, functional groups and growth habits using self-standards for each species (Wolfe et al., 2008). Herbage (leaves and fine stems) were collected at vegetative and early flowering and dried in a forced-air oven set at 55oC.

Main Results

Fourteen of the legumes, including all annuals and all cool-season species (not in Table 1), contained no detectible TCT or PP. Of the nine perennial warm-season species for which both TCT and PPP content were available, six contained less PPP than TCT while three contained more PPP than TCT.



Common Name	Latin binomial	Origin	TCT (%)	PPP (%)	TCT/PPP %
Herbaceous					
Nuttalls ticktrefoil	*Desmodium nuttallii*	NAm	1.13	0.0	0
Panicle tickclover	*Desmodium paniculatum*	NAm	15.0	14.1	94%
Pega pega	*Desmodium incanum*	SAm		3.66	
Catclaw briar	*Mimosa nuttallii*	NAm	0	0	
Illinois bundleflower	*Desmanthus illinoensis*	NAm	8.1	5.4	67%
Roundhead clover	*Dalea multiflora*	NAm		0.91	
Prairie acacia	*Acaciella angustissima*	NAm	9.20	4.29	47%
Powderpuff	*Mimosa strigillosa*	NAm	11.7	12.5	107%
Yellowpuff	*Neptunia lutea*	NAm	8.3	12.5	150%
Sericea lespedeza	*Lespedeza cuneata*	Asia	6.78	6.33	93%
Tall bushclover	*Lespedeza stuevei*	NAm	11.7	16.8	144%
Perennial peanut	*Arachis glabrata*	SAm	0	0	
Arboreal					
Goldenball leadtree	*Leucaena retusa*	NAm	7.23	1.28	18%
Mountain laurel	*Dermatophyllum secundiflorum*	NAm	0	0	
Honey locust	*Gleditsia triacanthos*	NAm		6.03	
Eve's necklace	*Styphnolobium affine*	NAm	0	0	
Honey mesquite	*Prosopis glandulosa*	NAm	0	0	

Table 1. Total condensed tannins (TCT; Terrill et al., 1993) versus protein-precipitating polyphenolics (PPP; Naumann et al., 2014) content in 17 warm-season perennial forage legumes originating from North America (NAm), South America (SAm), the Mediterranean (MED) and Asia.

Conclusions

Our preliminary results indicated that TCT did not equate to biologically active polyphenolics as defined by PPP assay. We will continue to assay other species to build a more robust dataset comparing further forage legumes to discern patterns related to functional groups, origin, or growth habit.

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606/188. REACTIVE OXYGEN SPECIES PRODUCTION AND ANTIOXIDANT ACTIVITY DURING PEA SEED COAT DEVELOPMENT

Authors:

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Summary:

Objectives

Reactive oxygen species (ROS) are well-known oxygen-derived molecules that participate in a number of physiological processes, but also in plant stress-induced reactions. However, in higher concentrations, they can oxidize biomolecules and thus can be toxic to cells. In order to ensure redox balance and proper functioning of biological systems in plants, including seeds, mechanisms involved in the regulation of ROS concentration must be present. These defense mechanisms include antioxidants, molecules of both enzymatic and non-enzymatic nature capable of detoxifying ROS. So far, however, almost no information is available on their production and role in seed maturation and abscission. A mature pea seed consists of an embryo and a seed coat (SC), which protects the embryo from the effects of the external environment. In our study, we focused on the localization of ROS production during SC development, specifically in the hilum region, the place where the seed is connected to the maternal plant via the funiculus and where intensive transport of substances takes place in the early stages of seed development. Subsequently, during maturation and desiccation, the seed separates from the funiculus at this point.

Concise description of the work (materials & methods)

In this work, spectrophotometric determination of total antioxidant capacity, the concentration of total phenols, and peroxidases (POX) and polyphenol oxidase (PPO) activity were analyzed in the developing SC of cultivated (Cameor, JI92) and wild (JI64 and JI1794) pea genotypes differing in SC pigmentation and dormancy (Balarynová et al., 2022, Krejčí et al., 2022). The activity of POX and PPO and ROS production were also localized histochemically.

Main Results

Significant differences in studied parameters were found between the SC of the Cameor (non-pigmented SC) and those of JI92, JI64, JI1794 (pigmented SC). In contrast to the other genotypes, the SC of the Cameor genotype had a significantly lower concentration of non-enzymatic antioxidants, and very low PPO activity. On the other hand, the POX activity in Cameor SC was significantly higher compared to the other genotypes. Differences in enzyme activities between individual genotypes obtained by spectrophotometric measurement were also confirmed histochemically. Besides, the localization of ROS production was also studied in the hilum region during SC development. ROS production was most intense in the early stages of SC development and localized in the counter-palisade cells and in the vicinity of the tracheid bar in all studied genotypes, but a significantly higher signal was detected in the SC with pigmented hilum (JI64 and JI1794). During maturation, the ROS signal gradually completely disappeared.

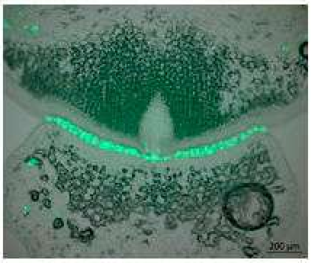
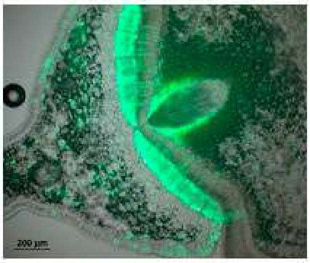
Species	*P. sativum*		*P. elatius*	
Genotype	Cameor	JI92	JI64	JI1794
	culture		wild	
	non-dormant		dormant	
Seed coat pigmentation	NO	YES	YES	YES
Hilum pigmentation	NO		YES	
				
Total antioxidant capacity	+	+++	++++	+++
Phenol concentration	+	+++	++++	+++
Polyphenol oxidase activity	+	++	++	+++
Peroxidase activity	++++	+	+	+
Reactive oxygen species localization (13 days after pollination)	 200 µm		 200 µm	

Figure: Properties and semi-quantitative comparison of monitored parameters in the seed coat of studied pea genotypes.

Conclusions

This work describes for the first time the antioxidant activity and ROS production during SC development of wild and cultivated pea genotypes. Thus, it was confirmed, that genotypes with pigmented SC are more similar in the studied parameters regardless of the degree of dormancy.

Acknowledgment: This work was supported by IGA _2023_22 and by Czech Science Foundation (19-07155S).

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606/226. ROLE OF THE SMALL INTERFERING RNAS IN EPIGENETIC REGULATION OF NODULE DEVELOPMENT IN MEDICAGO TRUNCATULA

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

The rhizobium-legume symbiosis is characterized by the formation of nodules. Different epigenetic actors are essential for nodule development in the model legume species Medicago truncatula, including the DEMETER (DME) demethylase and the DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) (Pecrix et al., 2022), the main DNA methylase of the RNA-directed DNA methylation (RdDM) pathway. RdDM mediates DNA hyper-methylation and subsequent transcriptional silencing of a subset of genes and many transposable elements through the production of 24-nucleotides heterochromatic small-interfering RNAs (24-nt hc-siRNAs). To date, two RNAseIII enzymes are known for playing a role in modulating 24-nt siRNA population in Arabidopsis thaliana: DICER-LIKE 3 (DCL3) and RNASE THREE LIKE 1 (RTL1). While DCL3 generates the 24-nt siRNA by specific cleavage of dsRNA substrates, RTL1 acts as a silencing suppressor by degrading those dsRNA, therefore indirectly impacting the population of 24-nt siRNAs (Shamandi et al., 2015). To unravel the role of 24-nt siRNAs in nodule development different genetic backgrounds affected in MtDCL3 have been generated. No obvious nodulation phenotype was observed in RNAi-MtDCL3 nodules. However, RNAi only triggered partial repression of MtDCL3 and 24-nt accumulation. To obtain DCL3 KO mutants we used CRIPSR Cas9 technology in transgenic roots. We screened for the mutagenesis efficiency of different guide RNAs, thanks to NGS. Phenotypical and molecular analysis of indel mutants are ongoing. In addition, Mt roots down expressing a nodule-specific orthologue of AtRTL1, MtRTL1b, develop abundant defective nodules, with dead bacteroids. Combined mRNA and small RNA-seq showed an over accumulation of 24-nt siRNAs associated to the repression of large set of genes (including several nodule cysteine-rich genes). Additional genome wide analysis of DNA methylation and chromatin conformation will allow to decipher to what extent the 24-nt siRNAs and related pathways are involved in epigenetic regulation of rhizobium-legume symbiosis.

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606/24. SEED-ASSOCIATED MICROBIOTA FROM LEGUMES: AN UNEXPLORED WORLD FULL OF NEW CHALLENGES AND OPPORTUNITIES

Authors:

Juan Ignacio Vílchez[1], Marla Niza Costa[2], Raquel Teixeira[2], Tatiana Gil[2], Andre Sousa[2], Ana Sofia Rodrigues dos Santos[2], Inês RebeloRomão[2], María Victoria Ferrer[2], Cristina Sequero López[3]

Work centre:

(1) Portugal, (2) iPlantMicro Lab, Instituto de Tecnologia Química e Biológica (ITQB)-NOVA, (3) GeoBioTec, NOVA School of Sciences and Technology, Universidade NOVA de

Summary:

Objectives

The study of biodiversity in plant-associated microbiota has allowed us to compile useful knowledge to develop new biotechnological approaches for agricultural management. Interaction areas such as the phyllosphere and rhizosphere are being intensely addressed; however, there are other tissues of special interest as the seeds. As a hosting location, seeds are challenging, making them a highly variable environment, where the microbiota is as well very few consistently happening. However, studies on large samples of seeds have shown interesting patterns (Simonin et al. 2022). Among them, the presence of pathogens is relevant to avoid dispersing. In addition, it's catching more attention the presence of beneficial bacteria (biocontrollers, biofertilizers and tolerance-enhancers) that mother plant may be actively selecting for the next generation. The hypothesis contemplates specific recruiting pathways and signaling traits by which the plant is preparing a core microbiota to better deal with stressful situations. This would participate in the natural variances that we can observe in nature, as a complement or a cause of the genetic background of each individual. Despite the complexity of the approach, seed-associated microbiota may be a very relevant way to discern evolutionary patterns, inheritance conditioners, and enhanced plant-microbe interaction events.

Concise description of the work (materials & methods)

Nowadays, we count on the necessary technology to discern these traits and benefit from them in agriculture management. Here, the use of high-performance metabolomics (QqQ-MS), full-sequencing technologies (such as metagenomics), and in vivo tracking methods are helpful in elucidating how these phenomena occur. Our proposal also includes the use of different sources to compare traits, the use of culturable communities to prepare treatments, and high-throughput phenotyping to complement the research and obtain significant insights. In this sense, our approaches include the study of microbiota in ancestors, sampling of wild varieties adapted to stressful conditions, and comparison of commercial varieties with locally adapted crops (Niza-Costa et al. 2022).

Main Results

From this perspective, we have been able to isolate core communities from wild, rainfed-adapted alfalfa (Medicago sativa) and pitch clover (Bituminaria bituminosa), screened the best candidates, and used them as treatments to enhance the performance of drought-sensitive lentil plants (Lens culinaris) (Fig. 1). Moreover, we compared the microbiota present in lentil, bean, and chickpea ancestors to understand the effect of domestication on the interacting populations of their seeds. These studies have allowed us to discern the lost biodiversity that may be recovered to help commercial crops perform better under harsh conditions. Finally, we are working with local varieties of leguminous (beans and peavines) in Aragón (Spain) and other regions that are better adapted to restrictive conditions to study their seed microbiota as future biotreatments and to understand the effects of targeted breeding.

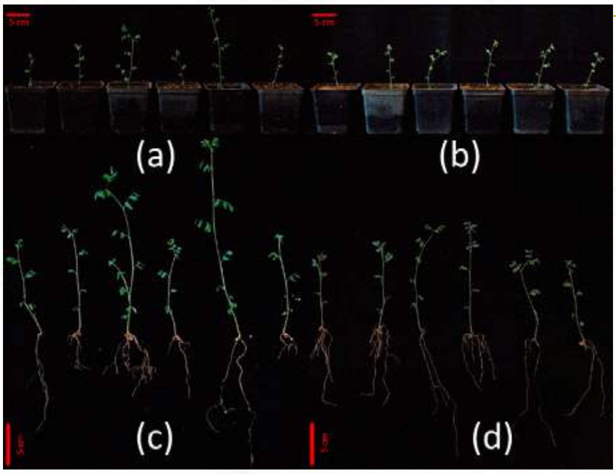

Figure 1. Effects of inoculating lentils with strains isolated from wild, rainfed-adapted legumes. The pictures show the side andfull-plant view of treated seedlings under irrigation (a, c), and under drought (b, d). The treatment, from left to right are labelled as 'Mock' for control set; 'PxM1C', for seedlings treated with Paenibacillus xylanexedens MS M1-C; 'SmM1B', with Stenotrophomonas maltophilia MS M1-B; 'PpOA', with Paenibacillus pabuli BB Oeiras A; 'PaB2A', with Paenibacillus amylolyticus BB B2-A; and 'EhB2C', with Enterobacter hormaechei BB B2-C.

Conclusions

Although much more research is necessary in the field and the incorporation of high-fidelity techniques, the potential of studying seed-associated microbiota has shown a remarkable potential to address some of the climatic challenges and pathogen spreading processes that our crops are going to be increasingly exposed to during the next decades.

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606/37. CATCHING RHIZOBIA TO INTRODUCE HIGH PROTEIN CONTAINING SOYBEAN FOR A SUSTAINABLE AGRICULTURE IN EUROPE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

To develop sustainable protein products, Europe would strongly benefit from soybean production at northern latitudes. However, soybean is not adapted to these environmental conditions and therefore the cultivation of protein-rich soybean is challenging. While several adapted soybean varieties have been bred, to guarantee consistent high-protein beans, plants need effective interaction with suitable soil bacteria that can fix nitrogen in root nodules. These nitrogen-fixing bacteria allow legumes to act as natural nitrogen fertilizers and green indicators of soil nutrition, and as such improve crop yield without the damaging effects of chemical nitrogen fertilization. Likewise, legumes contribute to solving major environmental challenges such as nitrogen pollution and declining soil quality. The current commercial inoculants are not adapted to cultivation under the local soil and environmental conditions of North-Western Europe. This hampers the interaction and leads to insufficient bean protein content for human food consumption. Local strains, adapted to our conditions may be more competitive and have a superior positive effect on soybean production. We set up a citizen science project to trap nitrogen-fixing bacteria that nodulate locally grown soybean. To have access to a large geographical gradient and different soil types in Flanders, over one thousand citizens across Flanders were recruited to grow and monitor soy plants in their garden, frequently entering data regarding plant phenotype into a platform specifically designed for the project. The outcome of the project in terms of microbial discovery but also correlations between soil parameters such as nutrient levels, soil texture, and nodulation performance will be discussed.

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606/41. NITROGEN FIXATION OF SOYBEAN AND LUPINE IN SWEDEN: DOES CULTIVAR MATTER?

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The cultivation of soybean and lupine in Europe has been on the rise in recent years. In Sweden, only a minor percentage of arable land is used to grow grain legumes. Colder and temperate climates, such as in Sweden, can limit the potential crop yield. Aside from climate, the presence of weeds can also act as a stress to the plant, for instance, by competing for resources. Abiotic stresses have been shown to negatively impact nodulation in several legumes and actinorhizal plants. As a consequence of the reduced nodulation, in terms of nodule size and number, it could be expected that nitrogen fixation is also reduced in such plants. We investigated the effect of climate and weed management on different cultivars of soybean, lupine, and faba bean grown in different sites in Sweden. Plants were grown in 2020 and 2021. The root systems of plants were collected, and nodulation was assessed based on total nodule weight, nodule number, and the individual nodule area. From the grains, the isotope ratios and mass fractions of C and N were measured, and the 15N/14N ratio was used to calculate the amount of nitrogen fixed. Our results show that both the field site, which differed in local climate, as well as weed management, affected nodulation, total nitrogen content, and amount of fixed nitrogen. Most importantly, different cultivars responded differently to these parameters. This indicates the importance of the choice of cultivar of soybean and lupine in organic farming.

606/69. DNA METABARCODING FOR THE CHARACTERIZATION OF THE SOIL BACTERIAL AND FUNGAL COMMUNITIES UNDER ORGANIC AND CONVENTIONAL FARMING SYSTEMS

Authors:

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Summary:

Objectives

The aim of this work was to compare the impact of management practices (conventional versus organic) of common bean (Phaseolus vulgaris) crops on the diversity of the bacterial and fungal soil communities.

Concise description of the work (materials & methods)

Two nearby soils dedicated to growing beans were analyzed and compared, one manages under conventional practices during the last 20 years and another manages under organic practices during the last 6 years. Five independent samples per soil were taken in the summer of 2022 during the flowering time of the bean crop. The composition and structure of the bacterial and fungal communities were assessed through the amplification and sequencing (Miseq) of the target genes 16S (bacteria; Barry et al. 1991) and ITS (fungi; Schoch et al. 2012).

Main Results

A total of 5166 phylotypes were detected in the 16S fraction, being significantly more abundant in the conventional soil (Figure 1A; Negative Binomial Model, $p<0.007$). In the ITS fraction 1214 phylotypes were detected, being significantly more abundant in the organic soil (Figure 1A; Negative Binomial Model, $p<0.001$). Taking into account the number and the abundance of phylotypes (Evenness), no significant differences between conventional and organic soils were detected for the bacterial fraction (Beta regression, $p=0.31$), while significant differences were observed for fungi (Beta regression, $p=8.3e-09$). Differences in community composition among soil types were evaluated by using the Bray-Curtis distance and visualized in a PCoA plot (Figure 1B). Differences between soils were observed for the bacterial community (PERMANOVA test; $p =0.008$) and for the fungal community (PERMANOVA test, $p=0.007$). Respect the taxonomic profile, in the conventional soil the most abundant bacterial phylum was Planctomycetota (16.91%), followed by Proteobacteria (15.53%). In the organic soil, the most abundant phylum was Proteobacteria (17.83%) followed by Acidobacteriota (15.38%). For fungi, Ascomycota (49.1%), and Morterellomycota (31.4%) were the most abundant in the conventional soil, while in organic soil Mortierellomycota (26.1%) and Basidiomycota (20.3%) were the most abundant. Based on the differential abundance of taxonomic profiles, 7 bacterial phyla showed significant differences between soils, 7 were more abundant in conventional soil (Actinobacteriota, Chloroflexi, Gemmatimonadota, Methylomirabilota, Patescibacteria, Planctomycetota, Sumerlaeota) and 8 more abundant in organic soil (Crenarchaeota, Acidobacteriota, Bacteroidota, Desulfobacterota, Myxococcota, Nitrospirota, Proteobacteria, Verrucomicrobiota). For the ITS fraction, 7 phyla showed significant differences between soils, 2 were more abundant in conventional (Ascomycota, Kickxellomycota) and 5 more abundant in organic (Basidiomycota, Glomeromycota, Chytridiomycota, Rozellomycota, Aphelidiomycota).

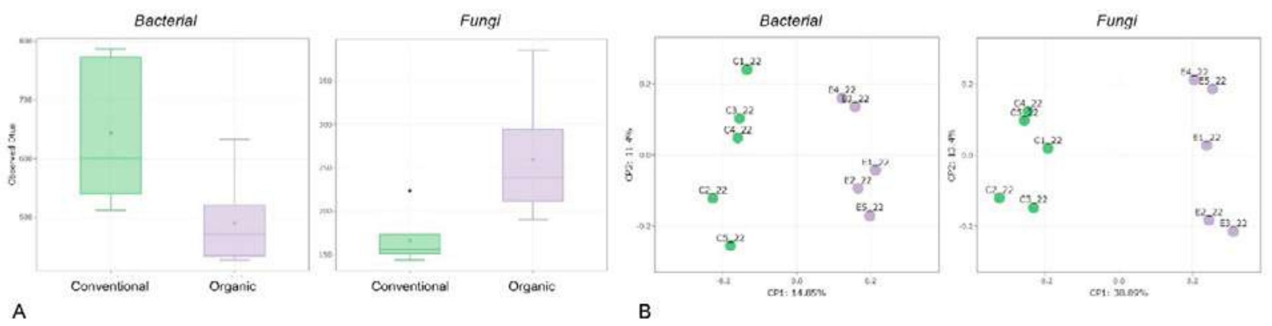

Figure 1. A) Alfa diversity. Boxplots showing the differences in observed OTUs between the experimental groups conventional vs organic management. B) Beta diversity. PCoA plots based on Bray-Curtis distances between samples. C, samples of the conventional soil; E, samples of the organic soil. Samples under the same management clustered together.

Conclusions

Results evidence that soil management conducted during the bean crop affected the alfa and beta diversity observed in the soil. In the organic fields, fungal diversity increased, while in conventional fields bacterial fraction is higher.

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606/104. NATIVE TRICHODERMA STRAINS TO IMPROVE DEFENSE RESPONSE AND DEVELOPMENT OF DRY BEAN (PHASEOLUS VULGARIS)

Authors:

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(1) Universidad de León. Spain

Summary:

Objectives, Description, Main Results & Conclusions

The bean is the most important leguminous grain for direct human consumption. The province of León produces approximately 59% of the dry beans in Spain. The yield and quality of the crop is threatened by phytopathogenic fungi. Trichoderma spp. are considered among the most promising and frequently studied biological control agent. In the case of bean diseases, some Trichoderma species have been shown to inhibit development of Rhizoctonia solani and to reduce the effects of this pathogen on plant growth (Mayo-Prieto et al., 2015). Among the Trichoderma secondary metabolites, trichothecenes are a family of approximately 200 toxic sesquiterpenoids that have attracted significant attention in recent years. The objective is select Trichoderma strains to bean defense. Trichoderma arundinaceum trichothecenes, e.g., harzianum A (HA), induce expression of plant genes related to salicylate (SA) and jasmonate/ethylene (JA)/(ET) defense pathways. In addition, HA exhibits strong antifungal activity against several important phytopathogenic fungi. Trichoderma strains were isolated from bean fields located in a region of northwestern Spain with the PGI designation Alubia de La Bañeza-León. Native Trichoderma isolates stimulated germination of bean seeds and the growth of above ground parts of the plants Trichoderma arundinaceum strains showed production of the trichothecene harzianum A (HA) and trichodermol, an intermediate in the HA biosynthesis. HA production by these isolates correlated with significant in vitro antifungal activity against R. solani and S. sclerotiorum. These results underscore the interest in searching for Trichoderma species capable of producing nontoxic trichothecenes to trigger plant defense responses without negatively affecting their germination and development.

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606/106. BIOPROTECTIVE ROLE AND ENHANCEMENT OF BIOLOGICAL NITROGEN FIXATION BY SAPROPHYTIC FUNGI IN PHASEOLUS VULGARIS PLANTS AFTER PENDIMETHALIN APPLICATION

Authors:

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Work centre:

(1) Universidad de Granada, (2) Estación Experimental del Zaidín -CSIC

Summary:

Objectives

The aim of this work was to study the effect of pedimethalin, one of the most widely used herbicides, on the growth and legume-rhizobia spp. symbiosis in Phaseolus vulgaris plants, and the role of two saprophytic fungi (Coriolopsis rigida and Fusarium lateritium) in degrading the herbicide and in promoting BNF in plants under herbicide application.

Concise description of the work (materials & methods)

Pregerminated P. vulgaris plants were grown in individual pots of 1.8 l filled with a mixture of soil:perlite (3:1 v/v) during 28 days in a controlled environmental chamber. Pendimethalin was applied one day after sowing following the manufacturer´s instructions at the recommended label dosage. Half of the pots were innoculated with two saprophytic fungi (Coriolopsis rigida and Fusarium lateritium) and the other half were not innoculated.

Main Results

After 28 days, P. vulgaris plants showed a significant growth reduction of 29% in control treatment (without fungi addition) when pendimethalin was applied. However, when fungi were added to the soil treated with pendimethalin they sharply decreased herbicide concentration in soil and protected plants from growth inhibition induced by its presence, especially in the case of C. rigida. The same was observed in nodulation of P. vulgaris plants, which was significantly inhibited by pendimethalin (about 50% NFW and NN reduction), while fungi addition prevented from the reduction in both NFW and NN in presence of the herbicide. Finally, nitrogen fixation rate was also inhibited in the presence of pendimethalin (37% reduction), although this difference was not statistically significant, whereas when fungi were present in soil nitrogen fixation rate was unaffected by pendimethalin.

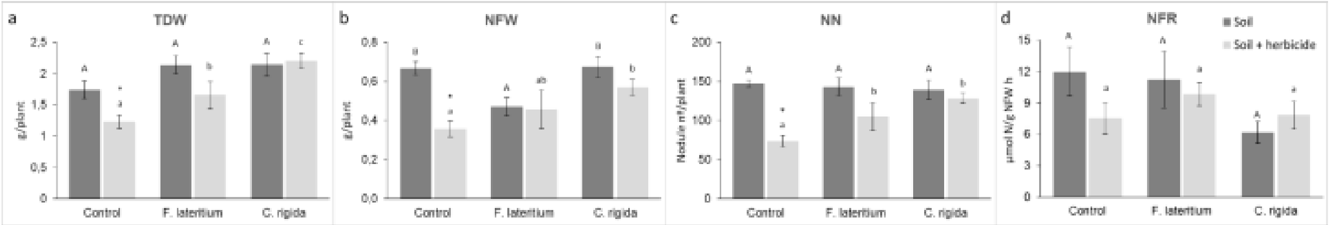

Figure. Main parameters measured in P. vulgaris plants treated with pendimethalin and in the presence of two different saprophytic fungi in a controlled chamber study. (A) Total dry weight (TDW), (B) Nodule fresh weight (NFW), (C) nodule number (NN), and (D) Nitrogen fixation rate (NFR) of P. vulgaris plants. Data represent means ± S.D. (n = 12) after a period of 28 days of growth. Mean values with different letters were significantly different (P <0.05; Duncan's test). Uppercase letters are used to compare differences between treatments without pendimethalin application (dark grey bars); lowercase letters are used to compare differences between treatments with pendimethalin application (light grey bars). Asterisks (*) indicate significant differences between the same treatment with and without pendimethalin application.

Conclusions

These results confirm that bioremediation of soils contaminated by herbicides using saprophytic fungi with the ability to detoxify herbicides present in the soil can be an effective strategy to reduce their toxic effects and to improve the biological nitrogen fixation process. Especially, Coriolopsis rigida induced a greater bioprotective role against pendimethalin toxicity in P. vulgaris plants.

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606/118. BIOLOGICAL NITROGEN FIXATION BY SOYBEAN (GLYCINE MAX [L.] MERR.), A NOVEL, HIGH PROTEIN CROP IN SCOTLAND, REQUIRES INOCULATION WITH NON-NATIVE BRADYRHIZOBIA

Authors:

Chrizelle Krynauw[1], Marta Maluk[1], Maddy Giles[1], Grace Wardell[2], Aminin Akramin[3], Francesc Ferrando-Molina[4], Ashley Murdoch[1], Marta Barros[5], Pete Iannetta[1], Euan James[1]

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Summary:

Objectives, Description, Main Results & Conclusions

It is currently not recommended to grow soybean further than 54° North, but climate change and the development of new high latitude-adapted varieties have raised the possibility of introducing it into Scotland to be used as a novel high protein crop deriving most of its nitrogen (N) requirements through biological N fixation (BNF). The feasibility of such an introduction was evaluated via field trials in 2017 and 2018 near Dundee (56.48°N). As there are no native soybean-nodulating bacteria (SNB) in UK soils, soybean-compatible inocula would be necessary. In 2017, three commercial inoculants containing elite Bradyrhizobium strains significantly increased plant biomass in plot trials with the soybean 000 maturity group variety, ES Comandor. Rhizobia isolated from the resulting nodules were identified as the original inoculant species, B. diazoefficiens and B. japonicum. One inoculant (Rizoliq Top) was used for larger-scale trials in 2018; inoculation doubled the grain yield to 1 t ha-1 compared to the uninoculated crop. The inoculated soybean obtained most of its N through BNF in both years regardless of plant genotype i.e. >73% Ndfa, with BNF contributions to aerial biomass exceeding 250 kg N ha-1 yr-1 in 2017 and that to grain 50 kg N ha-1 yr-1 in 2018. These data suggest that N-fixing soybean could be grown in Scotland without the addition of mineral N-fertiliser. The potential for the Bradyrhizobium inoculant strains to survive in soils was also demonstrated through the detection of the inoculant strain B. diazoefficiens SEMIA 5080 at relatively high populations (104 g-1 dry soil) using a qRT-PCR method designed with SNB-specific nodZ primers.

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606/135. GENETIC BASES OF VARIATION IN PLANT ARCHITECTURE AND RHIZOBIAL PARTNER CHOICE ALONG THE PEA DOMESTICATION GRADIENT

Authors:

Virginie Bourion[1], Jonathan Kreplak[1], Damien Ollivier[1], Mickael Lamboeuf[1], Marion Dalmais[2], Karine Heulin-Gotty[3], Stephane Boivin[3], Marc Lepetit[4], Maxime Bonhomme[5], Frederic Debelle[6]

Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes play an increasingly important role in sustainable agriculture due to their ability to form a beneficial symbiotic interaction with nitrogen-fixing Rhizobium bacteria. Legumes are also a valuable source of protein for both feed and food, but are not grown as extensively as expected in Europe due to their high yield variability. Many genomic approaches are being developed to improve stress tolerance traits. However, to date, little attention has been paid to improving the interaction between symbiotic partners. The establishment of the symbiotic interaction is a complex evolutionary process in which the interests of both partners are not always aligned. No evidence was found in pea for co-selection of competitiveness for nodulation and nitrogen (N) fixation efficiency (Bourion et al., 2018). Furthermore, several data indicated that N fixation and plant growth could be suboptimal in fields where pea is exposed to populations of heterogenous rhizobial strains with contrasting effects on nodule, root and shoot development (Laguerre et al., 2007). We performed Genome-Wide Association Studies to decipher the genetic determinants and relationships between the complex trait of pea choice between rhizobial strains in mixture and plant architecture. A large panel of 340 pea accessions including very diverse cultivars, wild accessions and landraces, all inoculated with the same mixture of 28 diverse rhizobial strains, was grown in two successive experiments, on a high throughput non-destructive phenotyping platform. The proportion of each strain in the nodules of each pea at harvest was determined by DNA metabarcoding, and 20 variables of nodulated root architecture or plant growth traits were estimated by image analysis or measured. The results highlighted differential variation and largely uncoupled genetic bases between rhizobial partner choice and architectural or growth traits, along the pea domestication gradient.

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606/141. EVALUATION OF SYMBIOTIC POTENTIALS OF ADAPTED BRADYRHIZOBIUM STRAINS ON SOYBEAN [GLYCINE MAX (L.) MERRILL] YIELD IN NORTHEAST GERMANY

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Grain legume production in Europe remains low despite their numerous benefits. They account for less than 2% of European cropland. In particular, soybean, a highly valuable grain legume, occupies only a small portion of total arable land in Germany, despite its potential and high demand. Commercial inoculants are commonly used to inoculate field-grown soybeans in Europe. However, nodulation efficiency has been consistently poor (Schmidt et al. 2015), necessitating the need to explore strategies to increase its growth and productivity. The study aimed: (i) to evaluate the ability of adapted Bradyrhizobium strain(s) to maximize soybean grain protein, grain and protein yields in field conditions in northern Germany; and (ii) to evaluate the extent to which the symbiotic performance of the strains is impacted by soil moisture. Field trials were set up in Müncheberg, Germany, at sites with no soybean cultivation history, from 2020 to 2022. Three Bradyrhizobium isolates from Northern Germany (GMF14, GMM36, and GEM96) plus a reference strain (Bradyrhizobium diazoefficiens USDA110) were tested in combination with three soybean cultivars (Merlin, Sultana, and Siroca). Among the strains, GMF14 inoculation resulted in slight increases in grain yield. The average grain yield ranged from 2,168 kg ha−1 in GMF14 to 1,902 kg ha−1 in GEM96. While irrigation did not significantly affect crude protein content, it resulted in a 27% and 16% increase in grain and protein yields, respectively. Although irrigation enhanced grain and protein yield in all strains, there was a more pronounced effect on the commercial USDA110 strain compared to GMF14 and GEM96. This implies that GMF14 and GEM96 are more adaptable to the drought conditions in Northeast Germany than the standard USDA110.

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606/147. DAT PROTEIN IS INVOLVED IN HOMOSERINE CATABOLISM AND CONTRIBUTES TO SYMBIOTIC PERFORMANCE AND COMPETITIVENESS IN THE RHIZOBIUM LEGUMINOSARUM/PISUM SATIVUM SYSTEM

Authors:

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Work centre:

(1) Universidad Politécnica de Madrid. Spain

Summary:

Objectives

Rhizobium leguminosarum bv. viciae (Rlv) is an α-proteobacterium capable of inducing nitrogen-fixing root nodules in association with Vicia, Pisum, Lens and Lathyrus. Although the mechanisms governing the exchange of signals and nutrients between Rhizobium and legume plants have been subject to detailed studies, little is known about the differences in the environment provided to a given microsymbiont by different legume hosts and how rhizobia adapt to them. Comparative proteomic analysis of bacteroids induced by Rlv UPM791 in lentil and pea plants showed a significant number of host-dependent expression proteins. One of these proteins, overexpressed in pea bacteroids, is encoded on the symbiotic plasmid and shows a high similarity to a diaminobutyrate-2-oxoglutarate aminotransferase (DABA-AT). This enzyme catalyses the reversible transfer of the amino group from glutamate to L-aspartate-β-semialdehyde (L-Asa), resulting in 2,4-diaminobutyrate (DABA) and 2-oxoglutarate. DABA-ATs have been described in several biological processes in endosymbiotic bacteria, including ectoine metabolism and the synthesis of siderophores, polyamines and pantothenate. The presence of DABA-AT activity was demonstrated in Rlv UPM791 bacteroid cell extracts and cultured cells, and also in purified protein, so this protein was named as Dat. The general objective of this work is to study the functional role of Dat protein in the adaptation of the endosymbiotic phase of Rlv UPM791 to different hosts (pea and lentil).

Main Results

The results obtained demonstrated that the expression of dat was strongly induced in the central zone of pea but not lentil nodules. In addition, analysis of a dat-defective mutant revealed that the protein contributes to symbiotic effectiveness and competitiveness in symbiosis with pea but not with lentil plants (Figure). A comparative metabolomic profiling analysis revealed that deletion of dat leads to significant differences in bacteroids from pea, but not from lentil. This analysis revealed that the mutation in dat was associated to the absence of DABA in pea nodules, suggesting that the DABA-AT reaction is directed towards DABA production from L-Asa. This analysis also showed the presence of L-Homoserine (L-Hse) in pea bacteroids, and reduced levels of β-alanine (a precursor of pantothenate) in pea bacteroids of the dat mutant. Interestingly, the mutant was impaired in growth in cell cultures with L-Hse as nitrogen source. Rlv strains have been described as auxotrophic for pantothenate, but inclusion of DABA as nitrogen source in minimal medium with glucose as carbon source completely suppressed this auxotrophy, and to a lesser extent when L-Hse was included as nitrogen source. These data indicate that Rlv UPM791 Dat is part of an adaptation mechanism of this bacterium to a L-Hse-rich environment such as the pea nodule and rhizosphere. In order to study the possible ecological role of Dat in soil rhizobial populations, a collection of 213 rhizobial isolates was obtained from Tierra de Campos (Valladolid) soils using pea and lentil as trap plants. The analysis of the presence of the dat gene in these isolates suggests that there is no positive selection for this gene in pea isolates.

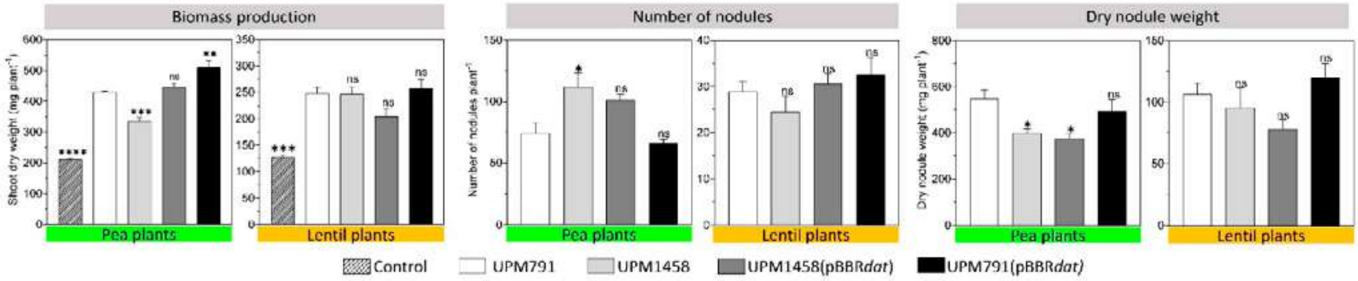

Figure. Effect of dat gene on the symbiotic phenotype of Rlv UPM791 in pea and lentil plants. Values (±SEM) of shoot dry weight, nodule number and fresh nodule weight. Differences were analyzed by one-way ANOVA with Dunnett`s for multiple comparisons in relation to UPM791. Strains: UPM791 (wild type), UPM1458 (UPM791dat). Plasmid pBBRdat contains an intact copy of the dat gene. Control: non-inoculated plants. Level of significance in statistical analyses: (****) P<0.0001, (***) P<0.001, (**) P<0.01, (*) P<0.05; (ns) no statistical significance (p>0.05).

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606/26. ISOLATION AND IDENTIFICATION OF EFFICIENT COMMON BEAN ENDOSYMBIONTS WITH REDUCED CAPACITY TO PRODUCE THE GREENHOUSE GAS NITROUS OXIDE

Authors:

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Summary:

Objectives

The ability of legumes to form N2-fixing symbiosis with soil bacteria globally named as “rhizobia” reduces the dependence of environment polluting nitrogen fertilizers decreasing greenhouse gas emissions, particularly nitrous oxide (N2O), as well as protecting ground water from toxicity while improving soil fertility. However, legume crops also contribute to N2O emissions by providing N-rich residues for decomposition and directly by some rhizobia that are able to denitrify under free-living conditions or under symbiotic association with legume plants (Delgado et al., 2007). Consequently, it is crucial to identify symbiotically effective rhizobia with a limited N2O output for a more sustainable agriculture. This study aims to select native, competitive and efficient common bean endosymbionts with reduced capacity to produce N2O study aims to select native, competitive and efficient common bean endosymbionts with reduced capacity to produce nitrous oxide.

Concise description of the work (materials & methods)

We utilized the “plant-trap” method to isolate native rhizobia strains from soil samples taken from Misión Biológica de Galicia (MBG-CSIC). Two common bean (Phaseolus vulgaris L.) varieties, Galaica and Matterhorn, served as model trapping plants. Genomic DNA from bacteria isolated from sterilized fresh nodules was subjected to 16S rRNA and nodC PCR analyses. Several isolates were identified, including Burkholderia alba and Rhizobium leucaenae. To check their N2-fixing capacity, both isolates were individually and jointly used to inoculate common bean (Matterhorn) plants that were watered with a N-free nutrient solution and cultivated in a growth chamber for 25 days, until the V4-R5 vegetative stage. Rhizobium etli CFN42 served as a control.

Main Results

We found that plants inoculated with R. leucaenae alone or with B. alba exhibited superior development, based on trifoliate leaves, flower count and shoot biomass than the control plants inoculated with R. etli CFN42 (Figure 1). The physiological response of the plants is currently being quantified by analysing; (i) nodulation (ii) plant dry weight (iii) total N content, and (iv) leghemoglobin content in nodules. N2O emission by root nodules will be also measured as previously described by Tortosa et al. (2015).

Figure 1. An overview of the P vulgaris plants inoculated with R. etli, B. alba and R. leucaenae

Conclusions

These results allow us to propose R. leucaenae as a symbiotically efficient biofertilizer for common beans.

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606/108. SELECTION OF NATIVE NITROGEN-FIXING RHIZOBIA FOR IMPROVED LEGUME-CACAO CO-CULTURE IN ECUADOR

Authors:

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Summary:

Objectives

The objectives of present research are i) to determine if soils of Ecuador dedicated to CN culture contain native rhizobia able of symbiotic nitrogen fixation with Phaseolus vulgaris, Cajanus cajan, or Vigna unguiculata; and ii) isolate, identify and select native rhizobial strains highly efficient in symbiosis with local varieties of these legumes.

Concise description of the work (materials & methods)

In Ecuador, the aroma fine Cocoa Nacional (CN) is grown mainly in low-input, average 2 ha size farms, located at the lowlands, and represents an important commodity for small-scale farmers. However, years of monoculture under traditional agricultural systems have caused a severe soil degradation, prompting the application of greater doses of synthetic fertilizers to enhance yields. Specifically, N-fertilizers are the source of greenhouse gas emissions (GGE). On the other hand, there is an enormous pressure from the UE for the imported CN, used in the chocolate production, to be of organic as well as of sustainable origin. For this, an increase in the overall N-use efficiency of the CN plantations via intercropping with N2-fixing legumes is one of the most effective approaches to recover eroded soils while decreasing GGE. The success of this approach requires that the N2-fixing capacity of the legume(s) is optimally harnessed.

Main Results

Soil samples were collected from a >10-year-old cocoa field (S1) or a nearby deforested area (S2), with no history of legume cultivation nor fertilization for over a decade. Black bean (P. vulgaris) cowpea (Vigna unguiculata) and pigeon pea (Cajanus cajan) plants were grown in 1 Kg plastic bags containing these soils, under open environment conditions. Plant root nodulation as well as aerial biomass were determined. Prolific nodulation in pigeon pea, black bean and cowpea plants grown in S1 was observed, correlating with a higher aerial biomass accumulation compared to plants grown in S2.

Bacteria from individual nodules were isolated and purified. Genomic DNAs from individual isolates were obtained and ERIC and BOX fingerprints obtained. In addition, 16S rRNA, glnII and recA gene fragments were PCR-amplified and sequenced. Phylogenetic analyses clustered most isolates in genus Bradyrhizobium, but some were related to genus Rhizobium.

Conclusions

This supports the existence in S1 of a great biodiversity of rhizobial strains capable of establishing effective symbiosis with these three grain legume species.

Differences in nitrogen-fixing efficiency also appear among isolates, suggesting there is room for selection of superior legume symbiotic rhizobia that can help to the establishment of high nitrogen-fixing legumes intercropped with CN.

Bibliography

- *This work was supported by grant COOPB20592 (CSIC).*

606/138. NOVEL SYMBIOTIC MICROBIOTA ON SEVERAL LEGUMES AT THE MBG-CSIC (SPAIN)

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes have capacity to stablish N2-fixing symbiosis with rhizobia reducing the use of polluting nitrogen fertilizers, decreasing greenhouse gas (GHG) emissions and protecting ground water from toxicity while improving soil fertility. The use of N-fixing bacteria is not generalized due to their specialization in the symbiotic association with legume species, being a priority the availability of promiscuous bacteria capable of nodulating with different legumes. Due to the interest that these bacteria represent for agriculture, being used as inoculants (bio-fertilizers) for crops, extensive research has been carried out on this symbiotic system.

The objective of this research was to study the nodulation capacity of Burkholderia alba (Lee et al. 2018) found in the soils of the MBG-CSIC in 2020. An experiment has been carried out in greenhouse with 17 different legumes (common bean, pea, chickpea, pigeon pea, cowpea, soybean, vetch, bitter vetch, yellow vetch, lentil, red pea, lucerne, faba bean, carob, grass pea, peanut and blue lupine) under 3 different conditions, a control 0, a control N and inoculated with B. alba.

B. alba was isolated from common bean, cowpea and pea nodule and identified by molecular techniques. In chickpea, soybean and lucerne no nodules were found in their roots. It seems that B. alba is a bacterium that persists in the seed since it also appears in the 0 and N control plants. Initial data indicate that B. alba could be a promiscuous bacterium, capable of nodulating at least three different legume species. Its efficiency for symbiotic nitrogen fixation and its ability to promote plant growth need further research.

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Session 9 Understanding and enhancing legume crop tolerance to abiotic stress

606/47. PROBING THE ROLE OF miRNAs IN THE EVOLUTION OF CLUSTER ROOTS IN WHITE LUPIN

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Work centre:

(1) Institute for Plant Sciences of Montpellier, Univ Montpellier, CNRS, INRAE, Institut Agro. France, (2) Institute of Plant Sciences Paris-Saclay, Gif-sur-Yvette-France.

Summary:

Objectives, Description, Main Results & Conclusions

Phosphorus (P) is an essential macronutrient for the production of food crops and the demand for P fertilizer is increasing worldwide. Earth's P is being depleted at an alarming rate urging the need to find solutions for crops to be more P efficient. White lupin can form specific organs constituted by numerous short lateral roots, the so-called cluster roots (CRs), as an adaptation to low phosphate availability in the soil, allowing an efficient acquisition of this nutrient, which is fundamental for plant growth and development. MicroRNAs are good candidates for the control of root development and its response to phosphate starvation by playing a pivotal role in regulating gene expression during development and in response to environmental cues. We performed a complete transcriptome analysis covering different stages of CR development in wild-type white lupin plants and in the constitutive cluster root (ccr1) mutant, which produces numerous CRs in the presence of phosphate (repressive conditions). The detailed analysis led us to identify both many conserved but also novel miRNAs with specific interesting profiles along the developmental series. Expression and functional analysis of candidate miRNAs revealed that the well-conserved miR396 would be playing a role in CR development different from its well-known function in root growth regulation. Hence, variation in gene regulation through miRNAs may be linked to the evolution of cluster roots in white lupin. A better understanding of the biological phenomenon that leads to the development of CRs will help to select regulatory networks with potential use in the improvement of relevant agronomic traits.

606/113. TRANSCRIPTOMIC AND ECOPHYSIOLOGICAL ANALYSES REVEALED CONTRASTED SOYBEAN MINERAL NUTRITION UNDER INDIVIDUAL OR COMBINED HEAT AND WATER STRESSESA

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

In a context of climate change, with more frequent drought events and heatwaves, it is predicted that soybean yields will drastically decrease in the near future. Soybean being the most widely grown legume crop in the world, there is an urgent need to improve its ability to sustain its growth under such conditions in order to guarantee high levels of productivity. The aim of this study was to explore the influence of heat and/or water stress on soybean growth and its water and mineral nutritions. Two soybean genotypes, displaying contrasted root architectures during their vegetative stage were grown under controlled conditions in the 4PMI high-throughput phenotyping platform where either optimal conditions, or heatwaves, or water stress, or both heatwaves and water stress were applied. Plants were characterized for their morphology, their water uptake, the mineral composition of their tissues and their root transcriptome (RNA-seq analyses). An ecophysiological structure-function framework, enabled us to link structural variables (leaf area, root architecture, biomass, etc.) to functional variables (water use

efficiency, element uptake and use efficiencies...) to understand the interactions between water and element fluxes at the whole plant level, and to quantify the overall tolerance of plants to each stress. Under combined stress conditions, one genotype appeared more susceptible. The genotypic difference was due to functional changes, particularly for water uptake and to differences in the content of certain elements in the roots (magnesium and calcium with their role in osmoregulation, and nickel, sulfur or sodium). This cross-analysis of the plant ionome, root architectural traits and root transcriptome under different stresses, is offering us new insights into soybean adaptation to climate change.

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606/120. SILICON SUPPLEMENTATION IMPROVES SYMBIOTIC NITROGEN-FIXATION EFFICIENCY IN LENTIL UNDER DROUGHT STRESS

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Drought stress is a major threat to global lentil production and decreases the symbiotic nitrogen-fixation(SNF)efficiency of lentil(Lens culinaris Medik.). Our previous research revealed that silicon (Si) supplementation improves drought tolerance in lentil plants(Biju et al.,2021,2023). However, these studies were focused on aboveground traits. Thus, the current study hypothesised that Si can positively regulate below-ground traits to enhance SNF under drought stress. A controlled-environment study was conducted in the growth chamber using selected lentil genotypes to test this hypothesis. Lentil genotypes were inoculated with rhizobia prior to sowing, supplied with or without Si and subjected to moderate(40-45% field capacity-FC)and severe drought stress(20-25% FC)at the early flowering stage. Drought stress treatments significantly reduced nodulation traits(nodule number, nodule biomass, nodule protein, leghaemoglobin, nodule Si, nitrate, aboveground biomass, leaf nitrogen content, antioxidants, amino acids, flavonoids, and seed yield)and activity of enzymes involved in nitrogen metabolism (nitrogenase, sucrose synthase, invertase, nitrite reductase and nitrate reductase) compared to the well-watered control($p \leq 0.001$). Conversely, Si significantly decreased the concentrations of carbohydrates and protease activity in nodules under drought stress. Principal component (PC) analysis revealed the reliability of the studied traits in assessing the SNF of lentils in response to Si with 86.56% total variance(PC1=70.89%;PC2=15.66%)with three distinct clusters in PCA biplot. A strong positive correlation was observed among amino acids, antioxidants, and flavonoids (r=0.83-0.88) which were the key traits in PC2 governing Si-mediated drought stress tolerance with a high factor loading of 0.5. However, in PC1, the loading contribution rate was mainly from nodule number, nodule biomass, nodule protein, leaf nitrogen, leghaemoglobin, nitrogenase, stomatal conductance and photosynthesis(0.21-0.25)with significant positive correlations (r=0.91-0.98). Thus, the results of this preliminary study showed that Si supplementation can potentially be an option to improve the SNF under drought stress in plants.

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606/139. GWAS AS A STALWART AGENT IN THE ENDEAVOR TO UNDERSTANDING WATER STRESS RESILIENCE IN GRAIN LEGUMES

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Grass pea is a grain legume of growing interest due to its outstanding robustness under adverse environmental conditions, like salinity, heat, drought, and flood, compared with other legume species .

The aim of this work was to unravel the genetic background of the grass pea water stress response, by conducting Genome-wide association studies (GWAS) on 20 physiological traits with single nucleotide polymorphisms (SNPs). The traits were measured in a collection of 193 grass pea accessions, grown under three different water treatments (well-watered, waterlogging and water deficit).

Phenotypic data (adjusted means for each trait and treatment taken separately) were tested for association with the genotyping dataset (5651 SNP markers), fitting markers as fixed and accessions as random terms, using restricted maximum likelihood.

Using a threshold of –log10(p) = 4, 37 SNPs were found associated with at least one trait by treatment combination; 7 SNPs were linked to the first four principal components of the full phenotypic data set. Some loci and candidate genes were also detected as putatively contributing to the observed phenotypic variation and water stress responses.

GWAS is as powerful tool for unravelling the genetic architecture of complex traits, and providing fundamental knowledge that ultimately contributes to developing targeted breeding strategies, preserving genetic resources, and addressing the challenges posed by climate change and food security.

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606/161. MINING AN INTERSPECIFIC CHICKPEA POPULATION FOR HEAT TOLERANCE

Authors:

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Work centre:

(1) University of Saskatchewan. Canada

Summary:

Objectives

Chickpeas (Cicer arietinum L.) are sensitive to heat stress at their reproductive stage causing significant yield loss at temperatures of 35°C or higher. Increasing global temperature and heatwave frequencies make heat tolerance an important breeding objective in chickpeas. However, chickpeas also suffer from a narrow gene pool. To find novel genetic sources for heat tolerance, we developed an interspecific population of 200 inbred lines by crossing CDC Leader (kabuli cultivar) with 19 accessions of C. reticulatum.

Concise description of the work (materials & methods)

This population was genotyped using CDC Cicer60K Axiom® SNP chip and was initially tested at three locations over two years under stress conditions (Kalve et al., 2022). Genome wide association analysis identified SNP loci associated with multiple stress indices (Kalve et al., 2022). The same population is currently being evaluated for its response to heat stress under controlled conditions in growth chambers. Two sets of temperatures were applied to the population. One chamber was set at normal temperature (22°C-16 hr day/14°C-8 hr night) and the other chambers were set at 35°C-16hr day/29°C-8hr night during flowering. Seed weight, seed number, pod number, immature pod number, seeds per pod, harvest index, days to flowering and days to maturity were measured and analyzed using ANOVA and used for genome wide association analysis.

Main Results

Preliminary results showed that the tolerant lines retain their seed weights under heat stress whereas the sensitive lines had lower seed weight at high temperature compared to control (Figure 1). Genome wide association analysis identified SNP loci associated with heat tolerance.

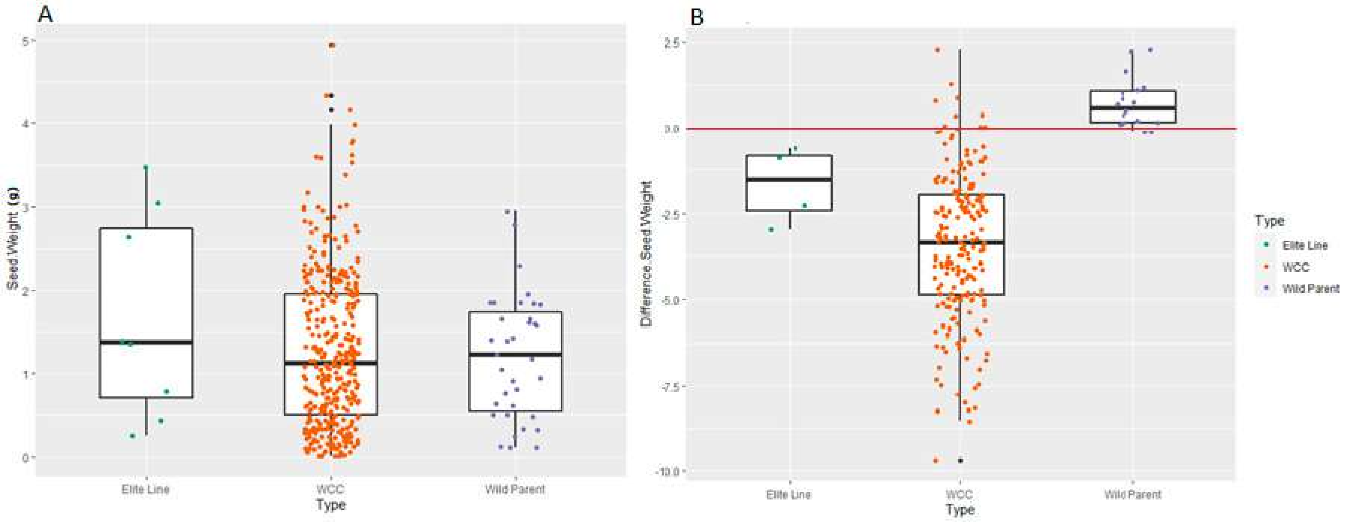

Figure 1. Seed weights of interspecific chickpea lines after heat stress treatment during flowering. Simulated heatwave was applied to the population at flowering to induce heat stress, with the temperature increasing gradually from 22°C-16 hr day/14°C-8 hr night by 2°C (1°C on final day of increase) for 6 days until 35°C day /29°C night, maintained for 14 days and decreased gradually for 6 days by 2°C (1°C on first day of decrease). Each dot represents a sample plant, and each box represents a type of line (elite line checks shown in green, interspecific WCC population in orange, and wild parents in purple). (A) Shows the seeds weights (g) in the heat stress treatment. (B) Shows the difference in seed weights between control conditions (22°C-16 hr day/14°C-8 hr night) and the heat stress treatment. Plants above the red line retained their control seed weights in heat stressed conditions.

Conclusions

Further research includes RNA-Sequencing on select lines under heat stress to identify candidate genes associated with the heat tolerance in chickpea.

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606/184. PLASTICITY IN STOMATA MORPHOLOGY AND THEIR IMPLICATIONS FOR DROUGHT ADAPTATION IN FABA BEAN (VICIA FABA)

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Faba bean (Vicai faba) is expanding in Northern European due to its high yield potential and high protein content. However, water stress causes yield instability. As spring- and early summer-droughts become more frequent, breeding efforts for faba bean must prioritize tolerance to water deficit periods. Stomata control transpiration at the leaf, where stomata conductance (gl) reacts to changes in soil water deficit. However, gl is variable, especially under field conditions with fluctuating environmental factors, making it difficult to measure accurately in large-scale phenotyping trials. Stomata morphology, i.e. stomata density (SD) and size (SS), determine maximum gl, and the reactiveness of gl towards changing environmental conditions (Bertolino et al. 2019). Its plasticity shows adaptation towards environmental condition. We aimed to investigate the potential of stomata morphology for high-throughput phenotyping to assess drought tolerance and adaptation at the field scale.We investigated six faba bean cultivars in a mesocosm experiment and a field trial, in a two-season project. Both experiments involved an experimental drought during the flowering period, and a well-watered control. Leaf imprints were taken before and after the treatment. Images of the leaf epidermis were obtained using a light microscope. The obtained images were analyzed using RootPainter, a convolutional neural network software, to segment stomata structures. Stomata physiology was assessed using a Li-cor 600 porometer, while drought response was measured through 13C discrimination analysis of plant samples. Our findings revealed significant variations in SD among

cultivars, with SS demonstrating significant adaptation to the drought treatment. Moreover, SD before treatment and SS after treatment exhibited a strong correlation with 13C discrimination. These results demonstrate genotypic differences in stomata morphology and their ability to adapt to environmental conditions. The plasticity trait in stomata morphology shows potential for inclusion in larger breeding programs.

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606/196. ROOT METABOLIC ADAPTATION TO IRON DEFICIENCY IN SOYBEAN: THE IMPORTANCE OF COUMARINS SECRETION

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Summary:

Objectives, Description, Main Results & Conclusions

Iron (Fe) nutrition is vital for legumes to establish symbiosis and nitrogen fixation. Moreover, Fe deficiency is a widespread nutritional disorder that severely affects soybean growth and productivity. In the last decade, several studies showed that Arabidopsis adapts to Fe limiting conditions by releasing redox-active coumarins with Fe-mobilization capacity [1]. Long before, it was described that a soybean genotype tolerant to Fe deficiency released Fe reductants into the rhizosphere [2]. However, their identity and the relevance of this phenomenon was later overlooked. Here, we investigate the effects of Fe deficiency on the root and root exudate composition in various tolerant (AR3, A7, Clark, A15 and A97) and sensitive (B219 and IsoClark) soybean genotypes by LC-MS-based metabolomic analyses. When we measured the Fe mobilization capacity of the exudates, those of A7 and IsoClark were the most and least effective, respectively. Up to 23 coumarins were found consistently accumulated in Fe-deficient samples regardless the genotype. Four of them (esculetin, fraxetin, sideretin and methylsideretin) were redox-active. Among all the coumarins the most abundant by far was methylsideretin, the only one not reported in Arabidopsis. The difference between tolerant and sensitive cultivars was not the quality but the quantity of coumarins secreted. Furthermore, an RT-qPCR study of the coumarin biosynthetic pathway was carried out in roots of a tolerant and a sensitive genotype: A7 showed a strong induction of all genes whereas only a marginal induction for some of them was observed in IsoClark. A gene encoding a methyltransferase enzyme, which could explain methylsideretin production, was found 5-fold induced by Fe deficiency, but only in A7. Our data reveal the importance of coumarins secretion in soybean tolerance to Fe deficiency.

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606/200. CHEMICAL DIVERSITY OF THE ROOT-SECRETED METABOLITES IN RESPONSE TO IRON DEFICIENCY IN THE FABACEAE FAMILY

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Iron is abundant in soils and required in small amounts for plant growth. However, Fe deficiency is one of the most important abiotic stresses that limits crop production worldwide. This nutritional disorder arises due to soil Fe availability problems, especially at high pH. A key feature of plant adaptation to stress is the synthesis and secretion by roots of spe-

cialized metabolites. When facing Fe deficiency, grasses synthesize and release into the rhizosphere up to nine different mugineic acids (MAs), metal-chelating agents with high affinity for Fe3+, for mining soil Fe. The Fe3+–MA complexes are then taken up via the YS1 transporter. Non-grass plants take up Fe as Fe2+ via the metal transporter IRT1. Although the fact that many dicot species tolerant to Fe deficiency secrete redox-active metabolites belonging to the phenolic and flavin compound classes has long been known (1), the identity of these metabolites is mostly unknown. This phenomenon has raised much interest as root secretion of the catechol coumarin fraxetin has been reported to be required for efficient Fe uptake in Arabidopsis grown at high pH (2). We have investigated the Fe deficiency effects on root exudates in several species of the Fabaceae family, especially medic species which are commonly found in high-pH soils. Medicago truncatula, M. scutellata, M. sativa and Glycine max plants were hydroponically grown with 0 or 25-50 µM Fe. The metabolite profiles of growth media and root extracts were obtained by LC-MS. An overview of the structural features and chemical properties of the metabolites identified, as well as intra-species and inter-species chemical diversity is presented. The capacity of the root exudates to mobilize Fe from ferric oxides at physiologically relevant pHs is also tackled.

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606/210. SUGAR AND AMINO ACID EXHIBIT DIFFERENT SPATIAL PATTERNS OF EXUDATION IN RESPONSE TO WATER STRESS AND N NUTRITION IN PISUM SATIVUM

Authors:

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Summary:

Objectives

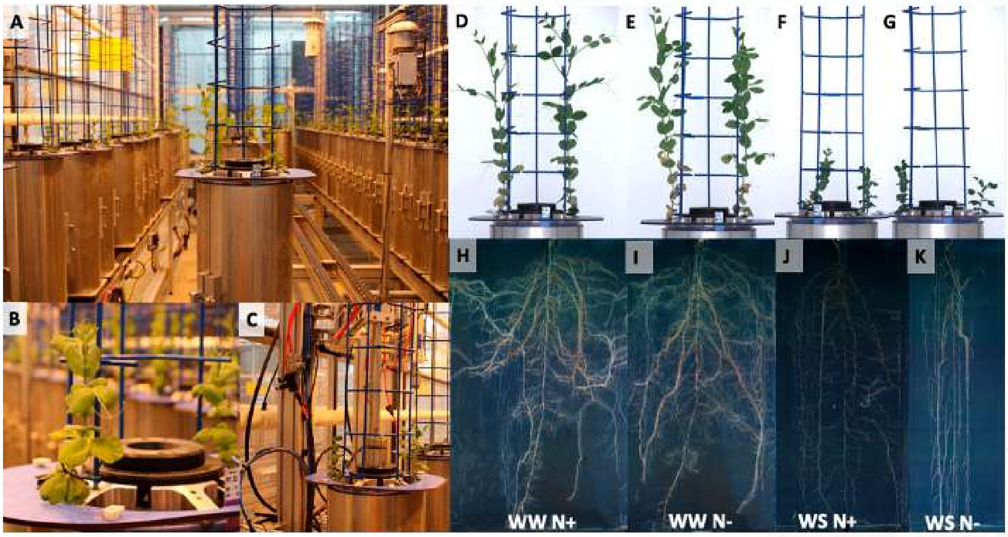
Present work focuses on the influence of root architecture and N nutrition on the interaction between plants and soil microbial communities in response to drought stress. This is especially relevant in legumes as N fixation is highly sensitive to water stress. In a context of increased intensity and frequency of drought episodes, it is important to maintain N fixation and thus legume productivity throughout the plant growth cycle. Root architecture and its plasticity can provide higher drought resilience in pea. Features such as deep rooting, nodule location and the interaction between emerging roots and microbial communities contribute to this resilience, conspicuously through N cycling in the soil (Prudent et al., 2020). Yet, little is known on the effects of root architecture on microbial communities and its consequences on soil N cycling and plant resilience. The objective of this project is to tackle this pluri-component question with a holistic approach integrating whole plant ecophysiology and microbial ecology, with spatial patterns of exudation as a cornerstone of the plant-microbiome interaction (Tixier et al., 2023).

Concise description of the work (materials & methods)

Pea plants were compared for their response to water stress (WS) and different sources of N nutrition (symbiotic fixation N- and nitrate fertilization N+) in terms of water relations, plant productivity, root structure (architecture, C/N) and function (growth, exudation, water and nitrogen uptake). In order to assess root architecture, the plants were grown in the RhizoTubes© of the 4PMI (Plant Phenotyping Platform for Plant and Microorganism Interaction). This innovative equipment is fairly unique for root phenotyping, allowing a quantitative non-destructive assessment of root growth and development using image analysis.

Main Results

Here we showed that water and N shortage decrease plant productivity and modulate shoot and root traits with a bigger impact of water stress on pea root structure and function than N shortage. Indeed, smaller root area was associated with smaller root growth in WS plants. WS plants root system showed a sinking architecture when compared to WW plants with significantly higher Depth/Width ratio. These structural root changes were concomitant to functional changes such as a significant decreased in specific nitrogen uptake, a significant decrease in root specific water uptake only observed in N + treatment and a significant increase of amino acids exudation in response to water stress, regardless of root location. Significant decrease of sugar exudation in response to water stress was only observed in N- plants. Further, while sugar exudation was significantly affected by spatial position on root architecture, no significant effect of position was observed for amino acid exudation.



Phenotyping of pea plant on the 4PMI (Plant Phenotyping Platform for Plant and Microorganism Interaction) which allows the high throughput phenotyping (Fig. 1A-B) and automated irrigation (Fig. 1C) of pea plants (Pisum sativum L. var. Rondo). Plants were grown in sandy soil in Rhizotubes which are cylindrical-shaped-rhizotrons that enable non-invasive image acquisition of plants shoot (Fig. 1D-G) and root systems (Fig. 1H-K). WW N+ : well-watered with nitrate; WW N- : well-watered without nitrate; WS N+ : water-stressed with nitrate; WS N- : well-watered without nitrate.

Conclusions

These results provide insights on the spatial regulation of exudation at the whole plant level, a first step to build a mechanistic understanding of exudation and its trade-off with productivity and resilience. Further, the correlation of these exudation patterns with microbial community structure and activity as well as soil C-N cycling will provide means to target and drive these communities in order to promote plant productivity and soil services such as C storage and N-cycling. Understanding these ecophysiological trade-offs and rhizosphere interactions is essential to develop ideotypes that are adapted to low-input agroecosystems facing climate change.

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606/57. GENETIC VARIATION FOR DROUGHT TOLERANCE IN SOYBEAN AND SELECTION OPPORTUNITIES

Authors:

Luciano Pecetti[1], Daniele Cavalli[1], Margherita Crosta[1], Nelson Nazzicari[1], Paolo Annicchiarico[1], Ivan Pejic[2], Tommaso Notario[1]

Work centre:

(1) CREA, (2) Centre of Excellence fir Biodiversity and Molecula Plant Breeding, Zagreb

Summary:

Objectives

Soybean cultivation is steadily increasing in the EU, responding to a growing demand for feed and food purposes. The unpredictable dry spells caused by the changing climate in most European regions, and the decreasing availability of irrigation water in Southern Europe, emphasize the need of selecting drought-tolerant cultivars, but there is limited knowledge on drought tolerance variation in soybean. Genomic selection may represent an efficient selection strategy for a complex trait such as drought tolerance, as shown for pea (Annicchiarico et al., 2020).

Concise description of the work (materials & methods)

A germplasm collection mostly encompassing European cultivars was evaluated in Lodi (northern Italy) under managed drought stress and moisture-favourable conditions, to gain knowledge on the germplasm variation for drought tolerance and on phenotypic and genomic selection opportunities. Grain yield (GY) was assessed for 59 early-maturing (MG 000/0) and 77 intermediate-maturity (MG 0+/II) cultivars grown in a phenotyping platform composed of eight large (24.0 m × 1.6 m × 0.8 deep), bottomless containers under a field rainout shelter equipped with a double-rail irrigation boom, adopting an alpha-lattice design with four replications per condition. Favourable and stress conditions implied soil moisture kept within 60-80% and 10-30% of the available water, respectively, starting from mean full flowering (R2 stage). The stress treatment received only 46% of irrigation water compared with the favourable one over the growing season. The cultivars were genotyped by the 50K Soybean array, which yielded over 19K SNP markers after filtering stages, to assess different genomic selection (GS) models for GY under both moisture regimes. A GWAS was performed concurrently.

Main Results

On average, drought stress reduced GY by 65%. Both early and intermediate-maturing groups of cultivars exhibited large genotype × environment interaction and fairly modest consistency of yield responses across conditions (as shown by genetic correlations for cultivar GY across conditions of 0.51 and 0.66 for early and intermediate groups, respectively). For both groups, cultivar genetic variation was definitely narrower under stress than under favourable conditions (16.6% vs. 22.7%, expressed as genetic coefficient of variation for GY). The GWAS suggested that GY is determined by many small-effect genes in both conditions. Genomic regions tending to association with GY showed inconsistency between stressed and favourable conditions, suggesting partly different genetic control of the trait in the two conditions. Poorer genetic variation under stress concurred to lower GS prediction ability observed for GY under drought stress relative to favourable conditions (Figure 1).

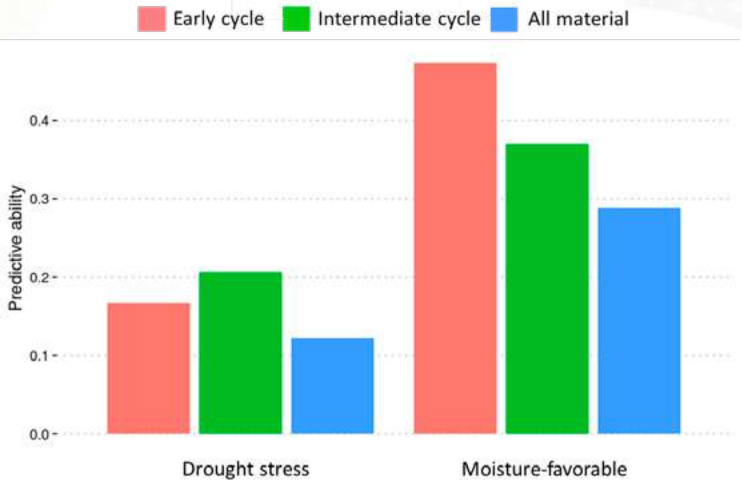

Figure 1. Predictive ability for soybean grain yield in drought stress and moisture-favourable managed environments according to the best-performing model among rrBLUP, Bayesian Lasso, Weighted GBLUP, for early-maturing (MG 000/0) and intermediate-maturity (MG 0+/II) cultivars groups and the whole set of cultivars.

Conclusions

Our results reinforce the crucial importance of specific selection for drought tolerance under stress in soybean, and the strategic importance of identifying novel genetic resources with greater drought tolerance than current cultivars.

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606/105. DROUGHT RESPONSE, SEED QUALITY AND YIELD OF BIOFORTIFIED COMMON BEANS IN A CONTEST OF CLIMATIC CHANGE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Common bean (Phaseolus vulgaris) is an herbaceous legume cultivated throughout the world and its seeds are appreciated for their organoleptic qualities: high energy value, considerable protein content and significant supply of minerals. They are considered a food with high nutritional potential and represent a strategic food in sustainable diets. However, in many countries in Africa, Latin America and South Asia, where bean seeds are usually consumed as a staple food instead of meat, nutritional deficiencies of minerals, mainly iron, arise. One of the main factors limiting common bean production is drought, which has serious consequences for food security. 14 experimental / commercial common bean genotypes constitute the genetic material of the BIO-BELIEF (BIOfortification of common BEan to promote heaLthy dIEt and Food security in a context of climatic variation) ERANET-FOSC project. These genotypes are very different in terms of: 1) morpho-agronomic traits such as plant habit, leaf size, flowering time, seed colour and size, yield and 2) nutritional quality of the seed (absence of lectins, reduced phytic acid content, increased iron content). The main objective of the BIO-BELIEF project is the development of biofortified and drought tolerant common bean genotypes. Here, we will present the results obtained in field and in greenhouse (in two locations: Italy and South Africa) from the morpho-agronomic analysis to the water stress experiments on the 14 genotypes. Preliminary data obtained in the first year of the project concerning plant and seed response to drought are being validated in the second year. The seeds of genotypes chosen for water stress tolerance and with a significant nutritional value will be exploited by preparing bean recipes, which will be characterised for their nutritional profile and micronutrient bioavailability.

606/158. SCREENING A DIVERSITY PANEL OF PEA AGAINST DROUGHT AND WATERLOGGING

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(1) Swedish University of Agricultural Sciences

Summary:

Objectives

The future climate is projected to experience an increased frequency of extreme weather events, including prolonged droughts, intense heatwaves, and heavy precipitation. These events will put a great burden on crops, for example, by the crops receiving too little water or too much water. To prepare for the increased frequency and intensity in extreme weather events, identification of plant varieties that have tolerance to these abiotic stress types is a prerequisite for breeding new adaptive cultivars. One crop that needs bolstering against both drought and waterlogging is the leguminous species, Pisum sativum L. (pea). Pea is globally one of the most harvested legumes, which is well adapted to colder climates and usually performs well in the northern hemisphere.

Concise description of the work (materials & methods)

From a germplasm collection of 300 pea accessions, we selected seeds 100 accessions—representative of the broad diversity within the collection (Figure A)—to evaluate their ion leakage and germination rates in a waterlogging experiment. Based on the outcome of this experiment, we selected 18 accessions (six good, six intermediate, and six poor, in terms of their performance on seed germination) for a subsequent experiment on three-week-old plants, testing both drought and waterlogging in a climate chamber. The drought group received no water for seven days, the waterlogging group had their roots immersed in water for seven days, and the control group was watered three times during the seven days (Figure B). Each accession was subjected to two stress conditions along with a control group, with a total of thirty plants assessed per accession (10 per treatment). After the stress period, the plants recovered for three weeks, and we recorded stress responses in plants using a LI-600 fluorometer/porometer and recorded the emergence of the first flower and the first pod. All accessions were markedly affected by the two abiotic stress types, but a few accessions showed some tolerance to both stress types. Among these accessions, we selected four accessions for yield analysis and investigated their water-use efficiency by means of carbon isotope ratios.

Main Results

As a first for pea, this study looked at both drought and waterlogging tolerance in parallel across the same accessions. In the waterlogging experiment on seeds, we observed that accessions with dark-coloured testa performed significantly better than accessions with light-coloured testa in terms of testa integrity and germination. In the seedling experiments, we observed that the semi-leafless accessions relative to most of the leafy accessions, stood well against the two stress types. Both these findings confirm observations from other studies on testa colour and semi-leaflessness (Zaman et al. 2019; González et al. 2002). However, we also observed that some of the leafy accessions were less affected in their ability to set flowers and develop pods. In three of the four accessions that were kept for yield analysis, the yield was not significantly reduced in the stressed plants. This indicates a potential for breeding pea cultivars adapted for adverse stresses.

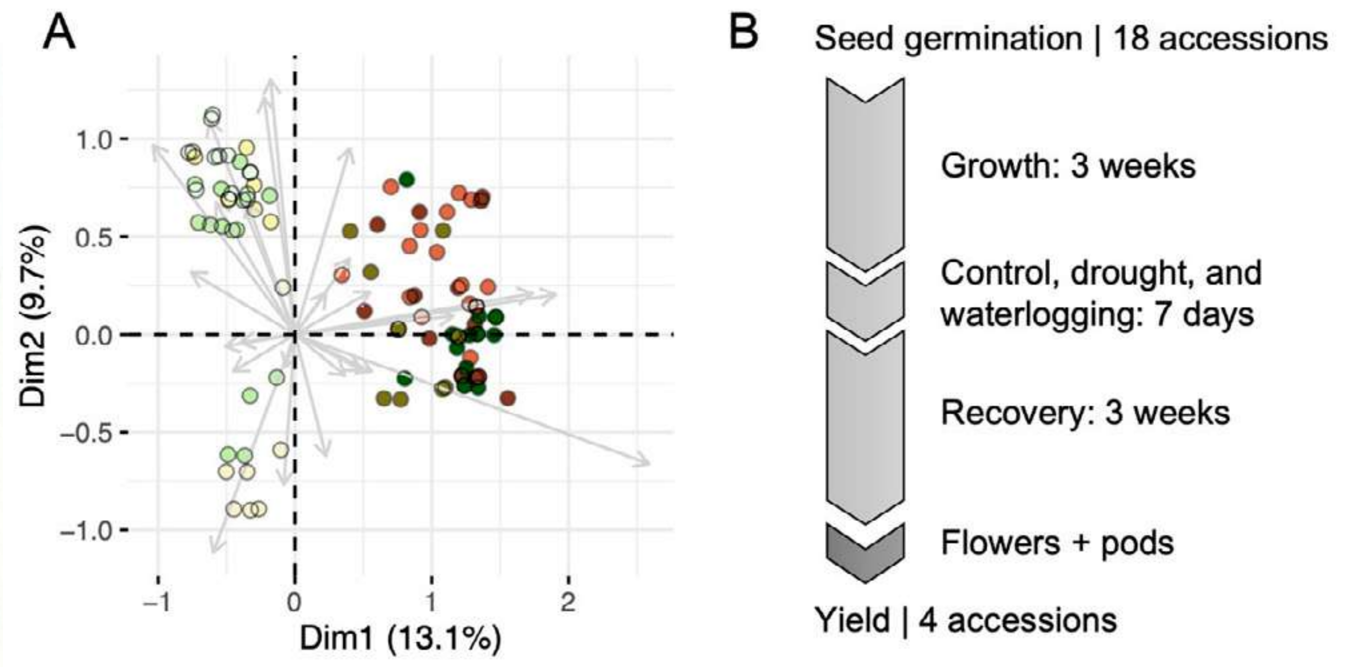

Figure. (A) Biplot for a multiple correspondence analysis depicting the 100 pea accessions used in the waterlogging experiment on seeds. (B) Timeline for the drought and waterlogging experiment on seedlings from 18 pea accessions. Four accessions were kept for yield analysis.

Conclusions

This study examined the drought and waterlogging tolerance of pea and identified specific traits associated with tolerance, such as dark-colored testa and semi-leaflessness. While most accessions in the study were affected by the stress conditions, a few accessions showed some tolerance to both drought and waterlogging, indicating potential for breeding multi-stress tolerant pea cultivars.

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606/168. DROUFGHT STRESS INDUCED CHANGES IN SPATIAL HETEROGENITY OF COMMON BEAN LEAF PHYSIOLOGICL TRAITS

Authors:

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Summary:

Objectives

Leaves represent an interface between plants' physiological processes with atmospheric and ecosystem processes (Hohmann-Mariot and Blankenship, 2011). Because of that, leaves are highly complex structures showing significant spatial heterogeneity of ecophysiological traits (Saudreau et al., 2017). For example, leaf spatial variability in stomatal density, stomatal conductivity, nitrogen content, net assimilation rate, and temperature were previously described in different plant species.

Drought is a significant stressful factor because it causes changes in CO2 and H2O fluxes and consequently affects plants' carbon assimilation and light and water utilization. Although the effect of drought on plant physiological processes has been widely studied, less attention has been given to its effect on leaf spatial heterogeneity of different physiological processes. This research aimed to assess the effect of drought on spatial heterogeneity of physiological processes and multispectral traits in common bean.

Concise description of the work (materials & methods)

Common bean seedlings with developed first pair of leaves were subjected to drought treatment (n=12) and control (irrigated) treatment (n=12) in a growth chamber. Drought treatment was applied by avoiding irrigation for six days. Leaf spatial heterogeneity was assessed by measuring the stomatal conductance (gsw), boundary layer conductance (gbw), total conductance (gtw) and transpiration rate (E) using LI-600 porometer (Li-Cor, Lincoln, Nebraska, USA) at three different positions on the leaf: base, the middle part and the leaf tip. Multispectral imaging and chlorophyll fluorescence imaging of the whole leaves were performed using CropReporter (PhaenoVation, Wageningen, The Netherlands), enabling analysis of leaf spatial heterogeneity at the pixel resolution. Finally, the stomatal density of the adaxial and abaxial leaf sides at the three above-mentioned positions was assessed by making the leaf impressions and counting the stomatal number under the microscope.

Main Results

A significantly higher number of stomata were found at the leaf abaxial side. However, heterogeneity in stomatal density was present only on the adaxial side, with the lowest stomatal density found at the leaf tip. Drought treatment increased the stomatal density per leaf area but did not affect its spatial heterogeneity. In addition, drought decreased gtw, gsw and E, and neutralized spatial heterogeneity of gtw and gsw which show a decrease from the leaf tip towards the base in control leaves. Drought decreased photochemical efficiency (effective quantum yield of PSII, relative electron transport rate, coefficient of photochemical quenching) but did not affect spatial heterogeneity of these parameters, which decreased from leaf base towards leaf tip in both control and drought treatment leaves. On the other hand, non-photochemical quenching (NPQ) increased from the base of the leaf towards the leaf tip in control but not in the drought treatment leaves. Most of the measured multispectral traits showed spatial heterogeneity with increasing or decreasing from the leaf base towards the leaf tip, whereas drought only neutralized heterogeneity of the near-infra-red reflection and the anthocyanin index.

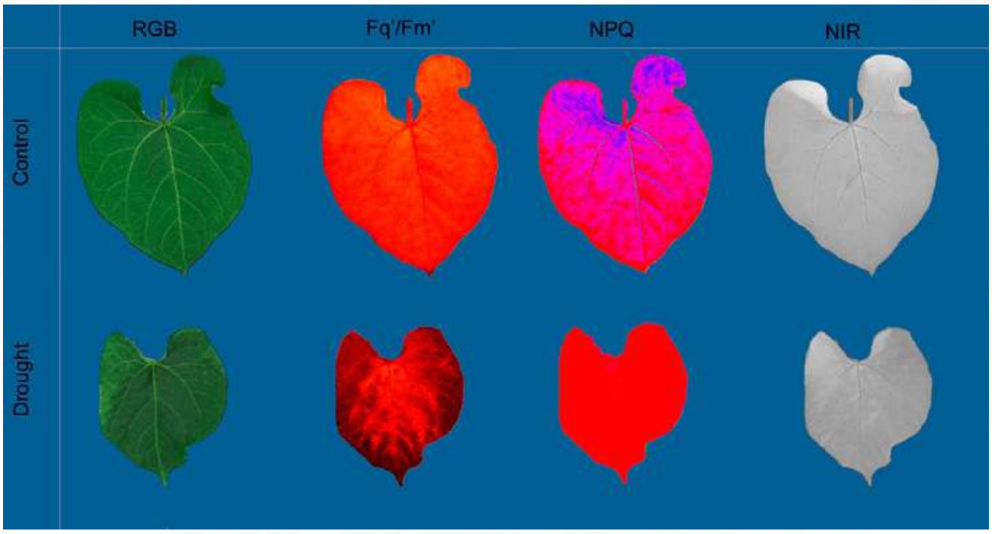

Figure. Colour (RGB) and pseudo-colour images of the effective quantum yield of PSII (Fq'/Fm'), non-photochemical quenching (NPQ) and near-infrared (NIR) reflectance captured by CropReporter in common bean plants grown under drought and control conditions.

Conclusions

Physiological traits show substantial variability within common bean leaf and are often affected by drought. Drought causes morphological, structural and physiological changes within leaf, and by studying their relationship and the within-leaf spatial heterogeneity we can improve our understanding of plant adaptation to drought.

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606/187. PHISIOLOGICAL CHARACTERIZATION OF DROUGHT RESISTANCE AMONG LENTIL CULTIVARS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Lentils are the fifth most important legume globally . Legumes are the main source of non-animal protein in human nutrition and, therefore, play a fundamental role in the establishment of a sustainable agriculture and a healthy diet. Due to the ongoing climate change, and the fluctuating atmospheric phenomena, the incidence and severity of drought periods will worsen. Drought is among the abiotic stresses causing more severe effects on the growth and yield of lentil crops. For instance, at the reproductive stage it causes reductions up to 70% of yield. The development of cultivated lentil varieties has typically focused on obtaining greater productivity or better organoleptic qualities. However, current priorities have switched to the development of varieties more resilient to climate change. To accomplish this, we first must design a reliable and consistent drought protocol. We first searched for parameters that allowed a fast and reliable phenotyping of drought stresses. Specifically, we studied 8 varieties of cultivated lentil (Lens culinaris Medik): Alpo, BG16364, BG16365, ILL1918, ILL7537, Lupa, Precoz and WA8649041, and the wild relative Lens odemensis ILWL235. Plants were kept at 40% field capacity (FC) for two weeks, and their physiological state analyzed, measuring every two days the leaf relative water content, plant water potential, photosynthetic rate, and stomatal conductance. The alteration of these parameters under drought conditions is well known; however, the correlation among them, as well as the best method to assess drought tolerance is yet to be stablished. Stressed plants were compared to well-water controls. All the parameters had significant variations in stressed plants, but genotype-dependent responses were observed. Results suggested that the changes in these parameters under drought stress conditions could allow the identification of resistant lentil varieties.

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606/206. TOOLS TO HELP BREEDING FOR FROST TOLERANCE OF WINTER GRAIN LEGUMES

Authors:

Adrien Sonnet[1], Benoit Decaux[2], Valérie Dufayet[3], Corinne Devaux[4], Blandine Raffiot[4], Hervé Demailly, Laurent Gutierrez[5], Pierre Delaplace, Benoît Mercatoris[6], Martine Leflon, Véronique Biarnès[4], Bruno Delbreil[1], Isabelle Lejeune-Henaut[1]

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Summary:

Objectives, Description, Main Results & Conclusions

Freezing is a major environmental limitation to crop productivity for a number of species including legumes. In the context of climate change, winter crops are experimenting milder autumn periods that may limit the achievement of cold acclimation, i.e. the ability for plants to increase their level of frost tolerance in response to low but non-freezing temperatures. Thus, even if frost events tend to be less severe, screening for frost tolerance remains a concern for breeders. In this context, screening tests in controlled conditions may provide an additional tool, with frost damages being evaluated by observations of whole plantlets or by electrolytes leakage assays on sampled tissues. Imaging techniques are also explored as they would open the way to a better characterization of the cold acclimation process and would allow to capture genetic variability before the application of negative temperatures. Parallely to these physiological assays, there is also a challenge in predicting frost tolerance with modelling approaches. Such a tool has been adapted to the winter pea crop, from a model formerly developed for wheat (Lecomte et al, 2003), using mean and minimum daily

temperatures to simulate cold acclimation and predict frost tolerance. This model allowed to evaluate the evolution of freezing risks under climate warming and to recommend new traits to be bred (Castel et al, 2016). A more accurate prediction is sought, taking into account the respective effects of incident global radiation and of floral initiation earliness on the achievement of cold acclimation.

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606/235. COMBINED BIOSTIMULANT EFFECT OF BACILLUS SP. AND BOTANICAL EXTRACT RICH IN HYDROXYTYROSOL ON CHICKPEA UNDER DROUGHT INDUCED CONDITIONS

Authors:

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Work centre:

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Summary:

Objectives

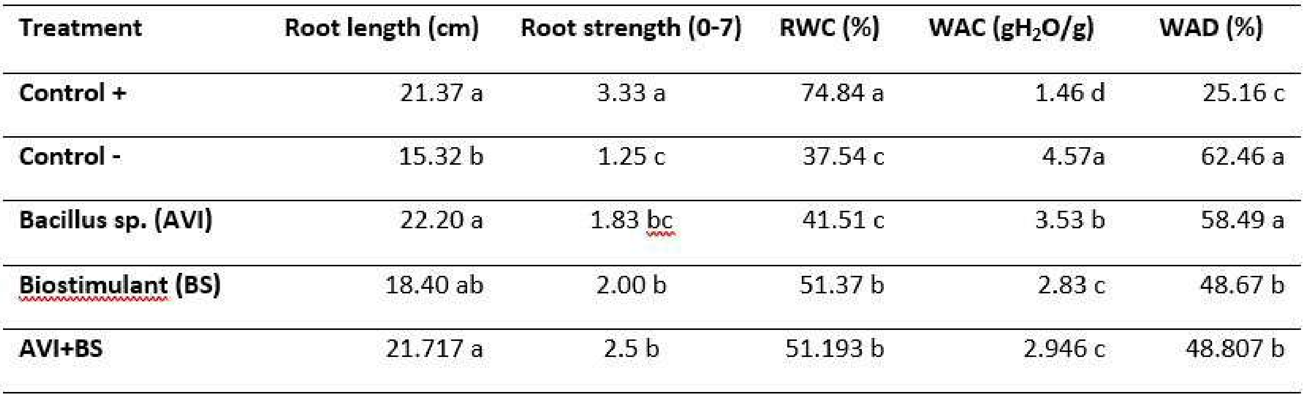
Chickpea (Cicer grietinum) crop is being severely affected by severe drought periods in recent years in the central-Southern area of Spain. Therefore, authors proposed the evaluation of a Bacillus sp. AVI (VitaNtech Biotechnology) and a biostimulant botanical extract rich in hydroxytyrosol (Biostimulant, Biopharma/Econatur) to induce resistance to water stress.

Concise description of the work (materials & methods)

Seeds of C. griteinum var. Garabito were disinfected and germinated for subsequent transfer to sterile universal substrate. Plants were grown in a climatic chamber (25 ± 2°C, 60-70% relative humidity in a 16 h light/8 h dark photoperiod) for seven days. At that time, the following treatments were differentiated: i) Bacillus sp (AVI: 3 ml plant-1 of a 108 cfu/ml broth), ii) biostimulant (BS: 100 µl plant-1), iii) Bacillus sp + biostimulant (at the same dose). After the irrigated-applications, the plants were subjected to 28 days without irrigation. A negative control (absence of water irrigation) and positive control (under normal watering condition, two daily irrigations of 50 ml plant-1 per week) were introduced. Samples were taken before the applications (T0) and at the end of the trial (T1, after 28 days). Morphological (root length and vigour) and physiological parameters were determined, such as relative water content (RWC), water content at saturation (WAC) and water deficit at saturation (WAD) (Bacelar et al., 2006; Borjas et al., 2015; Luna-Flores et al., 2012). The trial was repeated twice. Six replicates per treatment were arranged. An analysis of variance and comparison of means test (Fisher's LSD) was performed according to a significance threshold $p= 0.05$. Statistically significant differences were observed between treatments for all parameters (Table).

Main Results

The absence of irrigation did not reduce root length in the AVI and AVI+BS treatments ($p = 0.046$). Although root strength was reduced in all stress-induced treatments, strength of AVI and AVI+BS treatments were also statistically different from the negative control ($p < 0.001$). At the physiological level, the treatments that included the application of BS highlighted. Without reaching the values of the positive control, BS and AVI+BS obtained higher RWC values than the negative control ($p < 0.001$). In addition to these, AVI also reduced the WAC value ($p < 0.001$). The WAD values in the BS and AVI+BS treatments were lower than the negative control ($p < 0.001$).



Treatment	Root length (cm)	Root strength (0-7)	RWC (%)	WAC (gH_2O/g)	WAD (%)
Control +	21.37 a	3.33 a	74.84 a	1.46 d	25.16 c
Control -	15.32 b	1.25 c	37.54 c	4.57a	62.46 a
Bacillus sp. (AVI)	22.20 a	1.83 bc	41.51 c	3.53 b	58.49 a
Biostimulant (BS)	18.40 ab	2.00 b	51.37 b	2.83 c	48.67 b
AVI+BS	21.717 a	2.5 b	51.193 b	2.946 c	48.807 b

Table. Morphological and physiological growth parameters of Cicer grietinum under induced water stress. Different letters in the same column indicate statistically significant differences according to Fischer's protected LSD test (P = 0.05). RWC, WAC and WAD represent the relative water content, the water content at saturation and water deficit at saturation, respectively.

Conclusions

Therefore, we can conclude that AVI shows an effect on the root development of the crop. On the contrary, BS acts at the physiological level and allows greater tolerance to water stress. The combined treatment of plant and microbial biostimulants might be a viable strategy against drought, a limiting factor in world agricultural production.

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606/247. THE EFFECTS OF SULFUR DEFICIENCY AND DROUGHT STRESS ON GENERAL PERFORMANCE AND SYMBIOTIC NITROGEN FIXATION IN PEAS (PISUM SATIVUM L.)

Authors:

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Work centre:

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Summary:

Objectives

The symbiotic nitrogen fixation (SNF) of peas is limited by the nutrient- and water status of the plant. Sulfur (S) is an important component in nitrogenase and leghemoglobin activity and affects abscisic acid (ABA) induced stomata regulation (Ernst et al., 2010; Hungria & Nogueira, 2023; Zhao et al., 1999). The combined effects of S deficiency and drought stress on the yield stability of legumes are crucial to understand, as S deficiency is becoming more common, and we face drought periods due to climate change. The aim of this study was to investigate crosstalk between carbon (C) assimilation, S availability, and SNF in peas (Pisum sativum L.).

Concise description of the work (materials & methods)

This was done by assessing the combined effects of sulfur deficiency and drought stress on the general performance and SNF. The experiment was conducted under controlled greenhouse conditions, where plants were grown in cylindrical tubes containing sand and a soil inoculum. The plants were fertilized with inorganic nutrient solutions without nitrogen, with three levels of S (0.2mg, 49mg, and 99mg per plant), and with adequate amounts of all other plant essential nutrients. Ten days prior to harvest the S treatments were divided into a well-watered (with double deionized water) and a drought induced treatment (terminated irrigation). Measurements of general growth factors (leaf area, plant height, fresh and dry weight of shoots and roots, and number and dry weight of pods), drought related factors (shoot water content, stomata distribution, total C content, 13C/12C ratio), and SNF related factors (total N content, 15N/14N ratio, nodule count, and nodule weight).

Main Results

Sulfur deficiency and drought stress showed substantial negative effects on general growth, including number and dry weight of pods (Figure). Stomata distribution was not affected by either stressor. All treatments showed to rely on SNF for N-supply, suggested by the 15N/14N isotope ratio and S deficiency decreased the total fixed N and nodule number and weight per plant. Drought stress decreased biomass production and the total C content.

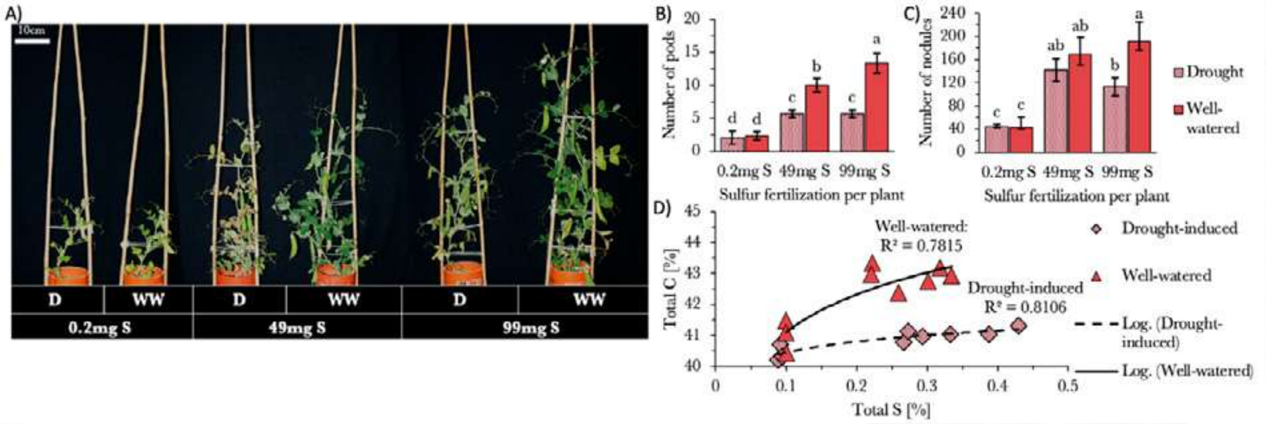

Figure. Effects of sulfur deficiency and drought stress on general plant growth, total carbon content and number of pods in peas. A) Representative plants from each treatment at harvest (75 days after sowing). WW = well-watered, D = drought induced. B) Total carbon content as a function of total S content. Regression lines are fitted to a logarithmic function. n=3. C) Number of pods per plant D) Number of nodules per plant. Bars present mean values, error bars present standard deviation, and letter are produced with Tukey's test (different letters = p<0.05). n=3.

Conclusions

It was concluded that there is a clear link between C, N, and S in relation to SNF and that S deficiency and drought stress are highly limiting factors in the SNF of peas. The study also emphasizes the need to investigate plant stressors in combination to improve the understanding of the interactions between plant nutrition and abiotic stress responses, and their impact on yield stability in legumes.

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606/257. IDENTIFYING DIVERSITY FOR DROUGHT STRESS TOLERANCE IN FABA BEAN (Vicia faba L.) BY DIGITAL AND PHYSIOLOGICAL PHENOTYPING

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Drought stress is one of the most crucial abiotic stress factors threatening the worldwide cultivation of faba beans. In the face of climate change, breeding for drought stress tolerant genotypes is growing even further in importance under increasing temperatures and longer drought periods. The aim of this study is to identify drought stress tolerant Vicia faba genotypes within a diverse genotype panel as a knowledge base for selection of crossing parents and for subsequent selection based on morphological and physiological traits associated with relevant drought stress tolerance characters.

A total of 56 diverse genotypes were grown in 240 large containers with a 90 cm deep soil profile in a DroughtSpotter XXL facility. This fully automated drought phenotyping platform automatically and accurately measures the weight of each container every five minutes 24/7, enabling precise gravimetrical recording of water use and stress responses. It simultaneously enables individual irrigation of each container, allowing simulation of specific drought stress conditions. In this experiment, the effects of drought stress were investigated under two contrasting irrigation regimes (normal irrigation at 60% field capacity, drought stress at 40% field capacity) with 3 replicates per genotype. Furthermore, non-destructive measurement of above-ground biomass was assessed twice weekly using a multispectral PlantEye 3D scanner. The scanner enables the capture of the normalized difference vegetation index (NDVI), normalized pigment chlorophyll ratio index (NPCI), plant senescence reflectance index (PSRI), and Hue) and their changes over time. Connecting precise phenotypic and plant performance data obtained under relevant drought stress conditions allows identification of target traits for efficient field selection and breeding lines capable of sustaining yield under limited water availability. The study revealed clear differences in plant indices between the irrigation schemes and between genotypes. Based on specific plant indices, significant variations were observed in relation to yield components, grain quality and water uptake as a basis to design future selection strategies.

Session 10 Understanding and enhancing legume crop tolerance to biotic stress

606/18. IDENTIFICATION OF NEW CHICKPEA GENETIC RESOURCES RESISTANT TO FUSARIUM WILT UNDER MULTI-ENVIRONMENTS

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

The aim of this study is to identify resistant genotypes that were used in ICARDA breeding programs to develop new cultivars with improved FW resistance and improved stability across different environments. Fusarium wilt (Fusarium oxysporum f. sp. ciceris) is an important biotic constraint to chickpea production worldwide. Host plant resistance is the most durable and economical way to manage these diseases. Fusarium wilt epidemics can devastate crops and cause up to 100% loss in highly infested fields under favorable conditions. The current study (funded by GRDC and ICARDA) aimed to identify new resistant genetic sources of chickpea against Fusarium wilt through multi-environment and multi-year screening. One hundred eighty chickpea genotypes were selected from the genetic resource section (GRS) of ICARDA based on the passport data using the focused identification of germplasm strategy (FIGS). The experiment was planted with an Alpha Lattice design in two locations (Terbol-Lebanon and Beja-Tunisia during the 2015, and 2016 seasons). The susceptible check (ILC482) was repeated every ten rows. The genotype and genotype × environment interaction has been conducted, and the GGE biplot analysis has indicated significant variations ($p \leq 0.001$) between genotypes and environments that are due to the genotype × environment (G×E) interaction. Six genotypes (IG74725, IG9050, IG6049, IG9631, IG70283, and IG8914) were highly resistant to wilt (<10% mean disease incidence) across the two locations. Also, there are nine moderately resistant genotypes for wilt (<20% mean incidence). IG70283 had the highest stability in two locations and years. The resistant genotypes identified in this study were used in ICARDA breeding programs to develop new cultivars with improved FW resistance and improved stability across different environments.

606/29. EARLY INDICATION OF A SECOND SHIFT IN THE PATHOGENICITY OF ASCOCHYTA FABAE ISOLATES IN THE PATHOGEN POPULATION IN SOUTHERN AUSTRALIA

Authors:

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Work centre:

(1) South Australian Research and Development Institute. Australia

Summary:

Objectives, Description, Main Results & Conclusions

Ascochyta fabae Speg. (teleomorph Didymella fabae) is the causal agent of Ascochyta blight of faba bean. The pathogen population is naturally variable and sexually reproduces meaning new genetic variants may be generated that overcome the resistance in current Australian faba bean varieties. In southern Australia, three pathogenicity groups (PG) are recognised, and PG-2 is aggressive on the formerly resistant variety Farah and dominant in the pathogen population (Blake et al 2022). We examined pathogenic variability of 71 isolates collected from 2019 to 2021 from commercial crops and field trials in southern Australia on a faba bean host differential set in ambient shadehouse conditions in three separate tests in a four-replicate split plot experimental design. Percent leaf area disease and percent stem area disease was assessed and percent whole plant disease calculated. A significant interaction between isolate and host was revealed in each experiment ($P<0.001$). Of the isolates collected in 2019 and 2020, 84% and 89% respectively were pathogenic on Farah and categorised as PG-2. For isolates collected in 2021, an equal number of PG-2 and PG-3 isolates was revealed, and no PG-1 isolates were detected. Cautiously, this suggests that second shift has occurred in the pathogen population towards isolates that are more pathogenic

on Samira and Nura, which are both rated moderately resistant to PG-2 (nvt.grdc.com.au). Some of the isolates causing higher than expected levels of disease on Samira, Nura and PBA Amberley have been deployed in the Australian faba bean program pre-breeding screens to ensure progression of elite varieties with resistance to Ascochyta blight. Continued monitoring will be critical to confirm the persistence of this shift in the A. fabae pathogen population.

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606/53. MULTIPLE ALLELIC VARIANTS OF THE NOVEL ALAVR1 EFFECTOR IN ASCOCHYTA LENTIS PROVIDE INSIGHTS INTO HOST SPECIFICITY OF ASCOCHYTA BLIGHT IN LENTIL

Authors:

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Work centre:

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Summary:

Objectives

Fungal pathogens pose significant threats to crop production and have devastating effects on global food security. In pulse crops, ascochyta blight (AB) is one of the major biotic stresses that leads to reduced yield and grain quality [1]. In lentil, Ascochyta lentis is responsible for losses from 5 to 50% in susceptible cultivars due to flower and pod abortion as well as stem damage. Thus, a better understanding of the molecular mechanism underlying host-pathogen interaction would support breeding of cultivars with improved AB-resistance.

Concise description of the work (materials & methods)

Pathogens secrete a suite of small cysteine-rich proteins known as effectors. Avirulence effectors are recognized by the plant and trigger a resistance response [2]. We previously reported the identification of the first avirulence effector, AlAvr1, for an Ascochyta species [3]. The AlAvr1 gene was identified by map-based cloning using isolates derived from a cross between isolate AlKewell (virulent on lentil cultivar PBA Hurricane XT and avirulent on cv. Nipper) and isolate P94-24 (PBA Hurricane XT-avirulent and cv. Nipper-virulent).

Main Results

DNA sequence polymorphisms and functional characterisation revealed two allelic variants, AlAvr1-1 and AlAvr1-2. Agroinfiltration of a AlAvr1-1 construct (P94-24) caused necrosis on PBA Hurricane XT but not on Nipper, while the AlAvr1-2 construct (AlKewell) did not elicit a response from either cultivar (Fig. 1A). Further gene knockout experiments showed that deletion of AlAvr1-1 in P94-24 led to virulence on PBA Hurricane XT, while deletion of AlAvr1-2 in AlKewell resulted in virulence reduction on PBA Hurricane XT (Fig. 1B). These results indicated that AlAvr1-1 was an avirulence effector that triggered a hypersensitive response in PBA Hurricane XT, while AlAvr1-2 was not an avirulence effector for Nipper, but may have a role in susceptibility in PBA Hurricane XT. Here, we have extended the research to include A. lentis isolates from different geographical regions. We sequenced the AlAvr1 gene in isolates from Canada, Syria, Morocco and Lebanon. All isolates contained a single gene copy of AlAvr1, and at least three allelic variants were identified, two of which were not present in the Australian population. Isolates from Canada shared 99.6% amino acid sequence identity with AlAvr1-1. We also identified the presence of an ortholog gene in the closely related species, Ascochyta viciae-villosae, a pathogen of hairy vetch (Vicia villosa). Sequence analysis showed all AlAvr1 variants exhibited the hallmarks of an effector, i.e. small cysteine-rich protein with a signal peptide needed for secretion into the plant cell. Phylogenetic analysis of the DNA sequences revealed three major clusters, where the AlAvr1 ortholog from A. viciae-villosae was the most divergent. The AlAvr1-1 cluster included allelic variants from both Canada and Syria, while AlAvr1-2 and a variant from Morocco formed a different cluster (Fig. 1C). Functional characterisation of the different AlAvr1 variants is in progress.

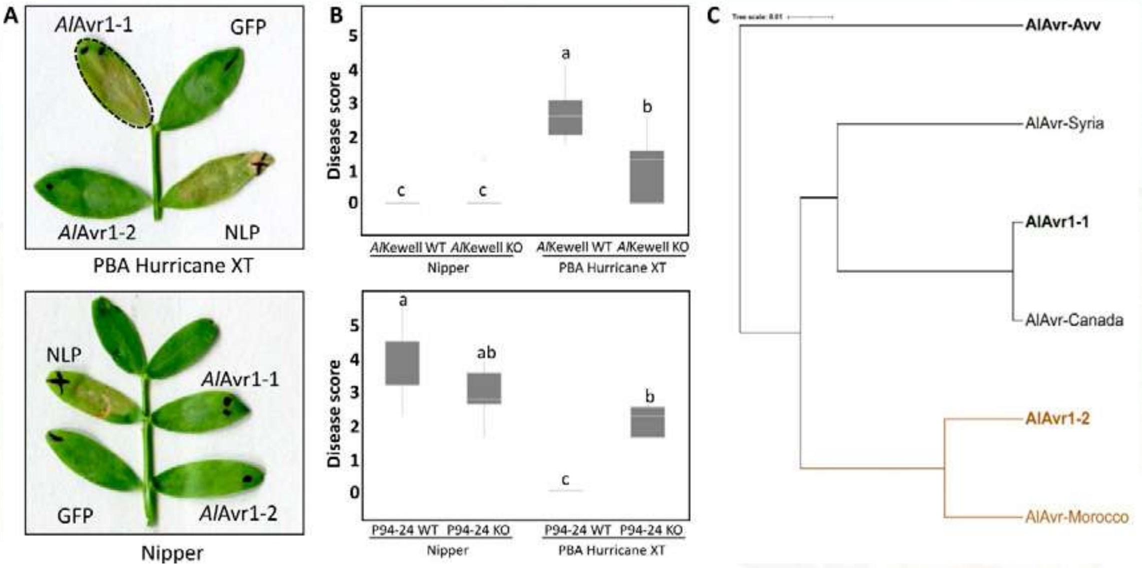

Figure 1. Characterisation of an AlAvr1 effector from Ascochyta spp. (A) Agroinfiltration of AlAvr1-1 and AlAvr1-2 in Australian lentil cultivars PBA Hurricane XT and Nipper; Green fluorescent protein (GFP) and Necrosis-inducing protein (NLP) were negative and positive controls, respectively. (B) Disease scores of A. lentis isolates AlKewell and P94-24 on two lentil cultivars and the corresponding AlAvr1-knockout mutants (KO). (C) Phylogenetic analysis of AlAvr1 variants from different A. lentis isolates and A. viciae-villosae (Avv).

Conclusions

The present study uncovered multiple AlAvr1 variants in different A. lentis populations, which opens the door for cloning of the corresponding R gene(s) in lentil. Characterisation of Avr gene variants in plant pathogen populations is vital to ensure the efficacy of R genes in cultivars destined for release in different geographical regions. Knowledge of the natural variations of Avr genes in diverse pathogen populations will also increase our knowledge of the arms race between plants and pathogens.

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606/76. EXPLORING PHYTOTOXIC METABOLITES FROM COLLETOTRICHUM SPP. INVOLVED IN LEGUME DISEASES BY APPLYING TARGETED AND UNTARGETED METABOLOMICS

Authors:

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Work centre:

(1) Instituto de Agricultura Sostenible - CSIC, (2) Universitá di Napoli Federico II, (3) Institute for Sustainable Agriculture. Consejo Superior de Investigaciones Científicas. Córdoba. Spain

Summary:

Objectives

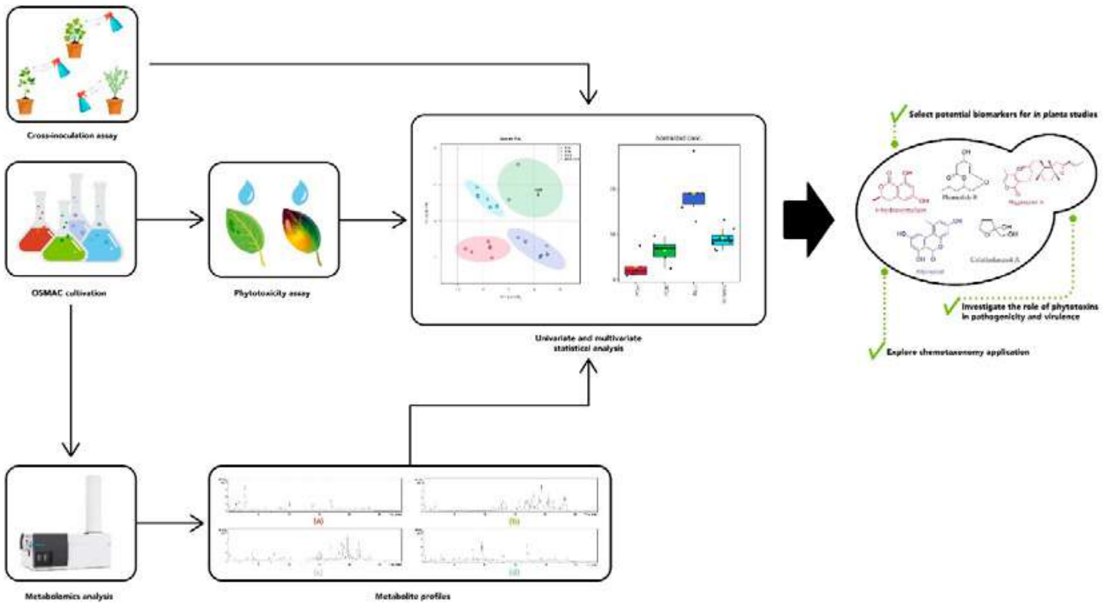
Anthracnose disease, caused by various species of Colletotrichum fungi, poses a significant threat to major legumes worldwide, resulting in substantial economic losses. The disease is characterized by dark, sunken lesions on leaves, stems, or fruits. Colletotrichum species have produced a wide range of biologically active and structurally unique metabolites that could be involved in the infection process. In this study, we aimed to uncover the phytotoxic secondary metabolite profiles produced by three isolates of Colletotrichum spp. involved in legume disease.

Concise description of the work (materials & methods)

We investigated the metabolites produced by two isolates of C. truncatum and C. trifolii employing the One Strain Many Compounds (OSMAC) approach integrated with targeted and untargeted metabolomics profiling. Four substrates were selected for in vitro growth: PDA, PDB, Richard's medium and rice. Additionally, we evaluated the phytotoxicity of crude fungal extracts on their primary host and related legumes, investigating possible correlation with the metabolite profiles generated under different cultural conditions (Figure)

Main Results

Our results showed that the phytotoxicity of organic extracts varies depending on legume species, fungal isolates, cultural conditions, and tested concentration. The highest phytotoxicity was found in Richard's medium extract. The metabolomics analysis and the chemometrics analysis pinpointed 84 discriminant metabolites. As a general result, these secondary metabolites produced by Colletotrichum species belong to numerous natural products classes, including alkaloids, terpenoids, coumarins, chromones, xanthones, polyketides, quinones, peptides, phenols, and macrolides lactones. Furthermore, our data show that their production heavily depends on the selected cultural media. The chemometric analysis of the untargeted metabolomics profiles of the organic extracts showed that the selected cultural media influence the production of specific secondary metabolites, even though the upregulated or downregulated compounds depend on the Colletotrichum species.



Workflow used to investigate secondary phytotoxic metabolites from Colletotrichum spp. involved in legume diseases.

Conclusions

In conclusion, studying secondary metabolites, particularly phytotoxic ones, is essential to gain information on the pathogenicity or virulence of a specific pathogen to develop more sustainable control methods. Indeed, this research could be beneficial for investigations dealing with fungal chemotaxonomy, host-pathogen interaction searching for potential fungal biomarkers or, more in general, investigations dealing with genomics, transcriptomics, and proteomics, to provide a more comprehensive understanding of fungal metabolism and physiology (Reveglia et al.,2023).

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606/94. ALTERATION OF TRANSCRIPTOME PROFILE IN CHICKPEA POD WALL DUE TO SIMULATED HERBIVORY

Authors:

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Work centre:

(1) Department of Agricultural Biotechnology. Assam Agricultural University. India

Summary:

Objectives, Description, Main Results & Conclusions

Chickpea suffers significant yield losses due to the pest Helicoverpa armigera which feeds on leaves, flowers, and green pods. Pod wall plays a significant role in protecting the developing cotyledons from external stimuli, including insect feeding. Therefore, we elucidated defense responses in the chickpea pod wall due to simulated herbivory by transcriptome analyses. The assays showed differential regulation of genes in the chickpea pod wall of a mild resistant (ICC 506 EB) line when compared with a commercial cultivar (JG11). We observed the expression of 1004 and 1271 genes in the cultivar ICC 506EB and JG11, respectively. In silico analyses revealed expression genes of the phenylpropanoid pathway involved in the production of compounds of a wide array of functions, including signaling molecules and secondary metabolites. In addition, the up-regulation of stress-induced genes and transcription factors of the WRKY, MYB, and ERFs families required for the process of induced defense was recorded. A significant change in the expression pattern of different defense genes was observed up to 48 h after the application of oral secretions. This study provides an insight of the transcript dynamics in chickpea pods due to simulated herbivory and may help identify and clone the desired gene(s) for protection.

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606/116. HIGH-DENSITY LINKAGE MAPPING AND GENETIC DISSECTION OF RESISTANCE TO BROOMRAPE (OROBANCHE CRENATA FORSK.) IN PEA (PISUM SATIVUM L.)

Authors:

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Work centre:

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Summary:

Objectives

Pea (Pisum sativum L.) is a widely cultivated legume of major importance for global food security and sustainable agriculture. Crenate broomrape (Orobanche crenata Forsk.) (Oc) is a parasitic weed severely affecting legumes, including pea, in the Mediterranean Basin and the Middle East. Previously, we reported the identification of the pea line 'ROR12', displaying resistance to Oc. This study was addressed to the genetic dissection of 'ROR12 resistance', based on the characterization of 148 F7 recombinant inbred lines (RILs) originating from a cross between 'ROR12' and the susceptible cultivar 'Sprinter'.

Concise description of the work (materials & methods)

Phenotyping of the RIL population was performed for two consecutive years, by scoring the average number of Oc floral shoots emerged aboveground at crop maturity. Broad sense heritability (H2B) was estimated by setting a random effect model in which genotype (G), genotype-by-year (GY), year (Y), and replicates (r) within years were set as random terms. RIL genotyping-by-sequencing (GBS) was performed to construct a high-density linkage map based on the minimum spanning tree algorithm, which was compared with the pea physical map reported by Kreplak et al. (2019). Genotypic and phenotypic data were merged by a composite interval mapping (CIM) approach, allowing the mapping and annotation of quantitative trait loci (QTLs) associated with resistance. Finally, Kompetitive Allele Specific PCR (KASP) assays were designed on the SNPs corresponding to the logarithm of odds (LOD) score QTL peaks.

Main Results

High broad sense heritability (H2B) was estimated (0.84), indicating a minor effect of environmental factors on the response to Oc infection. The application of GBS yielded 4,489 high-quality SNP, which were mapped onto seven linkage groups

(LGs), in accordance with the pea haploid chromosome number. Overall, good collinearity was found between the position of markers in the genetic map and the one in the pea reference genome, however a few exceptions were also highlighted. Three significant QTLs associated with response to Oc infection were detected. One of them, located on chromosome 4LG4 and named PsOcr-1, was associated with an extremely high LOD score peak (36.74), and explained as much as 69.3% of the genotypic variance (Figure 1). The other two QTLs, named PsOcr-2 and PsOcr-3, were positioned on the chromosomes 1LG6 and 5LG3 (Figure 1). The evaluation of the effects of different genotypic combinations indicated additivity between PsOcr-1 and PsOcr-2, and between PsOcr-1 and PsOcr-3, and epistasis between PsOcr-2 and PsOcr-3. The annotation of QTL confidence intervals allowed the identification of genes predicted to be involved in the biosynthesis of strigolactones, which are known to act as Oc germination stimulants. In addition, the SNP corresponding to the PsOcr-2 LOD score significance peak was predicted to cause a missense mutation in a phenylalanine ammonia lyase (PAL) gene, previously associated with resistance against broomrapes. The three KASP marker assays designed in our study proved to be reliable in distinguishing the genotypic combinations at PsOcr-1, PsOcr-2 and PsOcr-3.

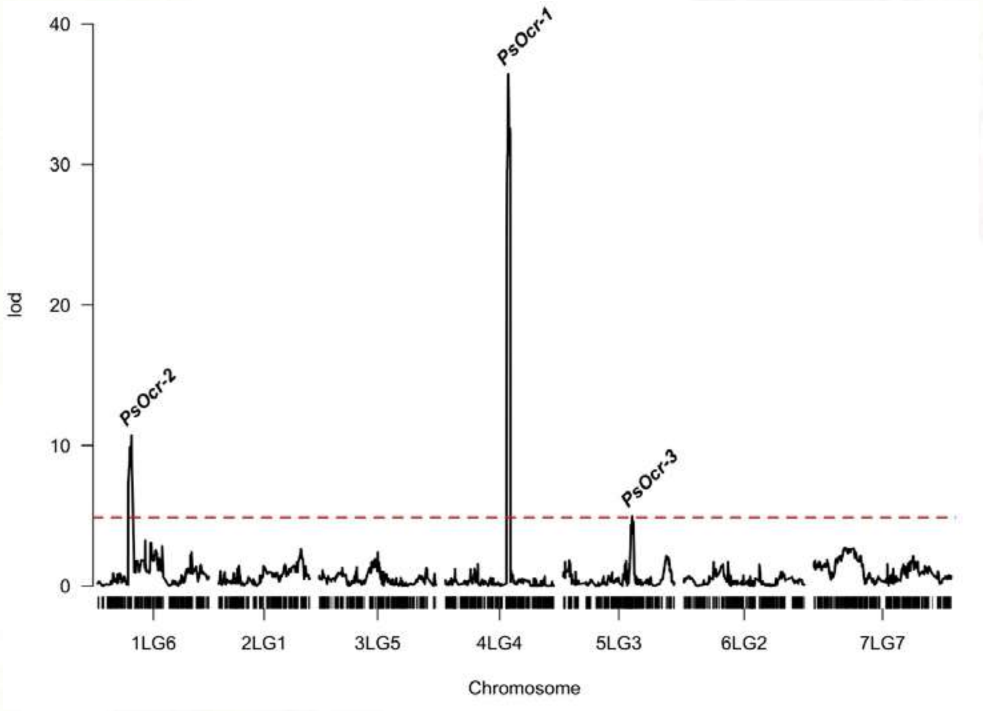

Figure 1. Logarithm of odds (LOD) score curves of quantitative trait loci (QTLs) associated with response to Orobanche crenata infection. The dashed red line indicates the significance threshold identified by permutation analysis. The three QTL above the threshold (PsOcr-1, PsOcr-2 and PsOcr-3) are indicated in correspondence of their LOD score peaks.

Conclusions

The results of this study might contribute to foster pea cultivation in the Mediterranean Basin and the Middle East, two areas in which Oc infestations discourage farmers from using legumes in crop rotations. Future research might be addressed to the refinement of the QTL mapping resolution. Pea is considered recalcitrant to stable genetic transformation protocols (Choudhury and Rajam 2021), therefore transient transformation or TILLING may be used for the functional characterization of candidate genes.

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606/174. ISOLATION AND IDENTIFICATION OF COMPOUNDS PRODUCED BY THE NECROTROPHIC FUNGI ASCOCHYTA FABAE UNDER IN VITRO GROWTH CONDITIONS ON ITS PRIMARY HOST AND OTHER RELATED LEGUME SPECIES

Authors:

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Summary:

Objectives

Ascochyta blight caused by Ascochyta fabae is one of the major constraints of faba bean and other legumes worldwide. The disease is characterized by the appearance of necrotic lesions of leaves and stems, and the latter can break leading to the loss of the upper part of the plant. Considering the economic importance of the pathogen, a deeper investigation of the phytotoxic metabolites produced by A. fabae and their role in pathogenicity, virulence and symptom development was performed. Therefore, the main objective of this work was to isolate and characterize chemically and biologically the panel of phytotoxic secondary metabolites produced by in vitro cultures of A. fabae.

Concise description of the work (materials & methods)

to explore the effect of culture media on the secondary metabolites production, the pathogen was grown in three different media (Czapeck-Dox, PDB media and Rice substrate). First, the crude organic extracts were bioassayed on its primary host faba bean, as well as on other related legumes of economic importance, at different concentrations (Fig. 1). The organic extracts were then purified by chromatographic method, and spectroscopic methods were used to characterize the most abundant secondary metabolites. The metabolites produced by the fungus in each culture medium were identified comparing their 1 H NMR and LC/MS spectra with those previously reported. Their purity was higher than 95% and was ascertained by 1 H NMR and HPLC analyses.

Main Results

The organic extract, showed differential phytotoxic activity depending on the culture media used for fungal development, on the concentration applied and on the host plant. All the three tested fungal extracts showed significantly increased phytotoxicity (measured as foliar damaged area), compared with the controls, as expected. In terms of legume species, faba bean was the crop with significant higher damage measured as necrotic area (cm2) caused by the pathogen, regardless of the culture media employed for its growth, followed by narbon and common vetches. Disease's symptoms were generally low on both lentil and pea leaves.

Seven metabolites were identified, as ascosalipyrone, ascosalitoxin, ascochlorin, ascofuranol, mevalolactone, tyrosol and benzoic acid. Finally, the phytotoxicity of the pure compounds was assessed in planta to gain insight into their role in fungal pathog

Figure 1. Symptoms developed on detaches leaves of several legume hosts as a consequence of the following treatments: (a) uninoculated, (b) water, (c) methanol (MeOH 5%), (d) A. fabae exudate from Czapeck-Dox medium, (e) A. fabae exudate from PDB medium, (f) A. fabae exudate from Rice medium.

Conclusions

Regardless on the host specie, the fungal exudate from Czapeck-Dox medium cause higher disease symptoms, followed by exudates from PDB and Rice. In addition, dose-dependent differences were also observed for all exudates on all legumes tested, being the higher dose applied (2 mg/mL) the most phytotoxic. As a result of our findings, fungal exudates from the pathogen A. fabae growth on Czapeck-Dox medium was deeply analyzed in order to ascertain is metabolite composition.

Further studies regarding the implication of the characterized metabolites on the symptoms development on each plant species are under development and will be presented during the congress.

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606/190. TISSUE - SPECIFIC EXPRESSION PATTERNS OF RESISTANCE GENES: A COMPARATIVE STUDY OF LEGUME NLR IMMUNE RECEPTORS

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Summary:

Objectives, Description, Main Results & Conclusions

Biotic stress severely constrains crop yield stability and threatens global food security. Plant resistance breeding is the most sustainable method to control pathogen pressure, providing plants with the necessary resistance features to fend off attacks. Nucleotide-binding leucine-rich repeat (NLR) proteins, representing the main class of characterized resistance genes, are intracellular immune receptors that detect and respond to pathogens. Whether and how tissue-specific expression of these NLRs can affect immunity is poorly understood. We aim to determine the degree to which NLR immune receptors display tissue-specificity. To achieve this, we employed a combined approach of phylogenomics and gene expression analyses across legumes. We characterized the NLR repertoires of 29 legume species using NLRtracker (Kourelis et al., 2021). We distinguish five classes of NLRs based on phylogenetic inference: coiled-coil NLRs (CC-NLRs), Toll/interleukin-1 receptor NLRs (TIR-NLRs), G10-subclade CC NLRs (CCG10-NLRs), RESISTANCE TO POWDERY MILDEW 8-like CC NLRs (CCR-NLRs), and TIR-NB-ARC-like-β-propeller WD40/tetratricopeptide-like repeats (TNPs). Our analyses reveal CC-NLRs and TIR-NLRs as the most gene-rich classes, consistent with their role in detecting and responding to pathogens. Additionally, using a patristic distance-based approach, we find that sequence conservation varied according to each class of NLRs, with TNPs and CCR-NLRs displaying a higher degree of conservation. Finally, we investigated the tissue-specific expression of NLRs across seven legume species by comparing RNA Seq data of leaf and root tissues. In total, we identified 1203 NLRs expressed across tissues, 72 leaf-specific NLRs, and 300 root-specific NLRs. While all different NLR classes are expressed in both tissues, we observed an average of 18% more NLRs expressed in roots compared to leaves. The legume NLR repertoires developed in this study will facilitate comparative resistance studies and legume disease-resistance precision-breeding programs. Our gene expression study offers insights for selecting new crop varieties with enhanced resistance to disease-causing agents in affected tissues.

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606/192. COMPARATIVE TRANSCRIPTOMICS REVEALS ACCESSION AND TIME-POINT-SPECIFIC GENE EXPRESSION PATTERNS IN GRASS PEA (Lathyrus sativus) UPON Erysiphe pisi INFECTION

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Grass pea (Lathyrus sativus) is a promising crop for more sustainable agriculture, combining dietary advantages with exceptional agronomic properties. Yield stability in grass pea is however constrained by foliar diseases, as the powdery mildew caused by Erysiphe pisi. Aiming to advance our knowledge on the grass pea defence molecular mechanisms against E. pisi, an Illumina RNA-seq study was performed for 48 cDNA libraries: four grass pea accessions (resistant, partially resistant, partially susceptible, and susceptible) in non-inoculated conditions and 12, 48 and 72 hours after inoculation (hai) with E. pisi. The LS007 grass pea genome (Edwards et al., 2023) was used for reference-guided transcriptome assembly. Differentially Expressed Genes-DEGs were identified across accessions and inoculation time-point, using the susceptible accession and the non-inoculated time-point as references, respectively. Bigger differences were identified among grass pea accessions than among infection time-points. At non-inoculated conditions, genes related to defence response, including innate immunity molecules, were more abundant in resistant and partially resistant accessions. A higher number of DEGs were identified in the early stages of infection (12hai) in partially susceptible and susceptible accessions. Later in the infection process, partially resistant and resistant grass pea accessions showed higher gene expression variation at 48 and 72hai, respectively. Innate immunity players, regulation of signal transduction, cellular detoxification, and response to hormones appear to be important biological processes in the grass pea molecular response against E. pisi infection. Moreover, specific legume proteins, like lectins, may have an important role in grass pea resistance. Candidate-resistance genes against powdery mildew will be selected from the transcriptomic data to be functionally validated and to develop new precision screening tools for breeding in grass pea and related species.

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606/204. A TRAIT-BASED APPROACH TO UNDERSTAND SOYBEAN AND LUPIN COMPETITIVENESS AGAINST DIVERSE WEED COMMUNITY

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Summary:

Objectives, Description, Main Results & Conclusions

Weeds constitute one of the major biotic stresses constraining organically produced grain legumes. For northern latitudes, crops such as soybean and lupin underperform against locally adapted arable weed communities. Understanding trait response to environmental changes, for example, plant growth under different weather, soil type or conditions that modify the plant micro-environment could enable a better understanding of crop-weed competition. We evaluate how soybean and lupin traits, and environment influence weed species traits and community composition shift. Field experiments with different cultivars of soybean and lupin were established in three locations across Sweden in 2020 and 2021. The locations represented different pedoclimatic zones. The crops were grown under weedy and weed-free conditions separated by fallow plots. Different crop traits were measured during the growing season and destructive sampling of the crops and weed species were done at crop flowering. Additional sampling to determine the crop yield parameters was done at crop maturity. Weeds were separated by species while crop trait values were averaged for each of the soybean and lupin cultivars. We assessed weed trait response to crop competitiveness and environment through RLQ and Fourth corner analysis methods. In general, the largest difference between the fallow and crops fields (weedy plots) was observed for the nitrophillic weed species Chenopodium spp. We also found a significant association between the weed growth form and the weed treatment. One cultivar of soybean was positively associated with the weed CSR strategy. The weed seed weight in lupin fields were found to be positively and negatively associated with the weedy and weed-free treatments, respectively. The environment mainly influenced seasonality of weed seed germination. We provide a better understanding of crop-weed interaction through integrating species abundance, plant traits and the environment influencing the interaction.

606/236. A REPERTOIRE OF PISUM IMMUNE RECEPTORS SUPPORTED THE DISCOVERY OF NEW DOWNY MILDEW RESISTANCE

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Downy mildew (DM) of pea caused by Peronospora viciae f. sp. pisi (Pvp) can result in significant yield losses (45-75%) and compromised seed quality during secondary infection[1]. Being a sexually reproducing oomycete, Pvp produces resting spores that persist in the soil and limit the availability of land for pea cropping. With recent regulations restricting seed treatments, breeding for DM resistance is crucial to mitigate the pathogen's impact. To identify new sources of resistance, UK Pvp pathotypes were screened on a set of differential cultivars, and three diverse pathotypes were then selected for screening the diversity panel of around 230 lines from the JIC Pisum collection chosen based on historic screens and the genetic diversity[2]. The phenotype screening revealed that 20% of the lines exhibited high resistance to all three pathotypes. To identify the genetic basis of this resistance, a repertoire of disease resistance genes was sequenced and assembled from the diversity panel using R gene enrichment sequencing and their pan-genome variation exploited using association genetics. We discovered a novel locus on chromosome 5 and a previously reported locus on chromosome 2. These loci were further elucidated by comparing haplotypes of the resistant and susceptible lines in the associated region using whole genome sequencing. For the chr2 locus, haplotype analysis reduced the mapping interval to a 300kb region harbouring three R-genes whose expression was validated by RNA sequencing of the host 48 h post inoculation with Pvp. One of the R genes in this region has significantly different expression and sequence structure between resistant and susceptible lines. For the utility of these candidate genes in breeding, KASP markers have been designed and tested in different populations. Future work will involve validation of these candidates using gene silencing and editing approaches.

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- *[2] Jing et al (2010) 10:44*

606/31. INTEGRATIVE GENETICS AND GENOMICS FOR MULTI-PEST RESISTANCE IN GRAIN LEGUMES

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Summary:

Objectives, Description, Main Results & Conclusions

Grain legumes are major key players of the agro-ecological and food transitions due to their nitrogen-fixing and diversifying qualities in cropping systems and their protein-rich seeds. However, grain legumes are susceptible to multiple diseases and pests, generating high crop yield instabilities. Aphanomyces root rot, Ascochyta blight, and seed weevils have been the most damaging and studied stresses in France over the past 20 years. Other pests, including weeds, aphids and sitona, are currently becoming problematic for grain legumes in the light of new regulations on pesticide use. Breeding varieties for multi-pest resistance is a major challenge for grain legume development. A major objective of the French national SPECIFICS (2021-2027) project is to generate and integrate knowledge on the diversity, genetics, genomics and mechanisms of resistance to multiple pests and protective traits in grain legumes. A Grin-Global database is deployed to manage the pea and faba bean INRAE genetic resources. Automated or manual phenotyping of exome sequenced pea or faba bean collections is performed for seedling emergence, response to water

deficit, plant root and nodule architecture and aphid resistance. A NoSQL graph database is developed to integrate QTL, RNASeq and gene annotation data in five legume species. The data base will connect data obtained in the project or previously for resistance to diseases and pests (A. euteiches, D. pinodes, Bruchus spp., A. pisum and A. fabae), seedling establishment and pest or disease-related root/aerial architectural traits to identify putative pleiotropic or syntenic genes. Pea mutant lines in susceptibility genes conserved from other plant species and speed breeding introgression lines are produced to identify and combine genes and loci for multi-pest resistance. Results will provide new knowledge and tools to accelerate breeding of grain legume varieties for pesticide-free cropping systems.

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606/62. HARNESSING WILD RELATIVES' DIVERSITY TO IDENTIFY RESISTANCE TO ASCOCHYTA BLIGHT IN CHICKPEA

Authors:

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Work centre:

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Summary:

Objectives

Ascochyta blight, caused by the fungus Ascochyta rabiei Pass. Lab. is a detrimental disease to chickpeas (Cicer arietinum L.) causing yield losses of up to 100% under conducive environments. With the emergence of more aggressive fungal populations and varying climatic conditions, stronger and more durable resistance to Ascochyta blight in chickpeas is needed. There is an abundance of genetic diversity present in the wild chickpea accessions, including resistance to Ascochyta blight. Previous research has shown strong resistance to Ascochyta blight in Cicer judaicum, however, no fertile progeny has been recovered from crossing C. judaicum and cultivated chickpea (Shah et al., 2005). Cicer pinnatifidum has successfully been crossed with cultivated species (Salaria et al., 2023) and is currently being used as a potential bridge species. The aim of this study is determine if novel QTLs for resistance are present in the interspecific population of wild chickpeas.

Concise description of the work (materials & methods)

An interspecific population consisting of 200 F6 lines derived from a cross between C. pinnatifidum x C. judaicum were screened for their reaction to Ascochyta blight under field and greenhouse conditions in 2021-2023

Main Results

The results demonstrated that there is moderate to high resistance present in the population (Figure 1). Progeny with higher levels of resistance than the two parents were identified suggesting potentially different alleles for resistance in C. pinnatifidum and C. judaicum. The most resistant F6 lines were selected and are currently being used in crossing with elite cultivars. The population was genotyped by genotyping-by-sequencing approach. After filtering for missing data and minor allele frequency of 20%, 6,000 SNP markers were used for QTL analysis. Preliminary analysis showed a total of 122 SNPs were significantly ($p<0.05$) associated with the reaction to Ascochyta blight in the population. Linkage analysis showed that these markers were clustered into two linkage groups with the highest R2 value accounting for 5.8% of variation for resistance.

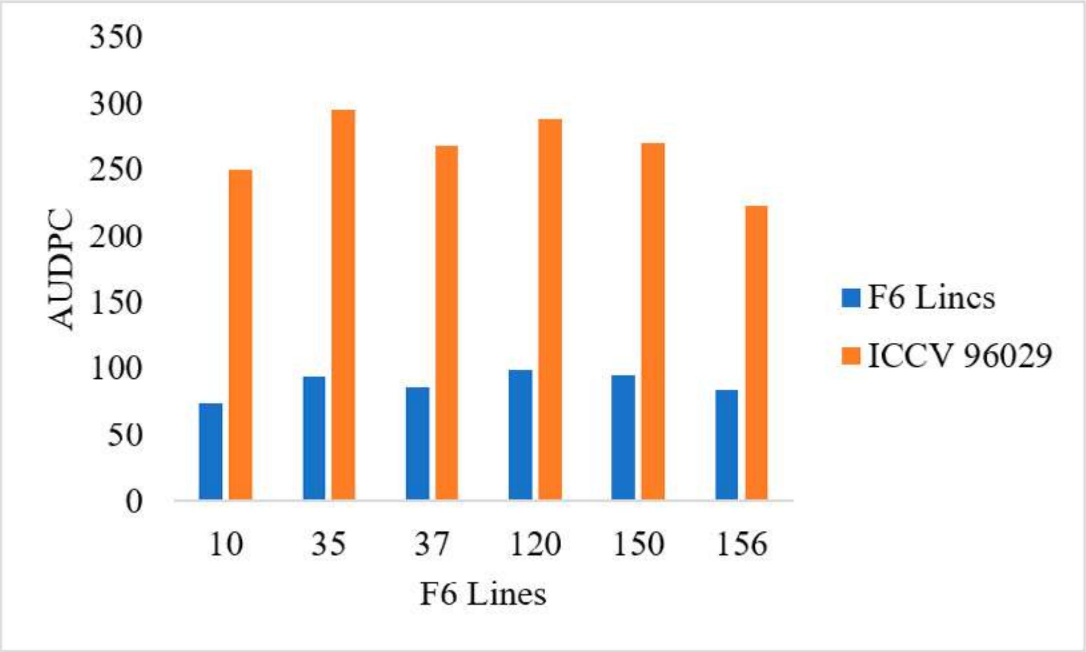

Figure 1. The Area Under the Disease Progression Curve (AUDPC) of the most resistant F6 lines determined by a best linear unbiased predictor (BLUP) test compared to the corresponding susceptible check (ICCV 96029).

Conclusions

Further analyses will be done to determine the alignment of the linkage map with the C. arietinum chromosomes, and to compare the current QTLs with all known QTLs for resistance to Ascochyta blight in cultivated chickpea.

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606/67. GWAS OF THE RESISTANCE AGAINST FOUR FUNGAL DISEASES IN A SNAP BEAN PANEL

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Summary:

Objectives

Snap beans are a group of common bean (Phaseolus vulgaris L.) cultivars grown for their edible immature pods. The objective of this work was to identify the genomic regions involved in the resistance response against four different fungal diseases, anthracnose (Colletotrichum lindemuthianum), white mold (Sclerotinia sclerotiorum), powdery mildew (Erysiphe diffusa), and Pythium in a snap bean panel (SBP) based on a genome-wide association study (GWAS).

Concise description of the work (materials & methods)

The SBP was constituted of 311 lines for snap consumption collected from European gene banks, working collections, and seed companies (García-Fernández et al. 2022). SBP was genotyped with 16,242 SNPs (missing values <20%; MAF>

0.05). The GWAS was conducted based on the multi-locus GWAS model FASTmrEMMA using the mrMLM package of the R project (R core Team 2022). Resistance test against each disease was tested in controlled conditions. The causal agent of anthracnose, C. lindemuthianum, is an extremely variable pathogen so three different isolates were tested, CL124 and CL18 from northern Spain, and C531 from Costa Rica. For the remaining pathogens, only one local isolate from northern Spain was tested.

Main Results

Figure 1 shows the Manhattan plots obtained. Concerning anthracnose, two well-known resistance clusters were identified in the SBP, the Co-2 cluster on chromosome Pv11 for isolates CL124 and CL18, and the Co-3 cluster on chromosome Pv04 for isolates CL124 and C531. In addition, new chromosome regions were identified for the resistance to anthracnose, located on chromosomes Pv02, Pv06, Pv08, and Pv10. For white mold, three regions located on chromosome Pv08 were identified. For powdery mildew, the two resistance genes (Pm) previously described on chromosomes Pv04 and Pv11 were identified in the SBP, but also new chromosome regions located on chromosomes Pv06 and Pv08. Concerning the response against Pythium, two main regions were identified, located on chromosomes Pv04 and Pv05.

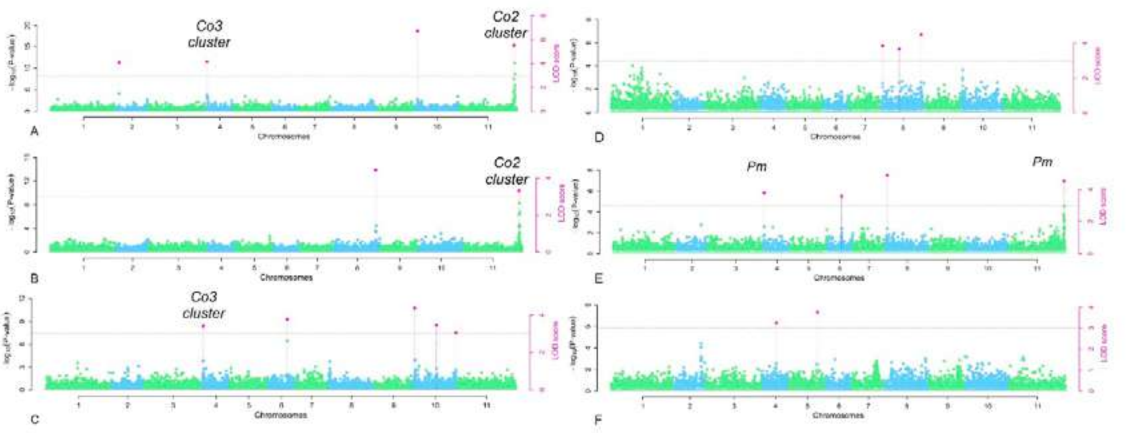

Figure 1. Manhattan plots obtained in the SBP. A) B) C) for C. lindemuthianum isolates CL124, CL18, and C531, respectively D) for a Sclerotinia sclerotiorum local isolate E) for a Powdery mildew local isolate, and F) for a Pythium local isolate.

Conclusions

Results obtained in this work highlight future opportunities of plant breeding programs for biotic stresses in the European snap bean market.

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606/77. ASSESSEMENT OF PATHOGENECITY/PARASITISM LEVELS OF DIFFERENT OROBANCHE/PHELIPANCHE SPECIES TOWARDS DIFFERENT LEGUMES SPECIES IN MOROCCO

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Legumes are among the most important crops worldwide, with a significant impact on agriculture, environment and human and animal nutrition (Graham and Vance, 2003). However, in Morocco, their production has decreased in recent years due to both abiotic and biotic stresses. Orobanche and Phelipanche genera are one of the major biotic constraints, causing considerable damage, particularly to legumes (Parker and Riches, 1993). To limit the negative impacts of these parasites, development of resistant cultivars remains a priority. The success of this control method relies on a thorough understanding of host plant/parasite interaction. The aim of this work was to assess the pathogenicity/parasitism levels by cross infestations performed with seven Orobanche/Phelipanche populations collected from different host plants (O.crenata/faba bean, O. crenata/lentil, O. crenata/chickpea, O. crenata/grass pea, O. foetida/alfalfa;Vetch, O. cumana/sunflower and P. schultzii/fennel) against seven legume species (faba bean, lentil, chickpea, pea, lathyrus, vetch and alfalfa). The monitoring of different infestation and agro-morphological parameters revealed a variable aggressiveness of these broomrape populations. Despite the existence of a close specificity between each of the parasitic species and its native host, some parasitic populations have shown a wide host range. In fact, O. crenata populations collected from faba bean, lentil, chickpea, vetch, and grass pea showed a broad pathogenicity and constitute a serious constraint on faba bean, lentil, chickpea, pea and grass pea. Nevertheless, O. cumana and P. schultzii populations don't exhibit a constraint for the host species tested. In addition, O. foetida population collected from vetch and alfalfa has no effect on faba bean, lentil, chickpea and pea and has a specific pathogenicity against alfalfa, vetch and grass pea.

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606/92. GENOTYPE × ENVIRONMENT INTERACTIONS AND IDENTIFICATION OF STABLE SOURCES OF RESISTANCE TO ASCOCHYTA BLIGHT IN CHICKPEA

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Summary:

Objectives, Description, Main Results & Conclusions

Ascochyta blight (AB) caused by Ascochyta rabei (Pass.) Labr. is a significant constraint that adversely affects the productivity and quality of chickpea (Cicer arietinum L.). Over the past two decades, numerous resistant genes/QTLs have been identified for this pathogen. To establish a comprehensive collection of chickpea genotypes with resistance to AB, the Global Ascochyta Blight Resistant Set Collection (GABRSC) was initiated through GRDC funding. The GABRSC comprises 200 Desi and Kabuli released resistant varieties breeding lines , and landraces. Field Phenotyping of GABRSC was conducted in 2022 and 2023 cropping seasons at six AB hot spot sites in India (Punjab), Turkey, Tunisia, Lebanon, Morocco, and Ethiopia. The experimental design was Alpha Lattice with two replications. The primary objective of this study was to identify sources of resistance that exhibit stability across multiple environments that favor AB epidemics. Genotypes were rated using 1–9 scale where 1= highly resistant and 9= highly susceptible. Analysis of variance revealed highly significant effects of season, location, genotype, and genotype × environment interaction. Genotype and genotype × environment (GGE) biplot analyses of multi-environmental results showed four genotypes (S0110227, S160454, S0110088, and S0110028) consistently exhibited resistance (<3) across all the environments. The four genotypes were derived from the ICARDA breeding program, which involved the pyramiding of various resistant genotypes through crosses and demonstrated higher levels of resistance across diverse locations. The resistant genotypes identified in this study hold significant potential for integration into breeding programs as stable sources of resistance, facilitating the development of agronomically desirable Ascochyta blight-resistant varieties. These findings contribute to the advancement of sustainable and resilient chickpea cultivation, ultimately leading to increased chickpea yields.

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- *DOI 10.1007/s13313-013-0219-x*
- *Doi: 10.1111/j.1365-3059.2004.01103.x*

606/96. GENOME WIDE ASSOCIATION STUDY REVEALS PEA CANDIDATE GENES INVOLVED IN RUST RESISTANCE

Authors:

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Work centre:

(1) Instituto de Agricultura Sostenible CSIC, (2) Institute for Sustainable Agriculture. Consejo Superior de Investigaciones Científicas. Córdoba. Spain

Summary:

Objectives

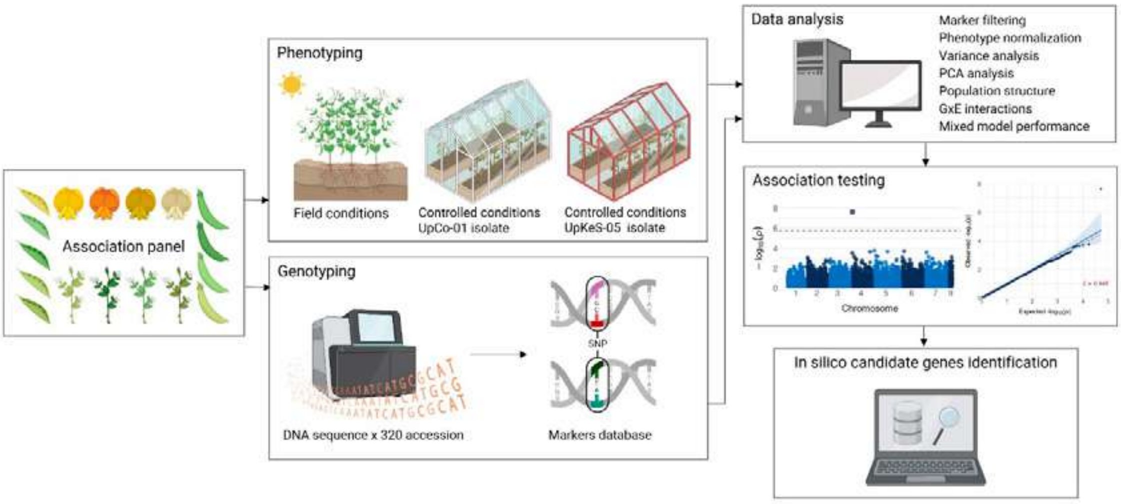
The aim of this study was to employ a GWAS (Genome-Wide Association Study) approach to identify candidate genes responsible for resistance to rust disease in pea plants. The investigation was conducted under both field and controlled conditions, targeting adult plants and seedlings, respectively.

Concise description of the work (materials & methods)

The study utilized a core collection of 320 pea accessions of worldwide origin and covering a wide range of genotypic and phenotypic characteristics. The collection comprised cultivars, landraces, breeding lines, and wild relatives representing the most prominent taxa. Field screenings for rust resistance were performed by artificially infesting the plants with a local rust isolate over three consecutive cropping seasons in Cordoba, Spain. Disease severity (DS%) was assessed 30 days after inoculation. Under controlled conditions (CC), two separate experiments were conducted using highly virulent U. pisi isolates: UpCo-01 from Cordoba, Spain, and UpKeS-05 from Kafr-El-Sheik, Egypt. In this case, infection frequency (IF) and DS% were evaluated visually or through image analysis from 8 to 13 days after inoculation, allowing estimation of disease progression parameters. The collected phenotypic data sets from field, CCCo-01, and CCUpKeS-05 experiments were analysed with a mixed model approach. The resulting estimated means from each dataset were used as phenotypic data for genome-wide associations. The genotypic dataset consisted of 26,045 DArTseq markers described by Rispail et al. (2023). The association analysis between DArTseq markers and phenotypes was conducted using a single-trait GWAS. This involved implementing a single locus mixed linear model (MLM) and a multi-locus Bayesian information and linkage disequilibrium iteratively nested keyway (BLINK) model. The analysis was performed using GAPIT 3.0, with the first 24 principal components (PCs) and the kinship matrix used as covariates to estimate family relatedness. Genomic inflations were also calculated for each trait/model to ensure adequate population structure adjustment in the models. Two LOD (Logarithm of Odds) threshold levels were applied to identify significant markers: 1) the Bonferroni corrected LOD threshold (-log10(0.05/n markers)), which represents a more conservative approach, and 2) a less restrictive LOD threshold based on the false discovery rate (FDR) method proposed by Storey and Tibshirani (2003). Candidate genes were considered when they contained a significant marker within their sequence or were in linkage disequilibrium with a significant marker. The location of candidate genes was determined using the pea cv. Cameor reference genome browser. Protein sequences were acquired and subjected to BLASTp analysis to identify homologs in closely related species. The potential function of each candidate gene was derived from annotations, literature, and in silico analysis.

Main Results

The GWAS analysis using MLM and BLINK models identified a total of 67 significant markers distributed across the seven pea chromosomes, along with four significant markers without an assigned physical position. Among the 67 markers, 53 candidate genes were annotated in the Cameor reference genome, of which 34 markers were directly associated with the candidate genes. These candidate genes encompass a wide range of biological processes, including genes involved in basal response to stresses and in defence against fungal pathogens.



Graphical abstract of the GWAS pipeline applied in this study to identify candidate genes to rust resistance in pea.

Conclusions

These findings shed light on the underlying genetic mechanisms involved in rust partial resistance and provide valuable insights for future breeding programs and genetic improvement of pea. The results contribute to the broader understanding of plant-pathogen interactions and hold potential for the development of more resilient and disease-resistant pea varieties. Further functional characterization of the candidate genes will deepen our understanding of their specific roles in disease resistance mechanisms. Overall, our research contributes to the advancement of legume research and has implications for sustainable agriculture and food security.

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606/117. THE CHICKPEA ROOT ROT COMPLEX IN SASKATCHEWAN AND SEARCH FOR RESISTANCE TO FUSARIUM ROOT ROT

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

In Saskatchewan, new chickpea health issue rapid decline of chickpea fields during the 2019 growing season. Root rot complex was identified as one potential contributor to the list of biotic and abiotic variables thought to be the basis of this health issue. In 2020 and 2021, field surveys were carried out in Saskatchewan to learn more about the pathogens responsible for chickpea root rot. The root samples were assesed using end-point PCR for the presence of 11 known chickpea root rot pathogens, some of which have not been previously reported in this area. While Fusarium redolens, F. solani and F. avenaceum were most prevalent, We also found Berkeleyomyces sp., Macrophomina phaseolina, and Verticillium dahliae in the root samples. Under controlled condition, a comparison of 16 isolates from six different Fusarium species with single isolates of V. dahliae, Berkeleyomyces sp., and M. phaseolina revealed that F. avenaceum and F. culmorum were the most aggressive Fusarium species on chickpea [1]. Based on preliminary screening of chickpea germplasm for resistance to F. avenaceum in 2018, it was hypothesized that seed coat thickness and pigmentation could be key contributor to disease resistance in chickpea. Screening for resistance to F. avenaceum was initiated

among 186 diverse accessions from a chickpea association mapping panel. Root rot severity ranged from 4% to 75% on desi types and 22% to 81% on kabuli types. The effect of seed coat on F. avenaceum infection was further evaluated by comparing root rot severity of a selected number of desi and kabuli chickpea with seed coat intact and seeds with the seed coat removed prior to sowing.

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606/133. HISTOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF GRASS PEA'S PARTIAL RESISTANCE AGAINST *Erysiphe trifolii* INFECTION

Authors:

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Work centre:

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Summary:

Objectives, Description, Main Results & Conclusions

Powdery mildew is one of the major airborne biotrophic fungal diseases compromising yield in grass pea (Lathyrus sativus). Although powdery mildew occurrence in grass pea is frequently attributed to Erysiphe pisi infection, Erysiphe trifolii was shown to induce increased susceptibility thus potentially constituting a more severe biotic threat. However, little is known about the underlying histological and biochemical mechanisms of this interaction. To overcome this gap of knowledge, our study characterized the histological response of grass pea to E. trifolii and reactive oxygen species (ROS)-scavenging enzymes activity and defence-related molecule contents at various timepoints, up to 72 HAI, Hours After Inoculation. This work follows a previous characterization of a worldwide collection of grass pea accessions for powdery mildew response, which identified partially resistant and susceptible accessions to E. trifolii infection (Martins et al., 2023). The current study found that histological differences among accessions were more evident at later stages of infection (48HAI onwards). Partially resistant accessions exhibited smaller colony sizes compared to the susceptible ones. Interestingly, the partial resistance response was not associated with host cell necrosis. From the characterization of the activity of ROS-scavenging enzymes, an increase in superoxide dismutase (SOD) activity, starting from 12 HAI, was observed in the partially resistant accessions. Additionally, there was an elevation in ascorbate peroxidase (APX) activity from 6 HAI onwards. Furthermore, we noticed an early accumulation of antioxidant phenolic compounds and a late accumulation of flavonoids, as additional defence mechanisms. Our findings suggest that grass pea mechanisms of powdery mildew exclusion may be associated with the restriction of pathogen invasion in host cells, while modulating the ROS-scavenging machinery and other secondary metabolites at different time-points of infection. These results provide valuable insights into the histological and biochemical mechanisms underlying partial resistance to pathogen infection, enabling more accurate selection for breeding purposes.

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606/177. THE MLO GENE FAMILY OF PEA (PISUM SATIVUM L.) AND ITS POTENTIAL INVOLVEMENT IN FUSARIUM WILT RESISTANCE

Authors:

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Summary:

Objectives

The MLO family is a highly conserved plant specific family of proteins with seven trans-membrane domains. Although the exact function of MLO is still unknown, phylogenetic analysis classified MLO proteins in seven clades. Mutation in one member of clades IV and V led to papila-based broad-spectrum resistance to powdery mildew in monocots and dicots respectively (Rispail and Rubiales, 2016). Potential involvement of additional MLO members in disease susceptibility or resistance is currently unknown. Papila formation in attacked root cells is a major defense mechanism of pea against the soilborne-pathogen Fusarium oxysporum (Bani et al., 2018). The aim of the present study was to explore the potential involvement of the MLO family in the susceptibility or resistance of pea against Fusarium wilt.

Concise description of the work (materials & methods)

Taking advantage of the recently published pea reference genome (Kreplak et al., 2019), we have identified the different members of the MLO family in pea throught Blast searches and characterised them with different in-silico prediction servers. Phylogenetic analysis was also performed using Neighbor-joining and Maximum-likelihood approaches to assign each pea MLO sequence to its clade. To detect the potential invovlement of MLO in Fusarium wilt resistance, we monitored the expresion level of some pea Mlo genes in roots of different accessions at different times post-inoculation by qRT-PCR. Mlo gene expresion was also monitored on powdery mildew inoculated pea leaves for comparison. Gene expression studies were performed on three biological and two technical replicates.

Main Results

Blast searches complemented by in-silico analysis identified 14 Mlos widely distributed onto 6 of the 7 pea chromosomes with 1 to 5 Mlo genes per chromosome(Figure A). Phylogenetic analysis revealed that these 14 pea MLO sequences belonged to 6 of the 7 MLO subfamilies (Figure B). Interestingly, PsMlo1 and PsMlo3 were asigned to clade V and PsMlo8 to clade IV. The gene expresion study revealed the differential expression of several Mlo genes in response to disease, suggesting their potential involvement in pea defense reaction.

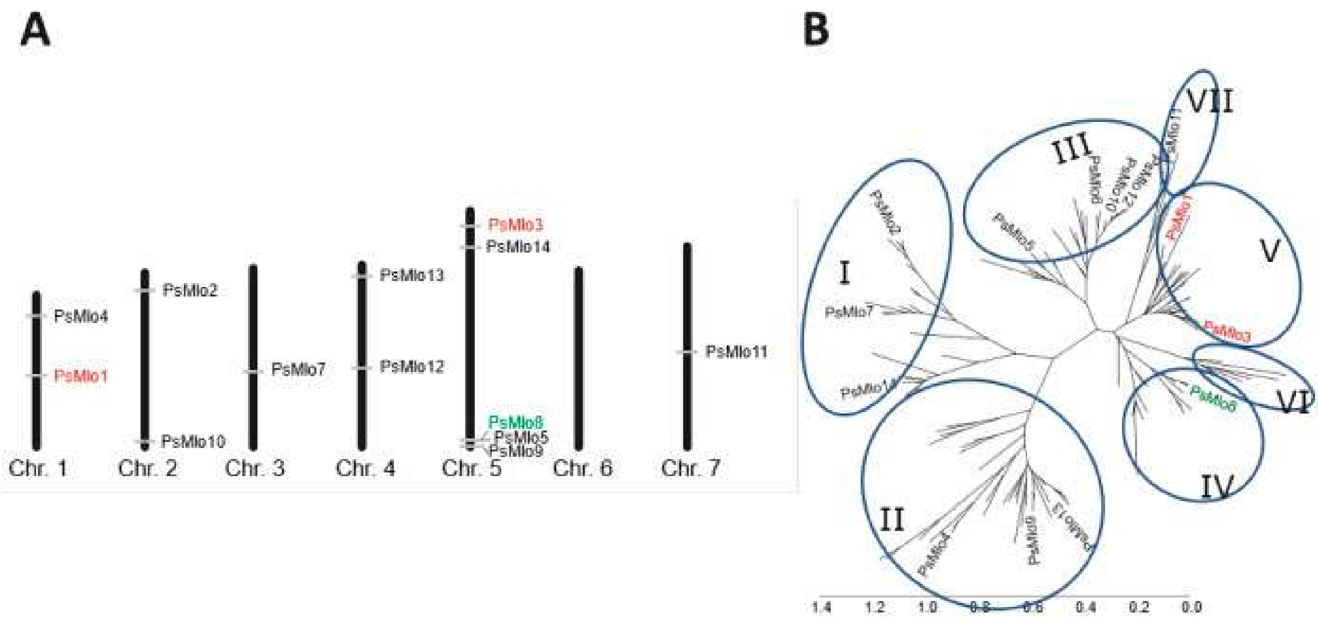

Figure. Localization of the Mlos onto pea chromosomes and their phylogenetic relationship. A. Localization of Mlo genes on pea chromosomes. B. Phylogenetic relationship of the 14 pea MLO protein sequences. The Neighbor-Joining tree was estimated based on the alignment of pea MLOs with legumes and non-legumes MLOs in MEGA with the Jones-Taylor-Thornston substitution model and 1000 bootstrap replicates. To simplify representation only PsMlos are shown. Clade V and VI MLOs are shown in red and green respectively.

Conclusions

This study provides further insight on the MLO family in legumes and its potential role in resistance which open valuable perspectives for breeding

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606/230. WEED MANAGEMENT OF LENTILS AND CHICKPEAS UNDER A NO-TILL SYSTEM IN SEMI-ARID REGIONS OF MOROCCO

Authors:

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(1) INRA MAROC. Morocco, (2) Research Institute of Organic Agriculture (FiBL). Morocco, (3) INRA Morocco, (4) IAV HASSAN 2. Morocco

Summary:

Objectives, Description, Main Results & Conclusions

Weeds pose a major problem in legume crops in Morocco as they compete with the crops for resources such as water, nutrients, and sunlight, significantly reducing yield and crop quality. Effective management strategies can help minimize the pressure exerted by these weeds and maintain legume crop productivity. This study evaluated the effectiveness of different weed control methods in lentil and chickpea crops under a no-till system in semi-arid regions of Morocco. A completely randomized block design with three replicates and seven treatments was used. The weed control treatments included pre-emergence herbicide application (pendimethalin and aclonifen), post-emergence herbicide (prydate + Haloxyfop-R Methyl Ester), mechanical hoeing with one or two passes, manual weeding, and a control group without any treatment. The results showed that six plant families contributed to two-thirds of the total number of species: Asteraceae (26%), Poaceae (13%), Brassicaceae (10%), Papaveraceae (6%), Apiaceae (6%), and Chenopodiaceae (6%). The floristic inventory conducted in both crops revealed the dominance of the following species: Centaurea diluta, Diplotaxis catholica, Lolium rigidum and Beta macrocarpa. The application of aclonifen demonstrated remarkable outcomes, including low weed density and dry biomass in both lentil and chickpea crops, measuring 9 and 11 plants/m^2 and 628.89 and 337.33 g/m^2, respectively. Furthermore, this approach led to the highest yields of 1200 and 230 kg/ha for lentil and chickpea crops, respectively, while simultaneously reducing weed control expenses by a substantial 90% compared to manual weeding. This finding suggests that the use of aclonifen can be equivalent to manual weeding and allow for effective weed control in lentil and chickpea crops under conservation agriculture practices.

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606/242. EXPLORING THE RELATIONSHIP BETWEEN COMMON BEAN SEEDLING ROOT ARCHITECTURE AND FUSARIUM ROOT ROT

Authors:

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Summary:

Objectives

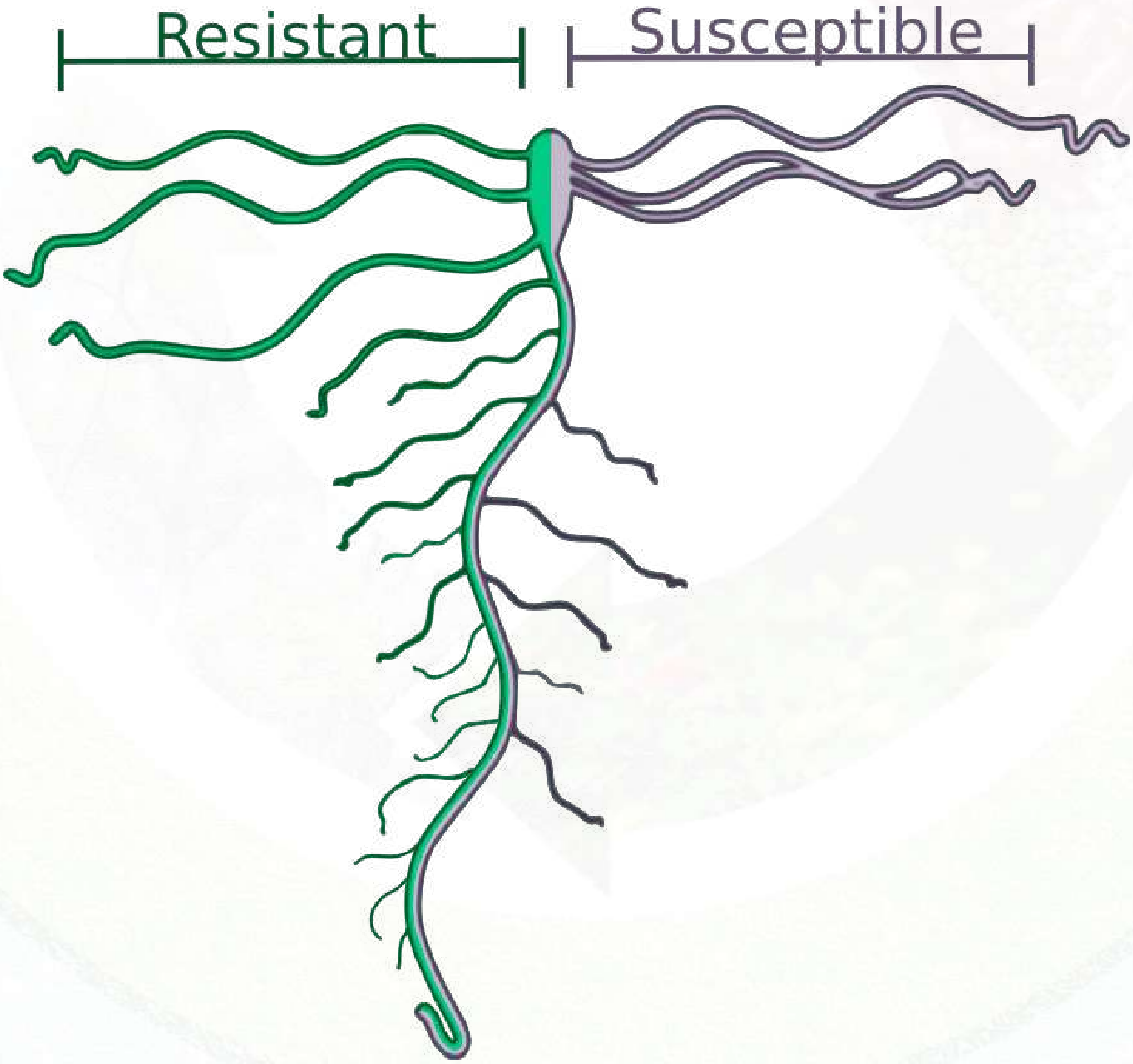
Common bean (Phaseolus vulgaris) production is severely hampered by Fusarium Root Rot (FRR), with the severity of this limitation presumed to be contingent on the host plants' gene pool. Despite over two decades of research suggesting the potential role of root architecture in conferring resilience to FRR, the identification of a root ideotype for FRR resistance has been a gradual process.

Concise description of the work (materials & methods)

We measured root traits of resistant and susceptible lines of a previously screened Recombinant Inbred Line (RIL) population, seeking to pinpoint critical root traits that could influence FRR resistance. The seedlings were inoculated with either a mock solution or Fusarium brasiliense liquid culture. The entire root system was measured before and after inoculation for developmental and root traits correlating with disease symptoms. Using shovel-omics techniques, we aligned our findings with those derived from field conditions.

Main Results

Interestingly, we discovered that FRR resistance is not solely determined by root traits, but rather by the developmental progression and rapid establishment of roots. Specifically, in resistant lines, basal root distribution was more consistent and the length of the unbranched apical zone (LAUZ) was markedly shorter, suggesting an association with FRR resistance. Therefore, the relationship between root architecture and FRR resistance transcends individual traits and necessitates an understanding of the intricate network of root phenotypes.



Summary figure of root system differences between resistant and susceptible common bean seedlings.

Conclusions

Ongoing research in the lab highlights these trends across larger populations of common bean. Future work includes using machine learning to identify necrotic regions of roots to evaluate systemic effects on root phenotypes.

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606/244. WEED COMPETITION AND DROUGHT STRESS EFFECTS ON PRODUCTIVITY OF SOYBEAN VARIETIES

Authors:

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Summary:

Objectives

Soybean breeding is usually concerned about alleviating the factors potentially limiting soybean productivity. However, non-optimal conditions for crop productivity are frequently met e.g. for organic farming, where weed competition occurs (Klaiss et al., 2022). This raises the questions whether the varieties selected in optimal conditions are also the most performant under limiting conditions and how to identify the most suitable varieties in stress conditions? The aim of this study is to test five contrasting soybean varieties for their tolerance to weed competition with and without drought stress.

Concise description of the work (materials & methods)

In 2022, soybean varieties were sown with 3 replicates in a field trial including 4 treatments: (i) watering without weed competition, (ii) watering with weed competition, (iii) no watering without weed competition and (iv) no watering with weed competition. Watering was done in order to maintain soil moisture at 70% to 100% of the field water capacity. Weed competition was generated by sowing a mixture of flax, lens and buckwheat within soybean rows whereas inter-row competition was mechanically controlled. The impact of the treatments on grain yield, yield components, and grain quality was analyzed. For the whole dataset (n = 60), grain yields were more affected by water stress (- 43%, adj R2 = 0.49, p < 0.001) than by competition effect (-33%, adj R2 = 0.24, p < 0.001), whereas the interaction between these two effects (p = 0.3) and soybean variety effect (adj R2 = 0.08, p < 0.08) were not significant.

Main Results

For each of the 4 treatments considered separately (n = 15), grain yields differed between soybean varieties with watering and weed competition (Fig. 1). The same soybean varieties remained the most and less productive whatever the stress conditions (Fig. 1). Nevertheless, the most productive soybean varieties has different strategies. Arnold has a high number of grains per m2 combined with a low thousand kernel weight whereas Obelix has a low number of grains per m2 and a high thousand kernel weight. The competition treatment decreased grain yields for all soybean varieties nearly similarly with watering (from -41% to -45%) whereas differences emerged without watering condition (from -37% to -54%). This was due to a higher decrease in grain yield of the most productive varieties compared to the lowest productive ones. The effect of soybean varieties on grain yields, thousand kernel weight, grain per m2 and protein concentration was maximal for watering treatments whereas it was reduced for drought treatments, particularly if combined with weed competition (see the adj R2 in Fig. 1). Thousand kernel weight and grain protein concentration were not affected by competition but dramatically influenced by watering. In contrast, number of grains per m2 was mainly affected by competition, while it was less affected by watering.

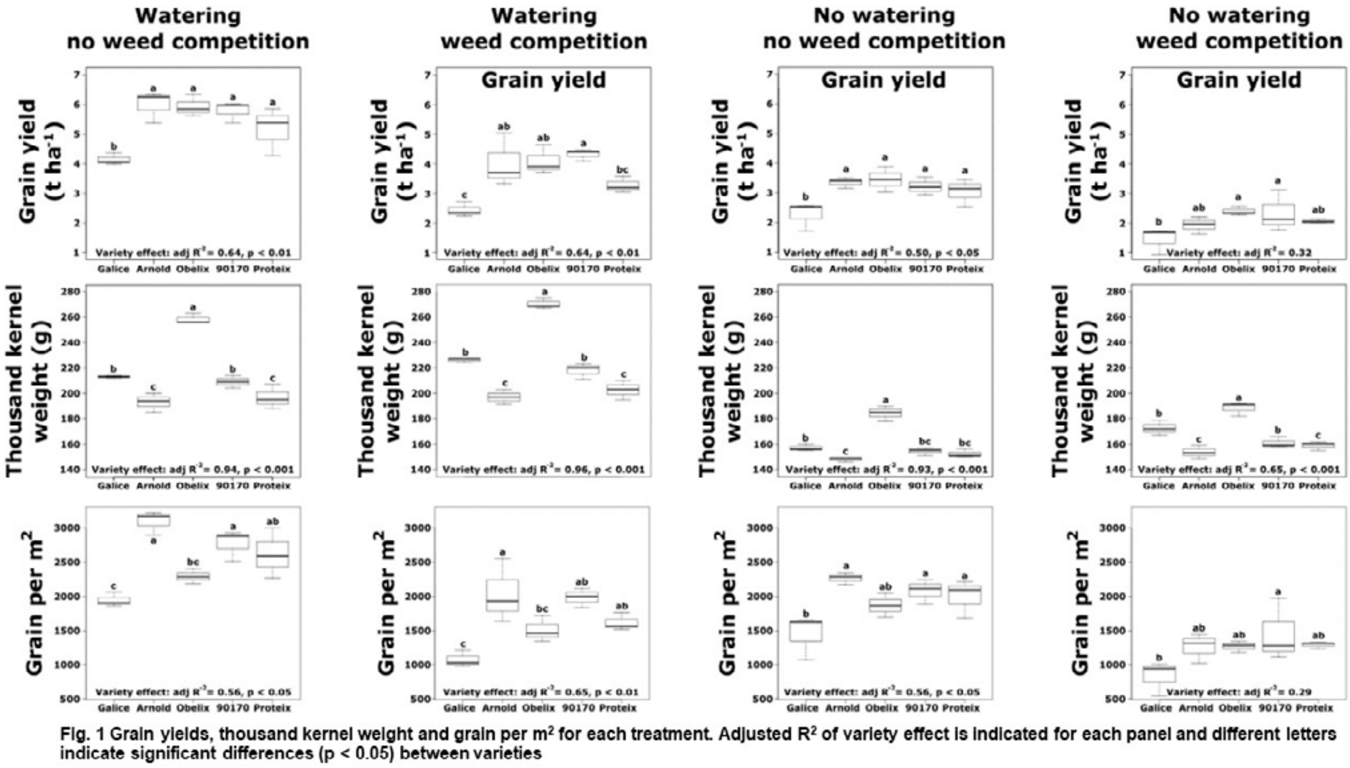

Fig. 1 Grain yields, thousand kernel weight and grain per m2 for each treatment. Adjusted R2 of variety effect is indicated for each panel and different letters indicate significant differences (p < 0.05) between varieties

Conclusions

Our data suggest that varieties selected for their high potential of grain productivity and/or grain protein concentration also performed best under limiting conditions while differences were less pronounced under stress conditions. In the following years, our trial will help to confirm that the decrease in yield due to weed competition results only from a decrease in grain per m2 while those due to drought is better explained by a lower thousand kernel weight than a decrease in grain per m2. A deep understanding of causal relationships between variety and environmental stress is needed to face the challenges of climate change and for developing varieties best adapted to organic farming.

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606/249. LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AS A RAPID DIAGNOSTIC TOOL FOR THE DETECTION OF THE ROOT ROT COMPLEX IN PEA

Authors:

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Summary:

Objectives, Description, Main Results & Conclusions

Pisum sativum provides essential dietary components, including proteins, vitamins, and minerals. Pea cultivation plays a vital role in sustainable agriculture through its ability to fix atmospheric nitrogen, reducing the need for synthetic fertilizers and enhancing overall soil health. However, one of the biggest threats to pea cultivation worldwide is the disease complex known as root rot, caused by various fungal and oomycete pathogens including Fusarium spp., Aphanomyces euteiches and Pythium spp. Its widespread distribution and persistence in soil present formidable challenges to pea production (1). As the global demand for sustainable agriculture intensifies, accurate and timely identification of pathogens becomes crucial for effective disease management. Recently, LAMP has gained popularity as a point-of-care alternative method for the detection of human, animal and plant pathogens. The major advantage of LAMP resides in its high specificity, quick readout and minimal required equipment (2). In this work, LAMP has been implemented for the detection of the root rot pathogens F. solani, F. oxysporum, A. euteiches and P. ultimum. The specificity of the LAMP primers was evaluated using a set of twenty different pea fungal and oomycete isolates, while sensitivity was assessed across different DNA concentrations. All pathogens were positively detected in less than 45 minutes even at the lowest DNA concentration of 0.02 ng. The next steps in this study are the creation of a calibration curve that correlates pathogen detection with disease severity and to deploy a portable system for field detection. My project highlights the urgency of integrating efficient disease diagnostics into the management strategies for root rot in pea. By enhancing our understanding of the complex composition of the disease, we can more effectively combat it, aiding in the sustainability and resiliency of pea cultivation.

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606/261. EVALUATION OF RUST DISEASE AND MORPHOLOGICAL TRAITS IN PEA USING AN IMAGE-BASED APPROACH

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Work centre:

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Summary:

Objectives

Rust is a wide-spread disease affecting a huge crops range, including peas (Pisum spp.). It is caused by the fungi Uromyces viciae-fabae and Uromyces pisi, with the latter predominating in temperate regions like Mediterranean environments, while the former is prevalent in tropical areas. Due to its detrimental impact, which can lead to yield reductions of up to 30%, rust is a major concern in pea cultivation. Therefore, there is a pressing need to identify resistant materials for integration into current plant breeding programs. Previous studies have identified germplasm with considerable levels of partial resistance, even in wild Pisum species, but complete resistance remains elusive1. Partial resistance entails a slowing of disease progression not associated with hypersensitivity, resulting in reduced severity (% of leaf surface covered by pustules) due to extended latency periods, lower infection frequencies (pustule count), and smaller pustule sizes. Visual severity estimation is subjective, and quantifying pustule number and size is laborious, making automation highly desirable.

Concise description of the work (materials & methods)

This study assesses the effectiveness of an automated method for evaluating pea rust using RGB image analysis and R programming. A collection of 100 pea entries was inoculated with two U. pisi isolates. Starting from day 8 to day 13 post-inoculation, third leaf petioles of each entry were transferred to square Petri dishes with agar:water (0.5% agar) and Benzimidazole (30 mg/L) to prevent foliar decay, with nine petioles per dish. Daily photographs of each dish were taken using a mobile device (3024 x 3024 pixels). Each RGB image was processed to individually analyse each petiole's severity percentage in the Petri dishes. Pustule count per genotype was estimated for each petiole and day, along with pustule size and leaf dimensions. This information allowed the calculation of rust damage in terms of disease progression, such as AUDPC, the severity ratio (DSr), and the latency period (LP50). The automated method faces challenges in distinguishing closely spaced pustules, determining the day of pustule emergence, and avoiding confusion with other types of foliar damage.

Main Results

To assess its efficacy, each image was traditionally evaluated, estimating pustule coverage percentage, and counting pustules on each leaf. Daily evaluations showed a strong correlation between the two assessment methods, with daily values consistently exceeding $\rho > 0.85$ and a higher correlation observed on days 10 and 11 ($\rho > 0.92$). Additionally, a strong correlation was observed between the traditional and automated methods for other estimated disease parameters (AUDPC, DSr, and LP50; $\rho > 0.80$). This study demonstrated significant potential for obtaining accurate measurements, providing additional information, and expediting research related to rust severity measurement in peas2. It is particularly effective for processing large batches of easily acquired RGB images and is applicable to other arial diseases affecting pea cultivation in different regions.

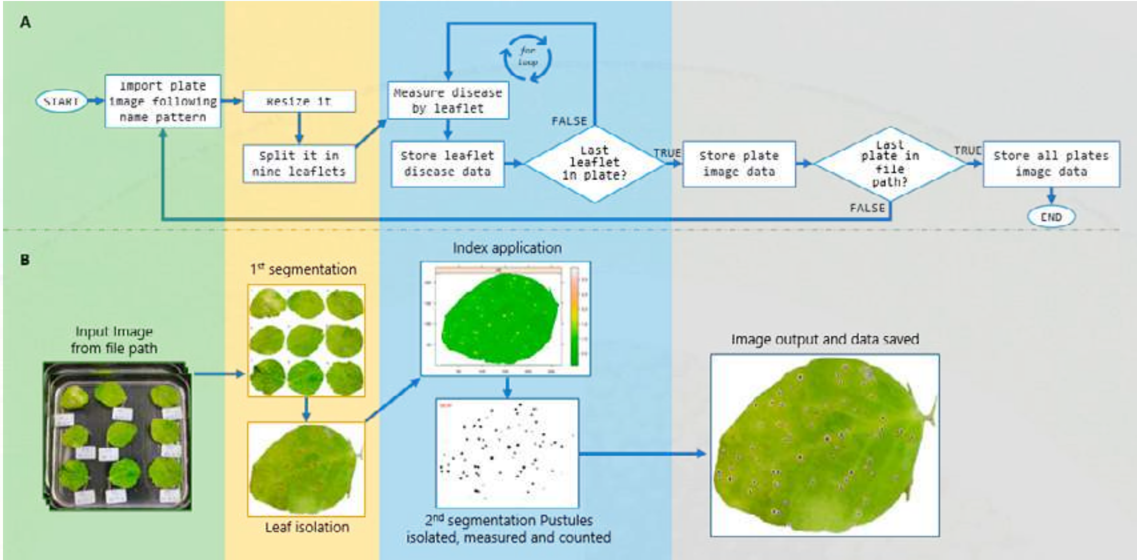

Image processing workflow. (A) correspond to the function pipeline summarized in the R script and (B) shows the image conversions from the raw image input to the single leaflet output.

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