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Cattle grazing *Gliricidia sepium*

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This is the 18th issue of *Legume Perspectives*, devoted to the 3rd Conference of the International Legume Society (ILS). The ILS aims to serve as the main hub of information and exchange on legume research and exploitation worldwide, linking together the different aspects of agricultural research on the genetic improvement, agronomy and utilization of grain and forage legumes. Its major activities include, *inter alia*, the organization of a triennial international scientific conference (we are reporting here on the 3rd one), and the dissemination of scientific and technical results by this *Legume Perspectives* journal and by ILS website (www.legumesociety.org/).

This 3rd ILS Conference was hosted in Poznan, Poland from 21-24 May 2019. It was attended by more than 200 legume scientists from 40 countries. Topics at the meeting included food quality, agronomic performance, breeding achievements and genetic tools for breeding and characterization of legume genomes. All these topics taken as a whole address many of the aspects of legume production that are important to producers, consumers and the public in general. Scientific developments in these areas continue to provide legume crops with greater sustainability for agricultural production and more nutritious crops for food and feed uses.

During this 3rd ILS conference, the ILS General Assembly the Scientific and Executive committees of the society were renewed. Also, the venue of the 4th ILS conference was fixed at Granada, Spain, 18-21 October 2022. Further developments in the coming years are expected to be reported in this next conference, and via future issues of *Legume Perspectives* and the web site. Your contributions are most needed.

Diego Rubiales
Publishing Director
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*Bogdan Wolko, Chair
of The ILS3 Local
Organizing Committee*

An Overview the Third International Legume Society Conference

As the world demand regarding plant protein is constantly rising, legume crops across the globe have had a unique opportunity to assure food security worldwide. Legume crops due to their biological role contribute greatly to sustainable agriculture. Therefore, the main topic of the ILS3 conference held in May 2019 in Poznań, Poland was: **LEGUMES FOR HUMAN AND PLANET HEALTH**. We welcomed almost 200 attendees coming from 38 countries of 5 continents. Our keynote speakers working on various legumes represented not only Europe (Czech Republic, France, Italy, Poland, UK) but also Canada, India and China. It shows that research on legumes is of worldwide significance.



Opening and Welcome addresses at the Third International Legume Society Conference

The ILS3 conference provided an opportunity to discuss the latest achievements in legume research. The conference was multidisciplinary and the following areas of legume research were tackled: Legume biodiversity and genetic resource exploitation, Advances in legume genetics, genomics and other –omics, New strategies and tools for legume breeding, Legume contribution to sustainable agriculture, Legumes for human and animal nutrition and health, Legume biochemistry and systems biology, Legume physiology, plant development and symbiosis, Biotic and abiotic stresses in legumes.

Altogether 57 oral presentations and 146 posters were contributed. The best presentations were awarded – as follows:

- *Valentina Caracuta (France)* - the best oral presentation award funded by the International Journal of Molecular Sciences.
- *Benjamin Péret (France) and Meghan Couchoud (France)* - two oral presentation awards funded by the Mayor of Poznań.
- *Ana Margarida Sampaio (Portugal), Alanna Orsak (Canada) and Ferawati Ferawati (Sweden)* - three ex aequo poster awards for students and PhD students, funded by Burleigh Dodds Science Publishing and the Mayor of Poznań.

The third ILS Conference was not only devoted to science, but it also allowed the legume community to socialize during Welcome Party, the Gala Dinner as well as excursions around Poznań or the Kórnik Castle located nearby. It was our pleasure to show our attendees some typical Polish folk dances presented by Łany ensemble during the Gala Dinner. The traditional Legume Football Cup was played out. The winners were the Starch Shuttles FC. All the cup players got medals and the challenge cup was brought to Canada by the winners' Captain, Tom Warkentin.

During the General ILS Assembly the new Executive Board was elected for the years 2019-2022 and Paolo Annicchiarico became the new President of ILS. 🌱



Group photo of the Third International Legume Society Conference participants at the Novotel & Ibis Poznan Centrum Hotel, Poznań, Poland

ILS3 highlighted nice results and challenging opportunities for innovative research on grain legume

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Keywords: grain legumes, feed, food, genetics, physiology, phenotyping, roots, modelization, abiotic constraints

The growth of the world's population, planned to be close to 9 billion humans in 2050, will lead to an increased demand for food provision. At the same time, a reduction of arable land area is occurring and there is a high consumption of non-renewable resources and fertilizers, and a high energy cost. As plant nutrient use efficiency remains suboptimal this results in a low energy efficiency of European and world agriculture. Because agriculture has a high reliance on pesticide use, biodiversity is decreased in agricultural landscapes, with health consequences for farmers and other exposed populations. Lastly agriculture is subjected to, but also responsible in part for, the acceleration of climate change.

We therefore need to conduct research in order to decrease environmental impacts, greenhouse gas emissions, avoid biodiversity loss and improve plant nutrient efficiency. To maintain simultaneous food productivity with healthy products, and an improved economic well-being of the farmers, is a major challenge. For this we need to design agroecological cropping systems based on a large range of biodiversity-based biological and ecological processes. Legumes have numerous merits in this context. In grain legumes both ways of nitrogen nutrition (i.e., root assimilation of soil mineral nitrogen and symbiotic fixation of N₂) are complementary. These however compete for carbon and energy supply. Symbiotic



Figure 1. Christophe Salon at the ILS3.

association with mycorrhizae allows grain legumes to meet their needs in phosphorus and access an adequate water supply. Some species such as lupin are even able to develop cluster roots displaying an original developmental program that allows plants to better acquire phosphorus without even relying on mycorrhizae. All these advantages of legumes lead to fertilizer reduction, improve soil structure and organic matter use, reduce fossil energy use and greenhouse gas emissions. Because grain legumes are also water-saving plants they are often used in rotations, associations, intercropping, and as cover crops. They increase associated biodiversity, as is the case of pollinating

insects. Many of these aspects have been discussed during the ILS3 Session “Legume contribution to Sustainable Agriculture” chaired by Dr Branko Copina (See Session 4).

Legumes contain protein-rich and digestible seeds with essential amino acids. Diets based on legumes and cereals produce a balanced supply of essential amino acids, which has long been exploited in traditional diets of South American or African populations. Seeds are rich in vitamins and minerals, fibres, and many have low fat contents without cholesterol. Moreover, grain legume seeds have additional health benefits because they contribute to

regulation of blood sugar, have protective effects against cardiovascular disease and improve colon health. Research underway is tackling the problems linked with grain legume antinutritional factors. In the ILS3 Session “Legumes for Human and Animal Nutrition and Health” chaired by Dr Ning Wang and Session “Legume Biochemistry and Systems Biology” chaired by Dr Tom Warkentin, talks were addressing part of these grain legume interests and remaining research bottlenecks (See Session 5 and 6).

However, despite all of these benefits, legume yields are still fluctuating, and their cropping area is decreasing (Source: UNIP, Terres Inovia). Legumes are underrepresented due to both biotic and abiotic constraints. We need to increase their profitability through higher and stable yield/protein content, finding new uses for legumes in a fluctuating environment (1). During the ILS3 conference all agreed that we need a multidisciplinary approach to work on various topics linked to adaptation and resilience of legumes to environmental constraints. Improving nutrient acquisition, and their storage and remobilization towards seeds are of tremendous interest. Nevertheless, the conference also highlighted plant- and soil-microbe interactions, not only focussing on symbiotic partners, which are increasingly studied in many institutes. Ranking, characterizing and selecting, among natural or induced genetic variability, the best allele combinations requires numerous genetics and genomic tools. In ILS3 Sessions “Legume Biodiversity and Genetic Resource Exploitation” chaired by Dr Diego Rubiales (See Session 1), Session “Advances in Legume Genetics, Genomics and other – omics” chaired by Dr C Clarice Coyne (See Session 2), and Session “New Strategies and Tools for Legume Breeding” chaired by Dr Maria Carlota Vaz Patto (See Session 3), high level speeches demonstrated that legume research is very active in these fields.

New phenotyping tools and methods for shoot and root characterization have to be developed (Opening lecture) as they are key for deciphering the mechanisms behind plant and microbiome interactions. Plant phenotyping, whatever the throughput, whether in the field or under controlled conditions in greenhouses or climatic chambers, is gaining more and more interest as it allows characterizing numbers of genotypes, species, dynamically and non-destructively. It allows measuring agronomic traits and environmental conditions precisely and at high speed, it also feeds models for

understanding and simulating plant functioning according to climatic conditions, contributing to the selection of plant varieties more tolerant to stresses. For this, we need to work on different organisational, spatial and temporal scales. Analytical studies, linked to phenotyping and modelling, allow one to dissect variables (growth, transpiration) of thousands of plants, and to carry out genetic analyses. Mechanistic models are appropriate for targeting key physiological processes but may need too many parameters for tackling a large genetic variability. On the other hand, integrative models which decompose integrative variables into elementary processes and vice versa feed our physiological understanding, generate genotypic parameters, and provide genetic analyses of “morpho traits” as a function of various environmental scenarios.

Legume-microbiome interactions, involving root system architecture and functions to improve plant nutrition, have also been widely discussed as they are key drivers of plant high temperature and/or drought tolerance. Legume-microbiome trophic interactions rhizodeposition need to be addressed more thoroughly, considering spatial and temporal dynamics, and the trade-off with productivity and resilience. All together this will contribute *in fine* to breeding pea varieties with improved symbiosis for N₂ fixation. This was discussed during ILS3 Session “Legume Physiology, Plant Development, and Symbiosis” chaired by Dr Alfonso Clemente (See Session 7) and Session “Biotic and Abiotic Stresses in Legumes” chaired by Dr Weidong Chen (See Session 8).

In this issue of Legume Perspectives, you will find many examples of very innovative research conducted with grain legumes, which show that we are making huge progress towards the above challenges but also that the road is still long before reaching them.



References:

- (1) Magrini M-B, Anton M, Cholez C, *et al.* (2016) Why are grain-legumes rarely present in cropping systems despite their environmental and nutritional benefits? Analyzing lock-in in the French agrifood system. *Ecol. Econ.* 126, 152-162.

Highlights from Session 1: Legume Biodiversity and Genetic Resource Exploitation

Chaired by Diego Rubiales (CSIC, Córdoba, Spain)

Soybean breeding for early maturity groups

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Keywords: gene bank, *Glycine max*, molecular marker

The scope of the protein strategy of the Federal Ministry of Food and Agriculture is the extension and improvement of soybean cultivation to support the production of domestic and GM-free protein feed. Due to the climatic conditions in (Southern) Germany, relevant breeding material has to be developed in the maturity groups MG00 to MG0000. A challenge as the development of early ripening varieties is not the focus of the big international breeding companies. Therefore breeding for climatic adapted, high and constant yielding varieties with high protein content is done in cooperation between the Bavarian State Research Center for Agriculture (LfL) and four private Bavarian breeding companies. To precede the breeding progress, we share winter breeding nurseries in Costa Rica and field test in Bavaria.

To enlarge the genetic diversity around 300 unselected soybean lines from gene banks (GRIN-USDA, GRIN-CA, IPK-GB) together with 80 elite varieties from France, Canada, Austria and Switzerland have been evaluated in several field trials for agronomic traits, especially for their performance in Southern Germany. Genotyping to determine genetic diversity of the pre-breeding material was done genome-wide using high-throughput genotyping (Soy6kSNPChip). Additionally selected candidate genes markers for breeding-relevant traits have been applied. Therefore molecular markers for maturity genes were successfully established and used for identification of very early flowering and ripening accessions. The results have to be

validated for further different environments.

Our results show a low genetic diversity of the currently used varieties in comparison to the studied gene bank material, therefore the narrow genetic base will be extended by crossing selected elite varieties with genetically divergent and good performing gene bank accessions.

In the last five years 20,500 offspring from 93 crosses were evaluated. As soybean has a high rate of self-pollination all crosses were tested by markers to exclude selfings.

In summary it was possible to identify breeding strains reaching a high total yield at early maturity or a higher protein yield than

current varieties. From 490 breeding lines tested in field plots three are now in the registration process for variety approval.

With our investigations the LfL laid the foundation for a powerful soybean variety development by optimizing the breeding and selection methodology, using efficient molecular marker approaches for breeders, farmers and consumers.



Acknowledgments: We are grateful to the financial support of the Federal Office of Agriculture and Food (FKZ: 14EPS028) and the Bavarian State Ministry for Food, Agriculture and Forestry (FKZ: A/14/11).



Figure 1. Evaluating *Glycine max* field trials

Highlights from Session 2: Advances in Legume Genetics, Genomics and other –omics

Chaired by Clarice Coyne (USDA ARS, Pullman, USA)

When complexity becomes fascinating: comparative cytomolecular and transcriptomic analyses of *Lupinus*

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Lupinus (lupins) belongs to tribe Genisteae of the legumes. Lupins, based on their geographical distribution, are divided into two groups. Most New World lupin (NWL) species, both annual and perennial, are distributed in the western part of North America and the Andean and Atlantic regions of South America. The Old World lupin (OWL) group is composed of twelve annual species that are distributed in the Mediterranean region and North Africa. It is also worth noting that there are three crop species: *Lupinus angustifolius* (2n=40), *Lupinus albus* (2n=50) and *Lupinus luteus* (2n=52).

Old World lupins, in contrast to the lupins of the New World, are characterised by a varied somatic number of chromosomes (2n=32, 36, 38, 40, 42, 50 and 52) and a basic number of chromosomes (x=5-9, 13). The variations in chromosome number and genome size raise questions about polyploidisation/chromosome loss processes in the Old World lupins (1,2). Attempts to explain the origin and evolution of lupins has been an active research area for many years. Recent research indicates that the genus *Lupinus* originated in the Old World (3).

Comparative cytogenetic mapping using BACs as probe for fluorescent *in situ* hybridization (BAC-FISH) have been used to identify homeologous chromosome regions and explore the karyotype variation of OWL (4,5). Based on our observation, we hypothesize that multiple and complex chromosomal structural rearrangements occurred in the OWL karyotypes during evolution. Chromosome fusion/fission



Figure 1. Karolina Susek at the ILS3.

rearrangements can shape the structure of plant karyotypes, leading to a loss in chromosome numbers. We assume that polyploidy might occurred along with aneuploidy events. Species with 2n=52 chromosomes could have evolved from an 'ancestral' species carrying the chromosome number 2n=54. Species with a chromosome number 2n=48, 2n=42 and 2n=36 might evolved through three rounds of Whole Genome Duplication (WGD). In addition, the accompanying events of aneuploidy led to the formation of karyotypes with the number of chromosomes 2n=52, 50, 40, 38, 32. We also hypothesize that the basic

number of chromosomes in the Old World lupins was likely x=6 (5).

We developed the first transcriptome database for all OWLs, several NWLs and eight species from the Genisteae tribe, an atlas of expressed genes than can be used to explore differential gene expression profiles and tissue-specificity. High-quality transcriptomes will enable us to track the evolutionary processes that led to the current diversity in lupins, but to also understand the mechanisms of the biological processes important for improving lupins and legumes.

Lupin seeds are characterised by a high protein content but also other important

nutrients such as fatty acids, fibre, minerals and vitamins that increased interest in lupins as a valuable source of human food. The use of lupins along with other well-studied and commonly consumed legumes can diversify the variety of food for an increasing plant-based diet.



References

- (1) Naganowska B, Wolko B, Sliwinska E, *et al.* (2003) Nuclear DNA content variation and species relationships in the genus *Lupinus* (Fabaceae). *Ann. Bot.* 92, 349–355.
- (2) Naganowska B, Wolko B, Sliwinska E, *et al.* (2006) 2C DNA variation and relationships among New World species of the genus *Lupinus* (Fabaceae). *Plant Syst. Evol.* 256, 147–157.
- (3) Drummond, CS, Eastwood, RJ, Miotto STS, *et al.* (2012) Multiple continental radiations and

correlates of diversification in *Lupinus* (Leguminosae): Testing for key innovation with incomplete taxon sampling. *Syst. Biol.* 61, 443–460.

(4) Susek K, Bielski WK, Hasterok R, *et al.* (2016) A first glimpse of wild lupin karyotype variation as revealed by comparative cytogenetic mapping. *Front. Plant Sci.* 7, 1152.

(5) Susek K, Bielski W, Czyż KB, *et al.* (2019) Impact of chromosomal rearrangements on the interpretation of lupin karyotype evolution. *Genes* 10, 259.

Can we identify agronomically useful and climate-smart characteristics for legumes by comparing grass pea with pea?

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Keywords: genomics, transcriptomics, resilience

Grass pea (*Lathyrus sativus*) is a hardy legume grown for food, feed and forage, often by poor farmers. It has some very useful climate-smart characteristics, being very tolerant to drought, flooding and saline soils, but it faces the constraints of low yield and containing a neurotoxin (known as ODAP) that can cause the disease, neurolathyrism, in poorly nourished people (1). By contrast, the closely related legume, pea (*Pisum sativum*), is a more widely grown cool season pulse crop with relatively high yield potential and it lacks major


antinutrients. It is, however, susceptible to many stresses that hinder its wider adoption by farmers in developing countries.

To compare the two crops (Fig. 1) at many levels, we have developed genomic resources for grass pea and pea. Both species are diploid, have a complement of 14 chromosomes and have large genomes. Analysis of the genome of grass pea (LS007, a European line) revealed that it has around 34,000 genes and an estimated genome size of 8.12 Gbp. To help us identify the functions of the genes, they have been annotated by comparing the genome with 2.5 billion transcripts from three lines of grass pea (LS007; LSWT11, a high ODAP

line; Mahateora, a low ODAP variety from India) as well as with other plant genome data. Furthermore, we have inter-crossed these three grass pea lines to produce 650 recombinant inbred lines, in which to map important traits, and generated populations of LS007 and LSWT11 that have been treated chemically to induce large numbers of mutations, which will help us with assigning function to genes. Since one of our targets for grass pea improvement is to remove its toxin, we have developed a sensitive method based on mass spectrometry to detect very small quantities of ODAP in plant tissues (2). For comparisons with pea, we have produced a

draft genome for a rapid-cycling John Innes accession (JI 2822), the parent of a JIC mutant population, and identified over 37,000 genes. Pea already has many other genetic resources in terms of inbred lines, mutagenized populations and molecular genetic markers (3).

Using these resources, we plan to identify genes involved in yield, resilience to physical and biological stresses, as well as in the production of ODAP. Additionally, the resources will be available for use by the

legume community to support trait discovery and genetic studies for the improvement of both crops. 

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References

- (1) Sarkar A, Emmrich PMF, Sarker A, *et al.* (2019). In: *Genomic Designing of Climate-Smart Pulse Crops*, pp 425-469.
- (2) Emmrich PMF, Rejzek M, Hill L, *et al.* (2019). *BMC Plant Biol* 19 (1):489.
- (3) Smykal P, Varshney RK, Singh VK, *et al.* (2016). From Mendel's discovery on pea to today's plant genetics and breeding. *Theor Appl Genet* 129, 2267-2280.

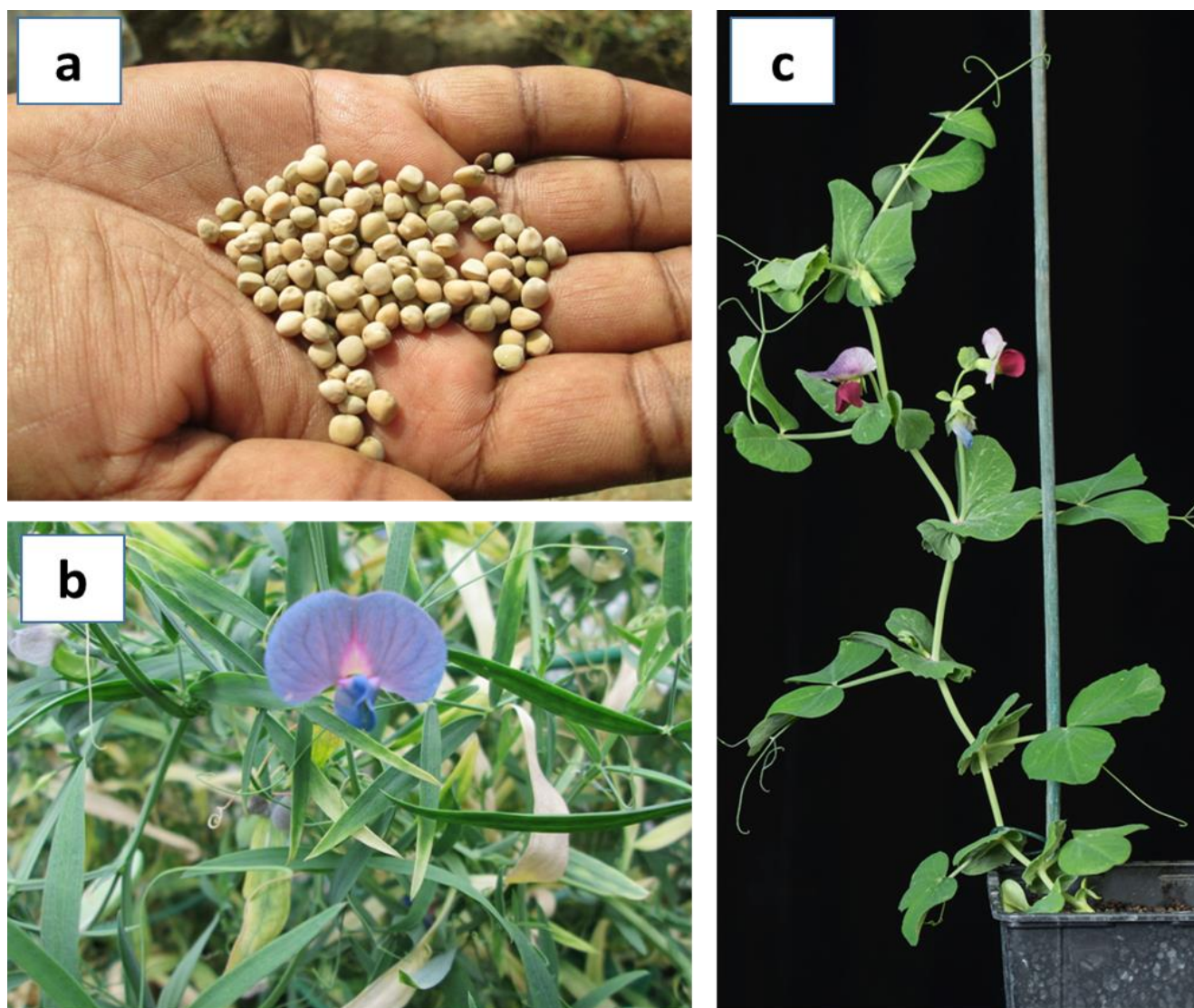


Figure 1. Seed (a) and flower (b) phenotypes of contrasting *Lathyrus sativus* (grass pea) accessions. The 'rapid cycling' *Pisum sativum* (pea) accession (c) has a purple-flowered phenotype.

Seed development in *Phaseolus vulgaris* L.: post-transcriptional regulation mediated by miRNAs

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Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for human consumption worldwide, as its seeds are high in protein, dietary fiber and essential vitamins and minerals. (1). The final characteristics of the seeds, such as their nutritional content, result from molecular mechanisms that act during seed development. While some of these mechanisms were previously described by us (2,3), it remains to be understood how gene expression is regulated, namely at the post-transcriptional level. MicroRNAs (miRNAs) are endogenous small non-coding RNAs (~21-22 nt) that regulate post-transcriptionally gene expression by cleaving target transcripts or repressing their translation (4).

To identify miRNAs expressed during seed development, we performed a small RNA sequencing analysis on seed samples from embryogenesis to desiccation. We retrieved 72 known miRNAs, already described in other species, and 39 new miRNAs. The overall analysis of the miRNA accumulation profiles during seed development highlights two major stages where post-transcriptional regulation is active: embryogenesis and seed desiccation. During embryogenesis, miRNAs, such as pvu-miR160a, are implicated in the regulation of cell division and patterning, which allows correct embryo differentiation. At the seed desiccation stage, miRNAs such as pvu-miR169c are implicated on the regulation of seed dormancy and stress responses.

In summary, our data indicates that complex post-transcriptional regulatory networks act during the common bean seed development. The modulation of miRNA abundances, and thus of their targeted transcripts, could be used to improve important seed traits like the seed quality and size.



Figure 1. Seeds of *Phaseolus vulgaris*. Credits of the photo: José Parreira (ITQB NOVA)

References

- (1) Leitão ST, Dinis M, Veloso MM, *et al.* (2017) Establishing the Bases for Introducing the Unexplored Portuguese Common Bean Germplasm into the Breeding World, *Front. Plant Sci.* 8 1–18.
- (2) Parreira JR, Bouraada J, Fitzpatrick MA, *et al.* (2016) Differential proteomics reveals the hallmarks of seed development in common bean (*Phaseolus vulgaris* L.), *J. Proteomics.* 143, 188–198.
- (3) Parreira JR, Balestrazzi A, Feveiro P, *et al.* (2018) Maintaining Genome Integrity during Seed Development in *Phaseolus vulgaris* L.: Evidence from a Transcriptomic Profiling Study, *Genes (Basel).* 9, 463.
- (4) Yu Y, Jia T, Chen X, (2017) The 'how' and 'where' of plant microRNAs, *New Phytol.* 216 1002–1017.

Highlights from Session 3: New Strategies and Tools for Legume Breeding

Chaired by Maria Carlota Vaz Patto (ITQB NOVA, Oeiras, Portugal)

Grain legume plant breeding in the EU

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The assessment of the grain legume breeding activities in the EU have never been done before the ILS3 conference in Poznan, and participant payed a lot of attention to the presentation. Most of the EU participants are now waiting for the publication of the database through the LegValue website (www.legvalue.eu). Indeed, no names were mentioned during the presentation. Moreover, the database is still improving with 25 additional breeding programmes since the conference thanks to the feedback from several participants.

Another database is under construction on forage legumes (alfalfa, clovers, sainfoin, etc.) and should be also published before the end of the year.

Next step will be the identification of current networks in breeding collaboration within the different grain legume species. This have been illustrated by the collaboration set up in France 25 years ago between public institute and private company to look after resistance against *Aphanomyces euteiches* genetic resistance in pea. It is quite relevant to notice that no question came from the audience concerning the successful improvement on Pea *Aphanomyces* tolerance in France, as these new varieties may have some potential development in other countries affected by the disease.

The challenge is now to strengthen the collaboration to improve the innovation of grain legume varieties well adapted to the wide range of pedoclimatic conditions in the EU. Anybody willing to participate in such a challenge is invited to contact the author.



Plant Breeding Database can be accessed at: <http://www.legvalue.eu/resource-section/european-pulse-breeding-programmes/>



Figure 1. Frédéric Muel at the ILS3.

Highlights from Session 4: Legume Contribution to Sustainable Agriculture

Chaired by Branko Cupina (University of Novi Sad, Serbia)

Keynote presentation: “Diversification of feed protein sources for the food security. An overview on Session 4”

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Legumes (Leguminosae) are one of the most important plant families (the Big Five). Covering many genera and species and showing a broad diversity of biological and useful features, the latent potential of legumes is an excellent subject matter for research. The practical value of legumes, particularly for sustainable agriculture, is based on two features – N fixation and the high protein content in seeds. Both these features should influence the grower to allot a larger share of land to legumes in a crop rotation (10–20%). Unfortunately, in most countries, only 2–3% of agricultural land is used for legume cultivation except for Lithuania and Estonia (about 14%), Latvia and UK (about 5%).

The fourth session of the Third International Legume Society Conference included eight oral presentations. Two were dedicated to highlighting the importance of legumes in human and animal consumption. Although legumes are directly eaten by humans, legume protein is generally used to feed animals. For a global production of about 240 million tons per year of pork and poultry meat, 179 million tons of soya meal was produced and used. In many countries, soya meal is the main (about 90%) source of protein in animal feed. The world market and availability of this raw material can be endangered as there are only three main producers and exporters of soya seed and meal – Argentina, Brazil and USA. This monopoly together with the fact that China imports over 60% of the world soya seed production can and should be a cause for



Figure 1. Evaluating indigenous protein sources such as winter peas

looking for alternative protein sources, for example, grain legume seeds, oil seeds meal, DDGS (distiller’s dried grains with solubles), fish meal or insect protein. The aim of any country should be to increase the share of indigenous protein sources, thereby ensuring a so-called protein security. A basic requirement of feed producers is a large input of raw material. In the case of grain legumes, this amounts to high yielding cultivars and cropping technologies

(competitive to cereals), new feeding technologies and a well-organized market and turnover system. Research studies show that using legume seeds for feeding pigs and poultry can be comparable or even better than using soya meal. In some European countries, the utilization of indigenous protein sources has clearly increased in the past decade (Table 1). To maintain this development in the European Union, it would be advantageous to establish a

European Target Indicator, which would measure the progress achieved by each European country towards the production of indigenous protein sources.

Interesting results have been achieved, as mentioned in one presentation, on applying arbuscular mycorrhizal fungi and plant growth promoting bacteria for chickpea yield under different water regimes. Underlined were all studies on increasing and stabilizing legume yield in abiotic, particularly water stress, conditions.

From time to time, attempts have been made on growing winter peas. Recently, factors such as mild winters due to global warming and improved cultivars have made conditions favorable for winter pea cultivation. There are two variations of growing peas in winter: 1) sowing seeds in autumn, germination and growth before winter, quicker plant development in spring, and possibly higher yield due to winter water or, 2) sowing seed coated in autumn, quicker germination and better usage of winter water in spring, resulting in higher yield.

Strategies for winter peas cultivation in Germany and Austria, including cultivar types for seed production and organic farming (mixtures with different plant partners), sowing terms and density, were discussed in the presentations. Also discussed were trial results conducted in Rensthal (Germany) in 2016/2017 and 2017/2018. In the first trial, the lowest temperature experienced in the test area was -13°C with a little snow cover. French cultivars presented the highest yield of peas and a total yield of mixtures. In the second experiment, the lowest temperature experienced was -17°C without snow cover. The French cultivars died completely and most long straw cultivars with white flowers were reduced in number after winter. A strong differentiation in yield was observed for pea cultivars as well as for mixtures with cereals. Moreover, different mixture partners were best in different years – rye in 2017 and triticale in 2018. Very good pea yield was observed in the 2018/2019 trial in Gostyń (Poland) with the lowest temperature -8°C (Picture taken 21.05.2019, sowing 15.10.2018). As weather conditions are impossible to envisage, the cropping technologies (including selection of locations and cultivars) for winter peas require further studies and improvements. Unfortunately, the biochemical and genetic mechanisms controlling pea plant resistance to low temperature are unknown yet.

The characteristics of legume (annual and

Table 1. Meat production vs. soya meal import, million tonnes (FAOSTAT)

	Year	Poland	%	Germany	%	Holland	%
Poultry	2007	1022		1120		763	
	2016	2257	+120	1550	+38	1105	47
Pork	2007	2151		4985		1290	
	2016	2009	-7	5590	+12	1453	13
Soya meal	2007	1908		3194		4456	
	2016	2243	+18	2989	-6	3052	-32

perennial) root, which include biologically fixing nitrogen and improving soil fertility, earn it an important place in crop rotations, particularly on light soils and in semiarid regions. There are many legume crops and usage types (e.g., cover crops, mixtures, intercrops, aftercrops) which can be adapted to local conditions. An example is the research conducted in the Vojvodina/Serbia province which showed the positive impact of winter legume cover crops on the soil water balance and water availability for the main subsequent crop, causing improvement in its yield.

The added value of legumes in different cropping technologies is their successive influence. It is accepted that crops grown after legume cultivation give 15–20% higher yield. Two presentations in this session were on legumes/non legumes intercropping as sustainable agriculture practices. In northwest part of China, productivity, N fixation, P utilization and soil fertility were

studied for the following intercropping: faba bean/maize, chickpea/maize, soya bean/maize, rapeseed/maize compared to monocropping. Intercropping increased many features like grain yield, aboveground biomass and NPK (nitrogen, phosphorus, potassium) nutrient acquisition as well as N fixation and soil N balance. The advantages of intercropping as an important practice for sustainable agriculture were presented by discussing the monocropping and intercropping experiments in Germany on faba bean, field pea, blue lupin and spring vetch.

The session confirmed the importance of two characteristics of legumes – N fixation and high protein seeds – for sustainable agriculture. It highlighted the necessity to increase research studies and promote usage of legumes in crop rotation and as food for human and animal consumption.



Legume-research and -innovation communities have a central role to play in helping to realise truly sustainable food- and feed-value-systems.

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It is troubling that, despite their many benefits, legumes are not yet cultivated to any great extent across Europe. This scenario has persisted for many years, and even despite the now urgent necessity to reduce greenhouse gas emissions and adapt to the impacts of climate change. Around the time of the International Legume Society's (ILS) 2019 meeting in Poznań (Poland), socio-political movements such as Extinction Rebellion (1) were disrupting centres of policy making. Such activism highlights the fundamental concerns of consumers throughout Europe, and indeed globally, and especially the hopes and fears of the young

who have inherited the current geological period - named 'The Anthropocene' (2), and its associated negative consequences for biodiversity, equality and a sustainable future.

Of course, there is hope and we have witnessed in the past year the response by consumers and policy-makers globally to the dangers of plastics (3). Yet the likely greater global risk of excessive reactive nitrogen leaking from our farmed systems is far less visible than littered beaches or marine animals as victims of carelessly-wasted plastics. Consequently, the role of well-managed legumes and diverse food- and feed-systems to encourage natural nitrogen

cycling, and thereby reduce greenhouse emissions, seems ignored by most consumers. However, an awareness of sustainable consumption is at an all-time high, and still increasing. While the purchase of vegan and vegetarian products account for only a small percentage of the total food market value, the potential of legume-based protein products to support demitarianism (the practice of reducing meat consumption for health, environmental and/or socio-political reasons) is large, and escalating year-on-year too. This is reflected by investors and the stock exchange flotation value of companies such as Beyond Meat™ (4).

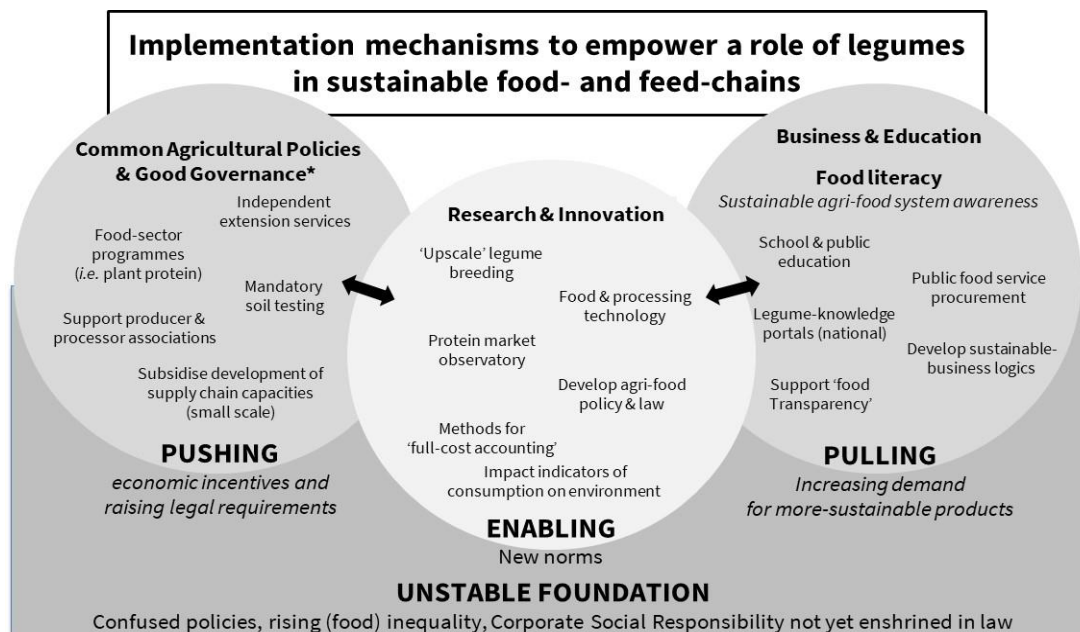



Figure 1. This figure has been informed by key articles (8-10) and highlights the central role for the 'enabling' efforts of the legume-research and -innovation focused communities. This figure also stresses that such effort needs greater support for integrated policies and at a range of levels – global, European, national and regional - to implement the necessary and complementary "pushing" and "pulling" mechanisms. Implementation efforts need based upon a foundation of linked policies (e.g. environment, health and climate change), which is more robust that currently facilitated.

* Good-governance is defined here as, "the implementation of mechanisms which ensure the creation, protection and fair-distribution of wealth".

Currently however, where grain legumes are cultivated in Europe, they are sold at the lowest of premiums, and mainly as a source of feed for animal production. Even then, much animal feed is usually not home-grown, and though the EU is reported to be self-sufficient for forage-based protein, around 75% of high-protein grain demand is still imported (5). Grain legumes are therefore not generally sold or developed for the highest premium markets offered by human consumers.

Shortly after the ILS meeting, we have also seen the EU and South American economic bloc Mercosur sign a large trade deal after 20 years of negotiations (6). It is argued that this development will increase EU demand for Latin American agricultural products, including meat. This policy seems likely to continue the restriction of grain legume crop production and benefits in Europe. This approach also seems likely to deliver yet more negative impacts in South America, compounding the effects upon their already denuded rainforest and cerrado regions - which are of global importance in terms of their biodiversity and ecological function (7).

Empowering diversified food- and feed-value-systems across Europe will remain very challenging when such systems are embedded in global trade markets. In addition, existing networks for legume production and processing in Europe are normally characterised by a lack of the necessary skills and capacities for effective commercialisation. Where capacities do currently exist, these are often large-scale and focus on mainly peas (and especially yellow- or white-pea), though some processors are about to realise value-systems processing large volumes of dry faba beans too. Commercial development of such grain legume-based value-systems are of course welcome, but will these approaches empower home-grown cultivation for a greater diversity of legume species and more nutritional intake of legumes and legume-based products throughout Europe? Or, will they simply expand large-scale industrial production for a narrow range of legume crop species, and for a similarly restricted range of countries or regions?

Whatever the future holds we can be sure that the opportunities to realise home-grown grain legume production in 'modern' times has never been greater. The legume-research community should be quick to capitalise on the role of consumer citizens to play a pivotal role in driving the shift towards legume-based agri-food and -feed systems throughout Europe, and globally. 

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Disclaimer: the opinions expressed here are my own, and do not (necessarily) represent the opinion of the James Hutton Institute.

References:

- (1) www.theguardian.com/environment/extinction-rebellion
- (2) www.science.sciencemag.org/content/347/6223/1259855
- (3) www.theguardian.com/environment/2018/jan/16/eu-declares-war-on-plastic-waste-2030
- (4) www.cnbc.com/2019/07/29/beyond-meat-hype-rivals-that-of-bitcoin-and-could-share-the-same-fate.html
- (5) www.ec.europa.eu/info/news/commission-publishes-overview-eu-feed-supply-2019-may-20_en
- (6) <https://copa-cogeca.eu/Download.ashx?ID=3667031>
- (7) www.bbc.co.uk/news/world-europe-48807161?fbclid=IwAR3OfNE0Z2IeFlizjITY6oChoWC Gj5yy_F2FN_KEZQb50gzD0vrhr_uY6bc
- (8) www.nature.com/articles/s41893-019-0266-6
- (9) www.ec.europa.eu/info/events/development-plant-proteins-europe-opportunities-and-challenges-2018-nov-22_en
- (10) www.ec.europa.eu/info/sites/info/files/food-farming-fisheries/plants_and_plant_products/documents/plant-proteins-study-report_en.pdf

Strategies for production of organic winter peas in Germany and Austria

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Keywords: winter peas, legume-cereal mixtures

In the beginning of the 2000s, winter peas were introduced into organic agriculture in Germany and Austria as a completely new crop. Meanwhile, winter peas are an important grain legume on many farms especially on poorer soils and under dry weather conditions. Introduction of winter peas helped to increase the amount of organic pea production.

There are two types of winter peas available in Germany and Austria: The first types are short straw varieties from France with relatively low winter hardiness, low weed suppression, white flower and large seed size. The second types are long straw forage type varieties with mostly good winter hardiness, good weed suppression, white or coloured flowers and often small seed size. This type is called Austrian field pea in North America. Conventional farmers prefer to grow French types as pure seed for seed production, while organic farmers prefer long straw types. Long straw types are always mixed with a cereal partner to reduce lodging. Partners are triticale, rye, wheat or barley. Sowing density depends on the region and location. An average quantity is 150 seeds of cereals with 40 seeds of peas. At dry locations the amount of cereals is reduced, and the amount of peas is increased. In wet regions or on soils with good quality, the amount of peas is reduced. Some organic farmers use the mixture with peas and cereals for silage. Limiting factor for production is winter hardiness of the varieties. Later sowing dates in October improve winter hardiness. Many forage types with coloured flower have got the best winter hardiness. French varieties have the lowest winter hardiness.

The yield potential of the French varieties is higher in some regions, but in other regions forage types have the higher yield potential. Mixing forage winter peas with cereals enhances total yield and yield stability. Weed suppression is very good.

The project DemoNetErBo supports production and supply chains of field peas



Figure 1. Winter forage pea Pandora with white flower.



Figure 2. Winter forage pea E.F.B.33 with coloured flower.

and faba beans in the German protein crop strategy.



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and Agriculture (BMEL/BLE) under the Federal Protein Crop Strategy.

For more information:

www.demoneterbo.agrarpraxisforschung.de (in German)

Legume cover crops for soil conservation

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Research on the effect of cover crops in crop rotation seems to be present everywhere—from organic farming to home gardens and to conventional cropping system. Cover crops are used to cover the soil between two cash crops or between the rows of existing crop bringing several benefits into the farm, such as maintain or improve soil properties, like fertility, soil structure, and water capacity. Legume cover crops are especially important for maintaining nutrient balance in cropping systems because they are one of the few organic inputs that supply nitrogen. In the temperate region, the uses of legumes or legume-based mixtures are showing an advantage having in mind that they also provide nitrogen to the soil and can be easily adapted to crop rotation. Field peas, common vetch, cereals (oat, triticale, rye) are usually planted as winter crops between two spring cash crops. Sometimes farmers are having doubts



Figure 1. Different aspects of legume cover crops evaluation for an improved soil conservation.



Figure 2. Different aspects of legume cover crops evaluation for an improved soil conservation.

whether cover crops will reduce soil moisture for main crops, creating difficulties for their initial growth and resulting in reduced yield. These suspicions are justified, especially in temperate areas where droughts are often in the spring and if there is no additional irrigation can make production uncertain. However, small and medium farms in south-eastern Europe are based on rain-fed systems and many studies have shown that legume cover crops have positive effect on soil properties and main crop yield and quality when precipitation is well distributed during the growing season. This is mainly related with a fact that with legume cover crops significant amount of plant residues are incorporated in the soil providing a base for building up soil organic matter and thus soil quality.



Figure 3. Different aspects of legume cover crops evaluation for an improved soil conservation.

Co-inoculation with plant growth promoting bacteria and arbuscular mycorrhizal fungi on chickpea (*Cicer arietinum* L.) increase grain yield under different water regimes

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Chickpea (*Cicer arietinum* L.) is widely cultivated and currently ranks second in the world's production of legumes. It is mostly grown in dry land conditions, and with ongoing climate changes, the severity, frequency and duration of drought in chickpea producing areas around the world are predicted to increase with negative impacts on grain yield.

Chickpea tolerance to biotic and abiotic stresses needs to be improved to allow plant growth that satisfies food demand under limited resource availability. The economic and environmental constraints from the use of synthetic fertilizers actively encourage the use of plant-associated microorganisms that enhance crop productivity and plant resistance to biotic and abiotic stresses. Among beneficial soil microorganisms, plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) can be used as biotechnology tools to enhance the availability of essential nutrients and improve water use efficiency.

Our research aims the selection of elite plant growth promoting bacteria associated with chickpea, including effective rhizobia, for improvement of plant growth and tolerance to drought stress. A field trial was conducted in Vila Real with control plants (non-inoculated), plants inoculated with a mixture of PGPB (*Mesorhizobium ciceri*, *Burkholderia* sp. and *Pseudomonas* sp.) through seed coating and plants inoculated with the same PGPB mix + commercial AMF inoculum. The irrigation regimes applied during the crop cycle were: 1) 100% of plant requirements (WR), 2) 50% WR, 3) 25% WR, 4) irrigation only during flowering (IF) and 5) rainfed.

This study revealed a 1.8% increase of the protein content in the grains of the inoculated plants when compared to the



Figure 1 - Chickpea root inoculated with PGPB mix.

non-inoculated plants. Single and dual inoculation with PGPB mix and PGPB mix+AMF, resulted in an increase of 142.16 and 267 kg ha⁻¹ of grain yield, respectively, when compared to the non-inoculated plants. The plants inoculated with PGPB+AMF with irrigation only during flowering presented the highest grain yield (1504 kg ha⁻¹), therefore, inoculated chickpea with efficient PGPB and AMF and irrigated only during flowering could be a good strategy to reduce water use.

In drought scenarios, inoculation with efficient PGPB and AMF has the potential to tackle problems arising from water scarcity and can be used to enhance grain quality and grain yield, leading, ultimately, to benefits in human nutrition and a more sustainable production of chickpea. 🌱

Highlights from Session 5: Legumes for Human and Animal Nutrition and Health

Chaired by Ning Wang (Canadian Grain Commission)

Chemical, nutritional and functional characterization of *Apulian black chickpeas*

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Chickpea (*Cicer arietinum* L.) is one of the most important legumes worldwide. Commonly, chickpeas are classified in two types, *kabuli*, characterized by large seeds with beige coat, and *desi*, characterized by small seeds with a dark coloured coat (Fig. 1). A third, uncommon type of chickpea has a centuries-long tradition of cultivation in the region of Apulia, in the south-eastern part of Italy, and was recently reported to be darker and bigger than common *desi* types. This *Apulian black* chickpea type is called “*cece nero*” in Italian, meaning black chickpea.

Our contribute to the ILS3 congress reports the chemical, nutritional and functional characterization of a collection of 21 *Apulian black* chickpea accessions, which were compared with 19 *kabuli* and 17 *desi* genotypes. Our results highlight that *Apulian black* chickpeas are nutritionally valuable, as they are richer in dietary fibre, bioactive compounds and polyunsaturated fatty acids. Whole meal flour of *Apulian black* chickpeas was suitable for mixing with cereal flours to produce cereal-legume foods, such as vegetable/vegan burgers, bread and other bakery products. On the other hand, our data show that flour obtained from *Apulian black* chickpeas is less suitable for food applications where oil incorporation is required, such as meat extenders.

Apulian black chickpeas have been traditionally used for animal feeding, as their fibrous nature causes difficulties in the cooking and preparation processes. Thus, in Apulia they are generally considered of less

value than *kabuli* chickpeas. The formulation of attractive, nutritious and convenient ready-to-eat legume-based foods represents, in our opinion, the best strategy to promote the consumption of the *Apulian black* chickpeas. For example, we produced ready-to-eat sterilized canned purée, legume-cereal burgers and, in collaboration with a local industry, some bakery products such as bread and pizza crust made with durum wheat semolina mixed with 40% *Apulian black* chickpea flour. Every product showed good nutritional, sensorial and textural properties, proving that it is possible to use the *Apulian black* chickpea flour to obtain

products characterized by high service value (i.e. ready-to-cook and/or ready-to-eat). The use of *Apulian black* chickpeas in the food industry also represents a solution to limit the genetic erosion of this traditional legume and increase the pulse consumption in general.



Fig. 1 - A trail of chickpeas, from beige to black

Grain legumes for human consumption: management of off-flavours

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Keywords: off-flavour, pea, saponins, TILLING

Grain legume production in Europe is currently dominated by the demand for animal feed. However, the increasing interest in locally grown pulses as a significant dietary protein source, and the overall decline in meat consumption, add a new dimension to the market. In order to supply this increased demand, new varieties optimized for human consumption are needed. The use of pulses as protein sources in transformed food products is often limited by the beany/grassy taste they can impart. This off-flavour is complex in origin and not completely characterized. Among the components implicated are saponins, which contribute to bitterness (1). The volatile flavour components arise from lipoxygenase action on unsaturated lipids present in the seed, yielding hexanal and other related aldehydes (2). Methoxypyrazines are also found in small quantities (3) and thought to be involved in beany flavour determination, although their origin and biosynthesis are less clear. Some of these components, notably saponins, may play a role in defence against pathogen or pest infestation whereas methoxypyrazines are thought to act as antifedants in fruits and seeds.

In food processing, pulses off-flavour is currently removed by selective extractions or by heat treatments, or masked by adding other chemicals (2). If a genetic approach could be used, these energy- and cost-intensive procedures could be avoided.

We have concentrated on eliminating the bitterness component, by targeting the biosynthesis of saponins in pea seeds. The first committed step in saponin biosynthesis is the cyclisation of oxidosqualene by a specific oxidosqualene cyclase, beta-amyrin synthase, to give the triterpenoid skeleton termed beta-amyrin (4). This molecule is subsequently modified by oxidations catalysed by Cytochrome P450 oxidases, and

by the addition of monosaccharides via UDP-dependent glycosyltransferases (UGTs), to give the final triterpene glycosides called saponins. We have recently identified a line no longer expressing beta-amyrin synthase in Pea (*Pisum sativum*), by screening a non-GMO TILLING (Targeting Induced Local Lesions in Genomes) (5) mutant population. The mutant plants accumulated less than 3% of wild-type levels of saponins in their seeds, but grow and yield normally. We are currently analysing the performance of mutant plants in more detail to ensure that the absence of saponins has no negative agronomic consequences. If so, these reduced-saponin lines will be used to develop more adapted pea varieties for the human food market.



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References:

- (1) Heng L, Vincken JP, van Koningsveld GA, *et al.* (2006) *J. Sci. Fd. Agric.* 86: 1225-1231.
- (2) Damodaran S and Arora A (2013) *Annu. Rev. Fd. Sci. Technol.* 4: 327.
- (3) Jakobsen HB, Hansen M, Christensen MR, *et al.* (1998) *J. Agric. Fd. Chem.* 46: 3727-3734.
- (4) Thimappa R, Geisler K, Louveau T, *et al.* (2014) *Ann. Rev. Plant Biol.* 65: 225-257.
- (5) Dalmais M, Schmidt J, Le Signor C, *et al.* (2008) *Genome Biol* 9:R43.

Pea breeding for value-added markets

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Field pea seeds, like those of other pulse crops, are rich in protein, slowly digestible carbohydrates, vitamins and minerals. However, we think it is quite feasible to improve this nutritional package further. One stream of research in our program over the past decade is related to increasing the concentration and bioavailability of important micronutrients. The main effort has been to develop and evaluate low phytate lines. Phytate is the natural storage form of phosphorus in seeds of many crops, however, it is not well digested by humans or monogastric animals. In addition, its negative charges bind valuable minerals like iron and zinc, which carry positive charges, and cause them to also be excreted. This is negative for the human or animal and causes pollution to the environment, as relatively low amounts of phosphorus can cause eutrophication of lakes and rivers. We

identified low phytate pea lines which have the majority of their phosphorus in a bioavailable (i.e., digestible) form. Therefore, the humans or animals are able to take up more phosphorus, iron and zinc, and much less becomes pollution. We have shown that the low phytate pea lines deliver 2-3 fold more iron to human cells in an *in vitro* assay compared to regular pea lines. We showed that peas with greater concentration of carotenoids may result in a further increase in bioavailable iron. We are currently evaluating several low phytate pea lines, in comparison to regular pea lines, in a chicken feeding study. Later we will conduct a study with female athletes as the 'final' test. Our initial low phytate pea lines carried a yield penalty of approximately 15%, but we have now closed that gap, so commercialization of low phytate pea varieties should be feasible soon.

A second major stream of research on field pea in our program is related to enhancing protein concentration and quality. Plant-based proteins are gaining popularity worldwide as many people chose to reduce their consumption of animal proteins in an effort to improve their health and reduce their carbon footprint. Pea protein fractions are being developed by fractionation using air classification or wet chemistry methods in several companies in North America and Europe. We are using genomic, biochemistry, and agronomic approaches to characterize and breed for high protein pea. In addition to high protein, our aim is to also improve the crop in the other important aspects including grain yield, lodging resistance, and general seed quality to make the crop more and more attractive to farmers.



Fig. 1 - Evaluating pea breeding trials before the coming rain (near Saskatoon, July 3, 2018).

Highlights from Session 6: Legume Biochemistry and Systems Biology

Chaired by Tom Warkentin (University of Saskatchewan, Saskatoon, Canada)

Improving nutritional and health-associated traits in *Pisum sativum* (pea)

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Keywords: germplasm, health, nutrition, mutants, pea, pulse breeding, seed protein, starch

The EAT–Lancet Commission has addressed the urgent need to examine the food systems adopted on earth at the present time to feed and nurture the human population (1, and citations therein). Its report has provided us with a comprehensive overview of the potential of our food systems to nurture human health and support environmental sustainability, while emphasising that our current trajectories threaten both (1). This conclusion is reflected in the current IPCC report (2) and is broadly in line with earlier analyses (3, 4). The need for the global population to have an adequate and healthy diet within sustainable food systems that will cause minimal damage to our planet is clear. A universal healthy reference diet, based on an increase of over 100% in consumption of plant foods and a decrease in consumption of unhealthy foods, is described in order to meet these goals (1, 5). Among plant foods which can sustain us, while promoting the health of humans and the environment, legumes feature highly. The seeds of many pulse crops (defined as legumes harvested as dry seeds for food or feed, and distinct from those food legumes grown primarily for oil) already provide a valuable source of protein, starch and micronutrients, but genetic

improvement of the concentration, composition and bioavailability of these seed components would enhance their ability to replace a higher proportion of animal food products (6).

In pea, seed quality traits have historically been studied at many levels, where their impact on improving the nutrition of farm animals was often a primary driver for research. More recently, there has been an increase in the adoption of pea and other pulse crops for producing widely consumed human foods, as a source of valuable nutrition for specialist diets, as high-value ingredients for sports nutrition, and as alternatives to animal-based products. This has resulted in a demand for increased diversity among the many constituents that are accumulated during seed development and stored within mature seeds.

Pea seed proteins include the major and distinct globulin proteins, vicilin and legumin, which have a low level of the sulphur-containing amino acids, methionine and cysteine. In addition, seeds contain an albumin fraction, which includes proteins with a more balanced amino acid profile but that may be comparatively poorly digested, and a few of these may be anti-nutritional proteins. We have screened natural germplasm resources and mutagenized populations of pea to identify null variants for genes encoding several seed proteins identified as anti-nutritional, poorly digested

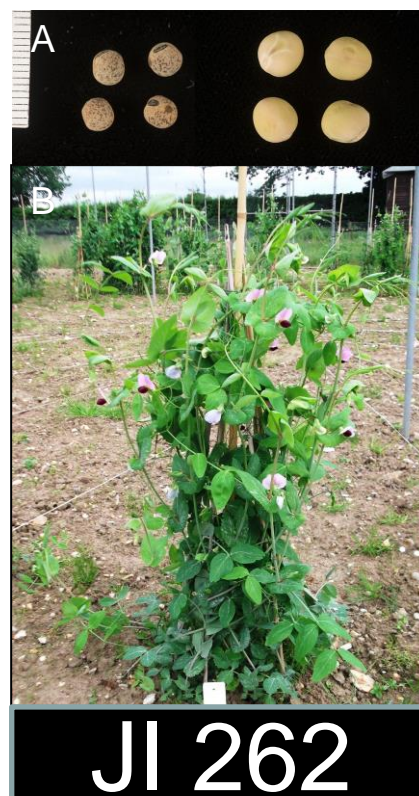


Figure 1. Seed (A) and plant (B) phenotypes of JI 262, a trypsin-chymotrypsin inhibitor null mutant line, identified from a germplasm screen. Four seeds of JI 262 are shown (left) compared with four seeds of cv. Cameor on the right (A).

or inferior in composition. These include pea albumin 2, lectin, trypsin-chymotrypsin inhibitors and five classes of vicilin proteins (7-9). A natural variant lacking the two major seed trypsin-chymotrypsin inhibitor proteins was identified from a screen of ~3000 *Pisum* accessions (8); this variant *Pisum elatius* line originated in Turkey and has small seeds with a thick seed coat (Figure 1). Combining the trypsin-chymotrypsin inhibitor null mutant with additional nulls for proteins with poor digestibility (pea albumin 2, lectin) has provided novel germplasm (Figure 2) with which the impact of major changes in protein composition on digestibility, functionality and overall amino acid composition can be investigated. An *in vitro* protocol mimicking human digestion conditions, developed by an international consortium (10), is enabling the screening of pea lines predicted to have improved digestibility.

A set of mutations derived by fast-neutron mutagenesis in JI 2822 (Figure 3) includes large genomic deletions, of which at least two affect additional unrelated genes (9, 11). The availability of a draft pea genome sequence for JI 2822 (12) has allowed us to estimate the minimum sequence deleted in every case. For the deletion mutation affecting convicilin, two closely related genes (*CvcA*, *CvcB*) are deleted in addition to the morphological marker, *k*, a locus encoding a TCP transcription factor which controls a flower phenotype (9, 13). The minimum deleted region is likely to be at least 152 kb, within which the direction of one convicilin gene is opposite to that of the second and the *k* gene. Within this affected region, one additional potential short open reading frame has been identified in the parental line.

New prospects for improving the nutritional profile of seed carbohydrates for food use are presented by discoveries made using near-isogenic lines of pea which differ in the structure and amount of starch deposited in seeds (14). A naturally occurring mutation affecting the carboxy-terminal region of the starch-branching enzyme I (*sbeI-ins*) results in a higher proportion of resistant starch, defined as starch with very low digestibility. Evidence suggests that resistant starch has a positive impact on controlling blood glucose levels in humans, possibly via fermentation in the large intestine and production of short chain fatty acids. The activation of receptors by these fatty acids is thought to lead to a variety of beneficial effects and positively regulate metabolic function in humans (14, 15).

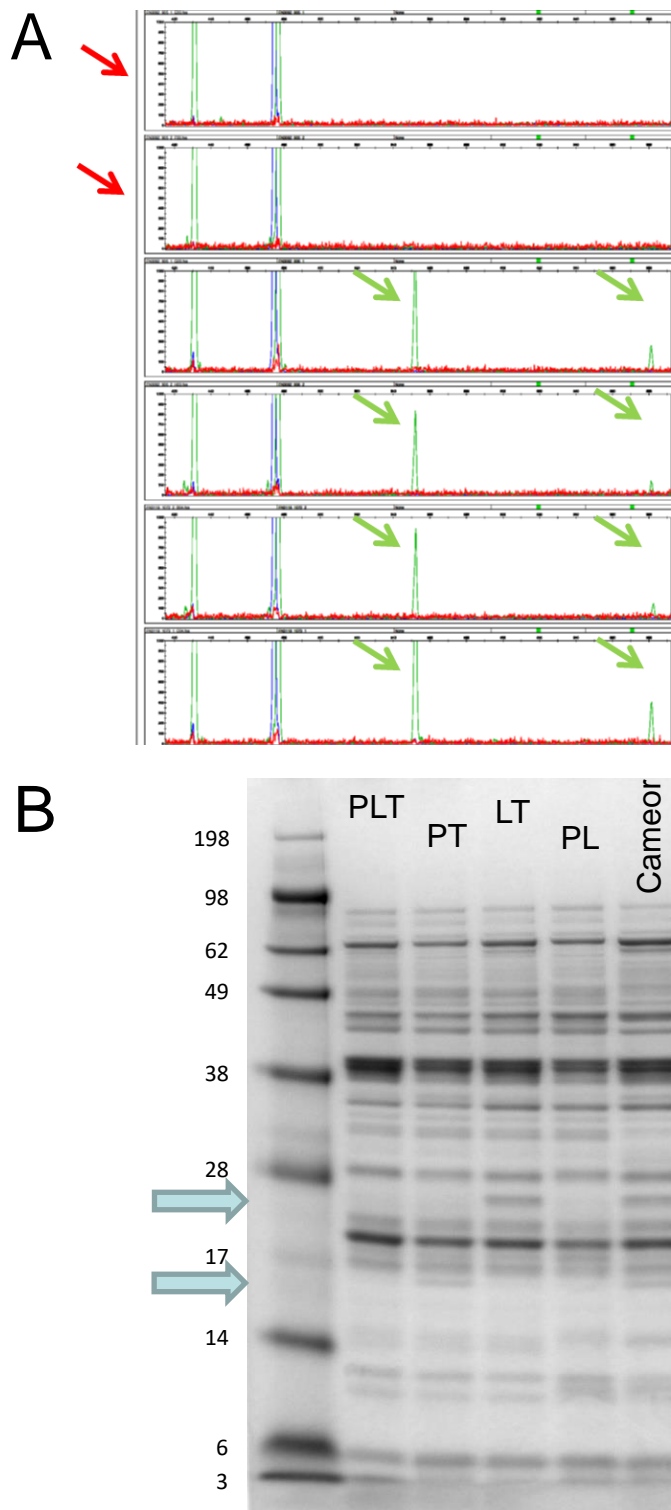


Figure 2. Identification of null mutant pea lines for convicilin, using a multiplex screen (A). Fluorescently-labelled primers were used to identify two amplicons corresponding to *CvcA* and *CvcB* genes (552 and 681 bp, respectively; green arrows). The two tracks labelled red lack both *CvcA* and *CvcB* amplicons. Analysis of seed proteins from a number of mutant pea lines by denaturing gel electrophoresis (B), highlighting the absence of PA2 and lectin (upper and lower blue arrows, respectively) in mutants lacking PA2 (P), lectin (L) or trypsin-chymotrypsin inhibitor (T) as triple (PLT) or double mutants (PT, LT, PL). The cv. Cameor was used as a wild-type control line. Molecular weight markers (x10⁻³) are shown alongside B.



Figure 3. Seedlings of fast neutron mutagenized lines generated in JI 2822 (A) before planting under field conditions (B). Microplots of JI 2822 (C) and a semi-leafless commercial cultivar (D) at flowering stages.

Human intervention trials have demonstrated the impact of changes in carbohydrate digestibility and pea seed structure on blood glucose levels (16). The data suggest that pea and other food plants, which carry a *sbeI* or equivalent mutation, may have a role in preventing Type 2 diabetes. These discoveries have prompted us to search for other *sbeI* variants which may show even more extreme differences in human responses. Although the *sbeI-ins* mutation is associated with a change in seed phenotype (seed wrinkling), substantial variation has been found within the carboxy-terminal region of *SbeI* among wild relatives of pea which are round-seeded (17). Advances in technologies for characterizing starch (18) and cellular structure are

facilitating the phenotyping of novel pea germplasm variants and developing our understanding of the consequences of variation in *SbeI*.

The acceleration of breeding programmes will be necessary for the rapid introgression of genetic variation, which impacts positively on the human health consequences of protein and starch quality, into breeding lines and cultivars of pea. There is already a demand for protein-rich foods as alternatives to meat, highlighted by studies showing inadequate intake of protein among older people (19). Changes to the starch component of commonly consumed foods using *sbeI-ins* pea germplasm will necessitate the introgression of the mutation into breeding lines suitable for combining (i.e.

harvesting as a dry crop). The mutation is currently used within vining pea crops, which are harvested as immature seeds, and have distinct flowering and plant architectural traits. The adoption of recent advances in ‘speed breeding’ (20) will facilitate rapid generation times which, when coupled with advances in selection, should reduce dramatically the cost and timescale for the development of new varieties. The impact of major changes to seed constituents on plant agronomic traits is being investigated, including the effectiveness of nodulation in lectin-less mutants (17) and the loss of trypsin-chymotrypsin inhibitors on predation by insect pests.



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References:

- (1) Lucas T, Horton R (2019) The 21st-century great food transformation. *Lancet* 393: 386-387.
- (2) IPCC report on agriculture: <https://www.ipcc.ch/report/ar4/wg3/agriculture/>
- (3) Poore J, Nemecek T (2018) Reducing food's environmental impacts through producers and consumers. *Science* 360: 987-992.
- (4) Tilman D and Clark M (2014) Global diets link environmental sustainability and human health. *Nature* 515: 518-522.
- (5) Willett, W, Rockström J, Loken B, *et al.* (2019) Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet* 393: 447-492.
- (6) Robinson GHJ, Balk J, Domoney C (2019) Improving pulse crops as a source of protein, starch and micronutrients. *Nutr. Bull.* 44(3):202-215.
- (7) Vigeolas H, Chinoy C, Zuther E, *et al.* (2008) Combined metabolomic and genetic approaches reveal a link between the polyamine pathway and albumin 2 in developing pea seeds. *Plant Physiol.* 146: 74-82.
- (8) Clemente A, Arques MC, Dalmais M, *et al.* (2015) Eliminating anti-nutritional plant food proteins: The case of seed protease inhibitors in pea. *PLoS ONE* 10: 1-24.
- (9) Domoney C, Knox M, Moreau C, *et al.* (2013) Exploiting a fast neutron mutant genetic resource in *Pisum sativum* (pea) for functional genomics. *Funct. Plant Biol.* 40: 1261-1270.
- (10) Brodkorb A, Egger L, Alminger M, *et al.* (2019) INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat. Protoc.* 14: 991-1014.
- (11) Moreau, C, Hofer JMI, Eléouët M, *et al.* (2018) Identification of Stipules reduced, a leaf morphology gene in pea (*Pisum sativum*). *New Phytol.* 220: 288-299.
- (12) Sarkar A, Moreau C, Cheema J, *et al.* (2019) Can we identify agronomically useful and climate-smart characteristics for legumes by comparing grass pea with pea? *Legumes Perspectives* (this issue).
- (13) Wang Z, Luo Y, Li X, *et al.* (2008) Genetic control of floral zygomorphy in pea (*Pisum sativum* L.) *Proc. Natl. Acad. Sci. U. S. A.* 105: 10414-10419.
- (14) Petropoulou K, Salt L, Warren F, *et al.* (2017) A seed trait studied by Gregor Mendel in *Pisum sativum* L. (pea): potential prevention of Type 2 diabetes. Chapter 6. In: *Legumes for Global Food Security* (Eds, A. Clemente and J.C. Jimenez-Lopez), Nova Science Publishers, pp. 129-155.
- (15) Nøhr MK, Pedersen MH, Gille A, *et al.* (2013) GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* 154: 3552-3564.
- (16) Petropoulou KA (2019) The role of plant-based food structures on gastrointestinal digestion, colonic fermentation and glucose homeostasis. PhD thesis. Imperial College London.
- (17) Rayner T, Moreau C, Isaac PG, *et al.* (2018) Genetic diversity and strategies for seed quality enhancement in *Pisum* (pea). *Asp. Appl. Biol.* 138: 141-147.
- (18) Perez-Moral N, Plankeel JM, Domoney C, Warren FJ (2018) Ultra-high performance liquid chromatography-size exclusion chromatography (UPLC-SEC) as an efficient tool for the rapid and highly informative characterisation of biopolymers. *Carbohydr. Polym.* 196: 422-426.
- (19) Stevenson EJ, Watson AW, Brunstrom JM, *et al.* (2018) Protein for life: towards a focussed dietary framework for healthy ageing. *Nutr. Bull.* 43: 97-102.
- (20) Ghosh S, Watson A, Gonzalez-Navarro OE, *et al.* (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nat. Protoc.* 13: 2944-29637.

Exploiting molecular diversity in legume Bowman-Birk inhibitors to investigate their potential role as colorectal chemopreventive agents

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Recent studies suggest that Bowman-Birk inhibitors (BBI) from legumes, such as soybean, pea, lentil and chickpea, could exert preventive and/or suppressive effects on carcinogenic and inflammatory disorders within the gastrointestinal tract (1). Physiologically relevant amounts of BBI can reach the large intestine in active form due to the resistance of these proteins to extreme conditions, including acidic pH, action of digestive enzymes, and the proteolytic and metabolic activity of intestinal microbiota. We have investigated the effect of a pea albumin extract enriched in BBI in the dextran sodium sulphate (DSS) induced colitis model in mice; the BBI-rich albumin preparation ameliorated DSS-induced damage in rodents significantly (2). In addition, we demonstrated a significant concentration- and time-dependent decrease in the proliferation of human colorectal cancer cells, following treatment with BBI natural variants from pea, lentil and soybean (3). In order to investigate the relationship between protease inhibitory properties, protein structure and possible health beneficial effects of BBI within the gastrointestinal tract, we have engineered a set of BBI mutants using a major pea BBI isoinhibitor, TI1, having both trypsin and chymotrypsin inhibitory activity, as a template. An inactive TI1 variant as well as modified TI1 variants, having trypsin or chymotrypsin inhibitory activity only, were generated. Although all the BBI variants were internalised by cultured HT29 human colorectal cancer cells (see example in Figure 1), only those with enzyme inhibitory activity decreased HT29 cell growth in a dose-dependent manner. Studies are in progress to identify the HT29 trypsin- and chymotrypsin-like proteases that may present as potential therapeutic targets of BBI proteins.



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References:

- (1) Clemente A and Olias R (2017) Beneficial effects of legumes in gut health. *Curr Op Food Sci*, 14, 32-36.
- (2) Utrilla MP, Peinado MJ, Ruiz R, *et al.* (2015) Pea (*Pisum sativum* L.) seed albumin extracts show anti-inflammatory effect in the DSS model of mouse colitis. *Mol Nutr Food Res*, 59, 807-819.
- (3) Clemente A, Arques MC. (2014) Bowman-Birk inhibitors from legumes as colorectal chemopreventive agents. *World J Gastroenterol*, 30, 10305-10315.

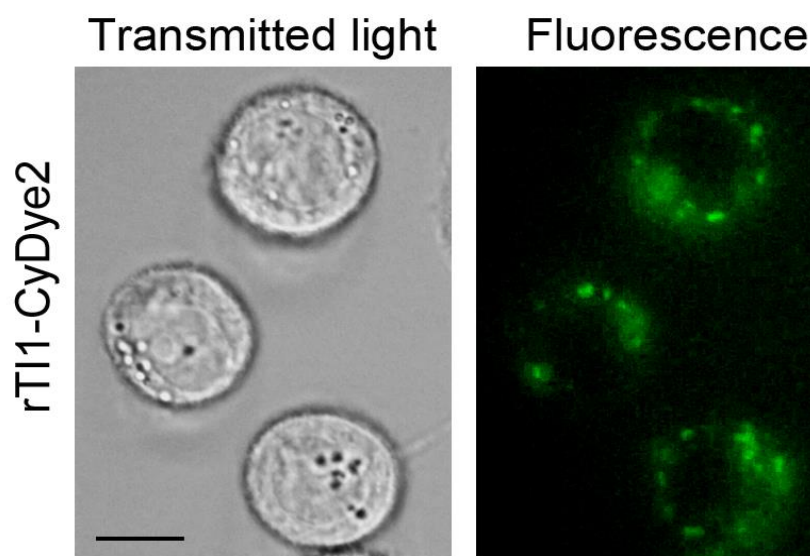


Figure 1. Example of an internalized TI1 protein variant in human colorectal cancer HT29 cells. Fifty micrograms of the CyDye2-labelled TI1 variant was added to the medium of HT29 human colorectal cancer 40 minutes before imaging. Photomicrographs were taken in an Eclipse Ti-U fluorescence microscope (Nikon) equipped with a blue LED light. Bar = 10 μ m.

Interactions between lupin seed proteins and native flavonoids

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Keywords: lupin seed, proteins-phenolic compounds interactions, flavonoids

Lupin seeds contain a number of valuable nutrients among which proteins play a relevant role. Moreover, similarly to other legumes plants, lupin is also marked by the high content of phenolic compounds. Recent research indicates numerous evidences of possible interactions that can take place between proteins and phenolic compounds. From food technological point of view, the formation of such complexes can be considered in two aspects. Firstly, these interactions have an influence on proteolytic enzymes activity and physicochemical properties of proteins, and thus leading to a reduction of the nutritional value of proteins. Secondly, the formation of such complexes can significantly reduce the potential health-promoting properties of phenolic compounds by masking their

antioxidant properties. Apart from a nutritional aspect, the interactions can play a key role during lupin plant development. Unfortunately, the detailed information about interactions between these compounds that can take place in the plant is missing.

During the presentation on Third International Legume Society Conferences (21-24 May, Poznan, Poland) the importance of interactions between lupin seed proteins and native phenolic compounds was highlighted. The presented results indicated that lupin seed proteins are capable to bind flavonoids that are naturally present in the seed. Simultaneously, analysis of digestion of lupin seed proteins by various proteolytic enzymes showed a release of these polyphenols. The most abundant compounds released from the digested proteins belong to apigenin C-glycosides. These flavonoids represent also the major

phenolic compounds present in seeds of different lupin species. Another important finding presented during the conference was that the unique flavonoid binding phenomenon is attributed to one of lupin seed protein fraction - γ -conglutin. Further analyses indicated that this protein is able to form a static complex with vitexin (flavonoid standard) with a stoichiometric ratio of 1:1 at cell physiological pH (pH 7.5).

The presented studies of lupin seed proteins-phenolic compounds interactions provided a better understanding of factors affecting the nutritional value of lupin seeds which is necessary to optimize the use of this plant for human nutrition. Simultaneously, the results are the first that indicate the importance of proteins-phenolic compounds interactions from lupin plant physiological point of view.



Alkaloid biosynthesis in lupins

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Keywords: lupin, alkaloids, biosynthesis

Plants of the genus *Lupinus* accumulate the alkaloids, which due to their toxic effects on human and animals as well as bitter taste are considered as antinutritional factors of food and feed (1). Low alkaloid content is, therefore, one of the pivotal seed quality traits in lupins and the accepted industry threshold for alkaloid level is currently 0.02% of the seed dry weigh (SDW) (2).

The lupins' alkaloid biosynthetic pathway is still obscure compared with other plants that produce alkaloids. Our current research has been devoted to understanding the biosynthesis and accumulation of alkaloids in three lupin crops: narrow leafed lupin (NLL, *Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus* L.) and white lupin (*Lupinus albus* L.), with aid of RNA-seq-based comparative transcriptome analyses of genotypes with contrasting seed alkaloid content. Recently, in NLL we have described APETALA2/ethylene response transcription factor, *RAP2-7*, which is likely to be involved in the regulation of alkaloid biosynthesis in NLL (3). *RAP2-7* was found to co-segregate with a major locus that confers reduced QA content in seeds, *iucundus*, and was located within a region containing highly significant QTLs that affect alkaloid composition (linkage group NLL-07). In the follow-up investigation we exploited a single-nucleotide polymorphism within *RAP2-7*, credibly associated with seed alkaloid content, to develop the co-dominant dCAPS marker *iuc_RAP2-7* (4). Marker validation in 199 NLL accessions encompassing different classes of origin and possessing the sweet *iucundus* or bitter *iucundus* allele demonstrated, that seed alkaloid content $\geq 0.9\%$ of the SDW was associated with the high-alkaloid marker band (*Iucundus* genotypes), whereas alkaloid content up to 0.5% of the SDW was associated with the low-alkaloid marker band (*Iucundus* genotypes). Within a given

detection limit, the overall matching rate of the *iuc_RAP2-7* marker was 100%. Therefore from a consumption perspective, the *iuc_RAP2-7* marker provides a powerful tool that will facilitate NLL marker-assisted selection by rapid rejection of distinctly bitter *Iucundus* genotypes ($\geq 0.9\%$ of the SDW) and thus will accelerate selection efficiency and development of new low-alkaloid cultivars. Preliminary results of transcriptome-derived investigation of alkaloid biosynthesis in white and yellow lupin suggest that the mechanism of accumulation in these two species differs when compared to NLL.

The finding of our study provide significant progress in the elucidation of alkaloid biosynthesis and its transcriptional regulation in lupins, providing insight into a poorly resolved aspect of secondary metabolism of these economically important, alternative crops.



References:

- (1) Wink M, Meißner C, Witte L (1995) Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. *Phytochemistry* 38, 139-153.
- (2) Cowling WA, Huyghe C, Świącicki W (1998) Lupin Breeding. In *Lupinus as crop plants: biology, production, and utilization*, Gladstones, J.S., Atkins, C.A., Hamblin, J., Eds. CAB International: Wallingford, United Kingdom, pp. 93-120.
- (3) Kroc M, Koczyk G, Kamel KA, *et al.* (2019) Transcriptome-derived investigation of biosynthesis of quinolizidine alkaloids in narrow-leafed lupin (*Lupinus angustifolius* L.) highlights candidate genes linked to *iucundus* locus. *Sci. Rep.* 9, 2231.
- (4) Kroc M, Czepiel K, Wilczura P, *et al.* (2019) Development and Validation of a Gene-Targeted dCAPS Marker for Marker-Assisted Selection of Low-Alkaloid Content in Seeds of Narrow-Leafed Lupin (*Lupinus angustifolius* L.). *Genes* 10, 428.

Highlights from Session 7: Legume Physiology, Plant Development, and Symbiosis

Chaired by Alfonso Clemente (Estación Experimental del Zaidín, CSIC, Granada, Spain)

Unravelling cluster root development in white lupin

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White lupin is the only annual crop that can produce cluster roots, which are developmental structures dedicated to efficient phosphate mobilization and acquisition. The development of these structures mimics that of lateral roots, starting with cellular divisions in the pericycle followed by further divisions in the endodermis and cortex, although it is not clear to which extend these two tissues contribute to the new organ.

We generated transcriptomics dataset at a high resolution to initiate Gene Regulatory Network approaches. As an example, we selected *LaLBD16*, a close homolog of *Arabidopsis AtLBD16* and we used *hairy root* transformation to determine its role during cluster root development. By using a fusion with the Superman repressor domain X (*SRDX*), under a strong constitutive promoter (*35S*), we were able to block cluster root development but not lateral root formation. This suggests that one orthologs member of *LBD16* in white lupin would be dedicated to the development of one organ rather than the other, a good example of subfunctionalization.

We also screened an EMS-mutagenized population to find mutants altered in cluster root development. We describe 4 *constitutive cluster root* mutants (*ccr*) that were identified in repressive conditions (high phosphate) and still produce numerous cluster roots. These mutants are still able to respond to low phosphate, either from a root architecture point of view (induction of even more cluster root) or in term of molecular adaptation (induction of phosphate starvation related genes such as the phosphate transporters from the *PHT1* family). The identification by Next

Generation Mapping of the causal mutation is now underway and should lead to a better understanding of the molecular mechanisms of cluster root induction.

This project should help answer questions about the evolutionary origin of cluster roots, since these structures can be found in 10 different botanical families (including one monocot) in which not all members can form cluster roots. Whether the ability to form these structures appeared several times independently or whether all these plants come from a common ancestor remains a

mystery. It is clear however, that the loss of mycorrhizal associations was a pre-requisite for the emergence of cluster roots, as a compensatory mechanism. In a modern agricultural context, these structures may however improve crop nutrient acquisition efficiency and should be considered as a key tool to reach this objective. 

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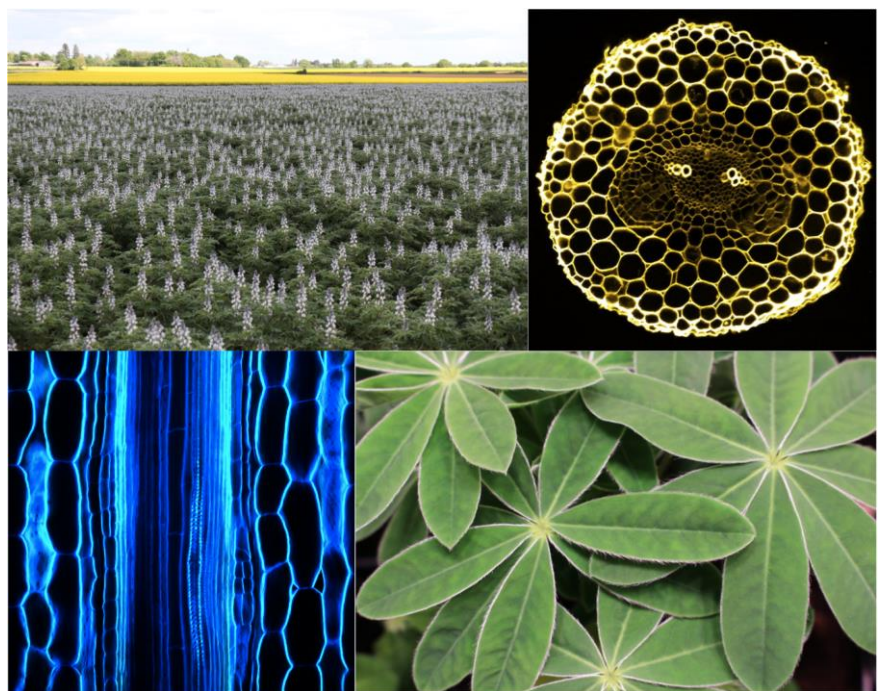


Figure 1. Various aspects of the project developed to unravelling cluster root development in white lupin

Local and systemic effect of cytokinins on soybean nodulation and regulation of their *isopentenyl transferase (IPT)* biosynthesis genes following rhizobia inoculation

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Nitrogen is crucial for optimal plant growth and yield of crops. However, synthetic fertilisers are polluting the environment through the production of potent greenhouse gasses, leaching and eutrophication of waterways resulting in the loss of biodiversity. Legumes have the potential to offset some of these negative climatic effects and increase agricultural sustainability through their ability to establish a symbiotic relationship with soil bacteria called rhizobia. These bacteria can fix atmospheric di-nitrogen and make it available to the plant. The interaction requires the production of new root organs called nodules, a costly process that needs to be tightly regulated by the host. The plant does this via systemic Autoregulation of Nodulation (AON) or via nitrogen-dependent regulation of nodulation when sufficient nitrogen is available in the soil for direct uptake.


The phytohormone cytokinin is essential for nodule primordia establishment via the LHK1/CRE1-mediated induction of the NIN transcription factor. Cytokinins were first characterised for their role in cell proliferation and differentiation but have since been attributed roles in shoot apical meristem maintenance, systemic communication of the plant's nutritional status, delay of senescence, response to biotrophic pathogens, branching and organogenesis. More recently, a role for cytokinin, and more specifically their biosynthesis *IPT* genes, was proposed in nodule control. To investigate this, cytokinin was directly fed into soybean, an agriculturally important legume, via petiole feeding to mimic the role of the shoot-



Figure 1. Cytokinin petiole feeding into soybean to mimic the role of the shoot-derived AON inhibitor.

derived AON inhibitor. While high concentrations do inhibit nodulation, this is likely due to cell toxicity at these levels. In contrast, low concentrations actually promoted nodule formation. In addition, all 17 soybean *IPT* gene family members were identified, and their expression was characterised in the shoot and root following rhizobia inoculation. *GmIPT5* is induced by rhizobia in the shoot independently of GmNARK, the receptor acting upstream of the shoot-derived inhibitor. Thus, *GmIPT5* might not be acting in the AON pathway,

but instead, its induction could be preparing the shoot for growth in anticipation of an influx of nitrogen expected from the developing nodules.

A better understanding of the molecular signals involved in nodulation control will enable genes of interest to be targeted in future through conventional breeding or via the latest molecular techniques, such as CRISPR. This might lead to the development of superior crops, improved nitrogen-use efficiency, and agricultural sustainability. 

Highlights from Session 8: Biotic and Abiotic Stresses in Legumes

Chaired by Weidong Chen (USDA ARS Washington State University, Pullman, USA)

Genotypic variation in Lentil (*Lens culinaris* Medikus) for transpiration response to Vapor Pressure Deficit

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Keywords: lentil, transpiration, VPD

Lentil (*lens culinaris* Medikus) is an annual food legume crop that plays an essential role in the food and nutritional security of millions, especially in the developing world. It is a great source of many nutrients including protein (22-35%), fibre and various minerals mainly iron and zinc. Lentil enhances nitrogen to soil through biological nitrogen fixation process playing an important role for diversification and intensification of cereal-based cropping systems worldwide.

Nevertheless, high temperatures and water deficit have become the major environmental constraints limiting grain legume production particularly in the arid and semi-arid tropics. Multiple strategies have been made to enhance the efficiency of selection for tolerant lines based on yield and specific physiological traits. Hence, the selection of robust genotypes characterized with limited transpiration, by reducing their stomata conductance, could be an effective approach to mitigate the risks associated with the increase of water deficit. This approach has been recognized as the key water saving trait under the atmospheric high vapor pressure deficit (VPD) conditions that influences the water balance, photosynthesis,

and the growth of the plants. Limited transpiration in response to high VPD has been demonstrated in several crop species (e.g. wheat, peanut, soybean, chickpea, and maize) as an efficient approach to increase crop yield by 75% in water-limited conditions. Furthermore, the selection of genotypes with a breakpoint in their response to increasing VPD have the possibility of using the conserved soil water to sustain physiological activity during seed fill and generate a greater yield than genotypes that are VPD insensitive.

The present study was carried out to compare transpiration rate in 20 selected lentil genotypes by whole plant measurement under controlled environments during a high VPD period. The results showed significant variation among lentil genotypes for transpiration rate in response to VPD. Eleven genotypes started limiting their transpiration under the increase of VPD and they exhibited a breakpoint at about 3.3 kPa. Two genotypes (ILL7835 and ILL7833) registered the lowest breakpoint at 2.8 kPa. These two genotypes with low breakpoint show the prospect of breeding lentil varieties with high yield and water use efficiency. The

remaining nine genotypes had a linear response to VPD and showed continued increase in transpiration rate as VPD increased. Outcomes from this study provide evidence for a plausible role of the limited transpiration trait under high VPD in future genetic improvement efforts for lentil.



Figure 1. Nouredine El haddad at the 31SLC.

How does pea (*Pisum sativum*) recover from water deficit?

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Keywords: water deficit, re-watering, symbiotic nitrogen fixation

Projections from model-simulated climate change suggest that severe and widespread droughts are expected in the next 30–90 years over many land areas resulting from either decreased precipitation and/or increased evaporation (1). Unfortunately, these climatic conditions will lead to dramatic consequences on pea yield and seed quality and may discourage further legume cultivation. We thus need to identify plant traits maximizing pea's resilience to drought events, in order to propose new ideotypes better adapted to fluctuating environments.

The ability of a plant to maintain its yield in response to a stress depends not only on its ability to resist during the stressed period, but also on its capacity to recover after this stress. Because most studies aim at identifying physiological and molecular mechanisms underlying plant resistance to drought, we decided, in the frame of the FP7-LEGATO project and with the support of Terres Inovia, to focus on plant recovery after a drought event.

During an experiment in controlled conditions (4PMI Platform, Dijon, France), we compared the ability of two pea genotypes to recover after a water deficit of 2 weeks occurring before flowering. While one of the genotypes, (Kayanne), was able to maintain its yield under these conditions, the other one (Puget) showed a 12% yield decrease compared to the well-watered plants. By analyzing in detail the dynamics of recovery of both genotypes, we observed that the mechanisms triggered after the water deficit that were related to N nutrition were critical in the ability of the plant to recover.

Pea, like other legumes, has the unique ability to fix atmospheric dinitrogen (N₂) via symbiosis with soil bacteria in root nodules. Although this mode of N nutrition is highly favorable under poor mineral N availability, it represents a high cost for the plant due to

the formation of nodules, their growth and their activity (2).

We demonstrated that during the recovery period Kayanne was able to quickly and strictly re-adjust the formation of nodules to its growth needs, allowing this genotype to fully recover from the 2-week water deficit period. On the other hand, in Puget, the initiation of new nodules after water deficit was delayed compared to control plants, and more nodules than necessary developed. We hypothesized that this energy cost, which occurred to the detriment of growth, would finally negatively impact yield. Now that we have detected the main traits underlying pea recovery efficiency, the next step will be to identify the molecular regulators controlling these traits, and altogether these results will contribute to the design of pea ideotypes better adapted to drought events. 🌱

References:

- (1) Dai A (2013) Increasing drought under global warming in observations and models. *Nat. Clim. Change*, 3:52-58.
- (2) Minchin FR, Summerfield RJ, Hadley P, *et al.*, (1981) Carbon and nitrogen nutrition of nodulated roots of grain legumes. *Plant, Cell Environ.*, 4:5-26.



Figure 1. Experiment conducted in the 4PMI Platform (Phenotyping Platform for Plant and Plant Microorganisms Interactions). INRA Dijon, France.

Improving black gram (*Vigna mungo* (L.) Hepper) against biotic stresses

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Black gram (*Vigna mungo* (L.) Hepper), also known as Black Matpe or Urdbean is grown over 3 million ha in South and Southeast Asia. It has a protein content of about 24% and is also a source for other minerals like iron and zinc. Major black gram producing countries include India, Myanmar, Bangladesh and Pakistan. One of the major uses of black gram is in the preparation of dishes like *idli* and *dosa*, which involves fermentation of the batter made from grains. Other black gram products include sprouts, *dal* (porridge), *vada* (a deep fried preparation from the batter) and sweets. Black gram easily fits into the cereal-based cropping systems, due to its short duration (70-75 days). The average productivity of the crop is still below 500 kg/ha. Major constraints in improving the productivity of black gram are susceptibility of commonly grown varieties to Mungbean Yellow Mosaic Disease (MYMD), dry root rot disease (*Macrophomina phaseolina*) and insect pests such as stem fly (*Ophiomyia phaseoli*) and bruchids (*Callasobruchus maculatus* and *C. chinensis*). The incidence of MYMD could be up to 80%. Incidence of dry root rot (35-40%) and infestation of stem fly (35-45%) on black gram grown as part of rice-based farming system have been observed. Bruchids cause considerable damage during storage. Efforts are in progress to develop varieties with resistance to the above diseases and pests by transferring resistance from resistant sources identified from screening trials.



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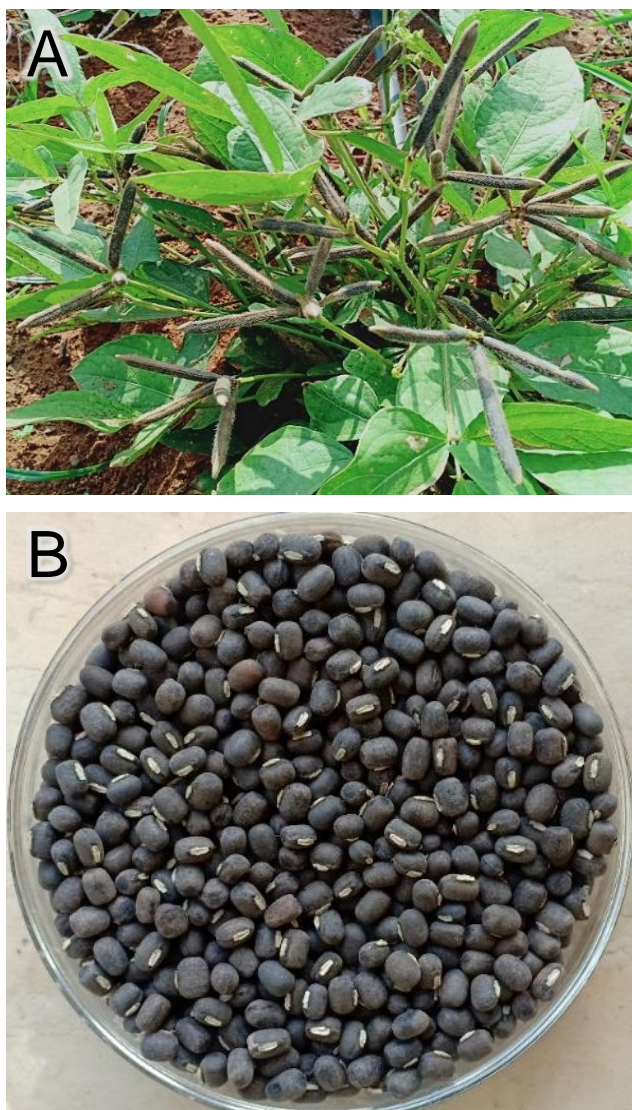


Figure 1. A) Black gram plant with mature pods; B) Black gram grains.

Resistance to aphid and weevil in wild peas

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Keywords: aphid, weevil, pea, pest resistance

Pea (*Pisum sativum*) is a widely cultivated temperate grain legume whose production can be constrained by a number of stresses. In an attempt to address the need of resistant cultivars we started at Córdoba in 1996 a research program to support resistance breeding paying particular attention to enlarging genetic diversity by identifying and exploiting resistances available in wild relatives. Like this, we identified sources of resistance in *Pisum* spp. to the parasitic weed broomrape (*Orobanche crenata*), and to the fungal diseases ascochyta blight (*Didymella pinodes*), powdery mildew (*Erysiphe pisi*), rust (*Uromyces pisi*) and fusarium wilt (*Fusarium oxysporum* f.sp. *pisi*). These were successfully crossed with elite pea cultivars and submitted to breeding. We also advanced in the understanding of the resistances by studying genes or QTLs, and in the characterization of the resistance mechanisms (1,2).

This program was recently enlarged searching for resistance also to insect pests such as pea aphid (*Acyrtosiphon pisum*) (Figure 1) and to the pea weevil (*Bruchus pisorum*) (Figure 2). This included from the screening of germplasm to the assessment of the mechanisms involved in the resistance. A multi-environment field screening allowed the identification of a number of sources of incomplete resistance to weevil (3). Both antixenosis and antibiosis mechanisms were identified against both pests under controlled conditions (4,5).

As only intermediate levels of incomplete resistance were identified we approached quantitative genetic analysis in an attempt to unravel the genetic control of resistance and the identification of molecular markers to assist breeding. Genetic studies were performed using two recombinant inbred line (RIL) populations, one developed from a cross between two *P. fulvum* accessions, and a second one developed from the cross between *P. sativum* ssp. *syriacum* and *P.*

sativum. Both RILs populations were genotyped by DArTseq, and the first one was phenotyped for aphid and the second for weevil resistance. This allowed the identification of a number of QTLs associated to reduced plant damage by aphid (6), as well as others associated with reduced seed infestation by weevil, and to reduced weevil larval development (7). These studies are being continued with the aim of developing of breeders friendly molecular markers to assist resistance breeding.



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References:

- (1) Rubiales D, Fondevilla S, Chen W, *et al.* (2015). Crit Rev Pl Sci 34:195-236.
- (2) Rubiales D (2018). Phytopathol Mediterr 57:170-185.
- (3) Aznar-Fernández T, Carrillo-Perdomo E, Flores F, *et al.* (2017). J Pest Sci 91:205-214.
- (4) Aznar-Fernández T & Rubiales D (2018) Ann Appl Biol 3:268-281.
- (5) Aznar-Fernández T & Rubiales D (2019) Front Plant Sci 10:491.
- (6) Barilli E, Carrillo-Perdomo E, Cobos MJ, *et al.* (2019) Pest Manag Sci 76(5): 1731-1742.
- (7) Aznar-Fernández T, Barilli, E, Cobos MJ *et al.* (2020) Scient Rep 10:33.



Figure 1. Pea aphid



Figure 2. Pea weevil

The Third International Legume Society Conference Social Program and Photographic Overview



Coffee breaks at the ILS3



Highlights of the Gala Dinner at the ILS3





The new Legume Society executive committee at the Legume Society General Assembly, from left to right: Diego Rubiales (Secretary), Paolo Annicchiarico (New President), Maria Carlota Vaz Patto (Vice-President), Kevin McPhee (Past President), and Tom Warkentin (Vice-President)



Poster and plenary sessions at ILS3

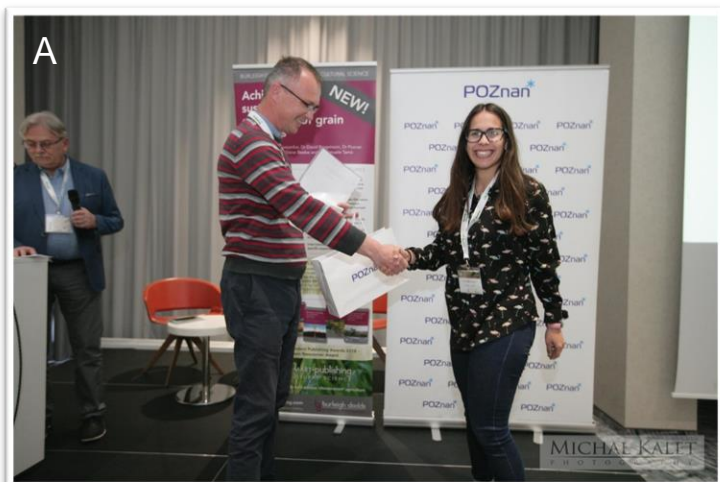


The Four Teams

<p>1. Starch Shuttles F.C.</p>	<p>2. Oil Orbiters F.C.</p>
<p>3. Protein Propellers F.C.</p>	<p>4. Biofort Builders F.C.</p>



Selected moments of the fourth ILS Football Cup – Poznań 2019 – and the winning team, the Starch Shuttles F.C.. From left to right: Tom Warkentin (Captain), Lars Kamphuis, Toby Newman, Robert Kempster, Federico Ribalta, and Davide Martins.



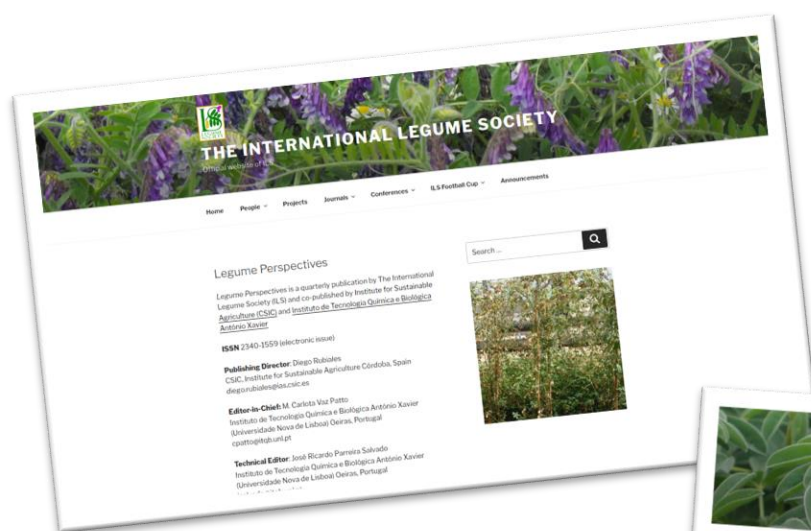
Ex-aequo best poster awardees at ILS3 for students and PhD students, funded by Burleigh Dodds Science Publishing and the Mayor of Poznań:

- (A) Ana Margarida Sampaio (ITQB NOVA, Oeiras, Portugal)**
- (B) Ferawati Ferawati (Linnaeus University, Kalmar, Sweden)**
- (C) Alanna Orsak (University of Saskatchewan, Saskatoon, Canada)**

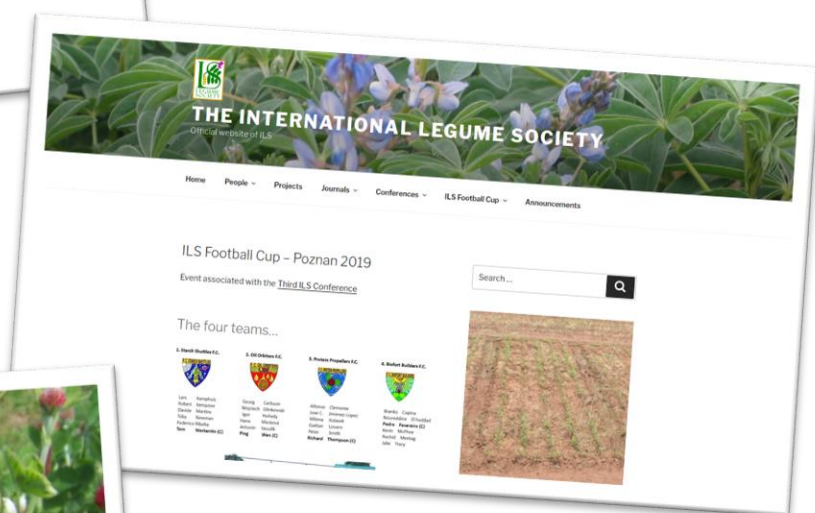
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