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ILS2 Sessions 14 & 16 overview: Frontiers in legume agronomy

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This is the 14th issue of our journal *Legume Perspectives* devoted to the 2nd International Legume Society conference. The conference was truly interdisciplinary with a special attention given to foster the interaction of researchers and research programs with stakeholders. As a result, the conference was also the site where a number of consortia organized satellite events, namely the EU-FP7 ABSTRESS, LEGATO, EUROLEGUME and REFORMA projects and a Global Pulse Confederation write shop. Third ILS conference is already planned at Poznan, Poland in 2019. With this we can proudly state that ILS is no longer a dream but a solid and reliable society, with a committed board being able to maintain and expand the well consolidated series of triennial conferences and *Legume Perspectives* magazine, with the final goal of serving as a platform for networking and as dissemination tool for the legume community worldwide. It should be noticed that these first years of ILS run with absolutely lack of any kind of financial support. Achievements made were therefore possible only thanks to the voluntary work of a number of committed people. A new ILS board was elected at Troia ensuring the renewed ideas and energies needed to continue ILS endeavor.

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Carte blanche
to...



*M Carlota Vaz Patto,
Diego Rubiales,
Pedro Fevereiro
and Susana Araújo*

The Second ILS conference

In a world urgently requiring a more sustainable agriculture, food security and healthier diets, the demand for legume crops is on the rise. This growth is fostered by the increasing need for plant protein and for more sustainable and environmentally friendly sound agricultural practices. Food, feed, fiber and even fuel are all products that come from legumes – plants that grow with low nitrogen inputs and in harsh environmental conditions.

In 2016, the FAO International Year of Pulses, we were glad to host the second ILS Conference in Portugal. In the wonderful peninsula of Tróia, we welcomed more than 370 attendees coming from more than 50 countries. This highlights not only the international recognition of the work developed by ILS but, importantly the worldwide significance of research in legumes.

The meeting was a fruitful forum to update and discuss the recent achievements in legume research. The multidisciplinary and international environment brought to the participants the last updates on legumes genome sequencing, development of new genomic and genetic resources, agronomy, plant and crop physiology, economy, biotic and abiotic stresses adaptation and how this knowledge could be applied in breeding and agricultural systems and on the adaptation to changing environments.

Social activities were also not neglected during the second ILS Conference. The welcome cocktail, the traditional Legume Football Cup and the Gala Diner were privileged events to foster interaction among participants, in a relaxed and fun environment. Worth to mention the spectacular moment, during the group photo, in which a happy group of Dolphins, likely fan of legumes, rewarded us with an unique show. 🥒



ILS2 Session 1 & 5 overview: Legume Value Chain: market requirements and economic impact

Chaired by Pietro P.M. Iannetta¹, Frédéric Muel², Adrian Charlton³ and Eduardo Rosa⁴

The first session of this year's convergence of the International Legume Society was dedicated to talks and discussion on "Legume Value Chain: market requirements and economic impact". This choice was very significant since 2016 was the Food and Agriculture Organisation of the United Nations, "International Year of Pulses" (see <https://www.youtube.com/user/FAOoftheUN>). This initiative aimed to highlight the role of pulses as sustainable global superfoods, as they can be grown without reliance upon inorganic nitrogenous fertilisers. This focus reflects many years of scientific evidence which shows that effective legume agronomy, linked to optimised crop rotations can deliver ecosystem services and minimise inorganic fertiliser use, lowering costs to the producer whilst also delivering highly nutritious commodities that may increase the gross margins for producers.

The Plenary talk of the session was delivered by Hakan Bahceci, Chief Executive Officer of Hakan Foods, and former President of the Dubia based *Global Pulse Confederation* (www.cicilsiptic.org). His presentation may be viewed on YouTube (https://www.youtube.com/watch?v=QzNQG_VAbNA). The Federations mission is to lead the global pulse industry to major crop status by facilitating free and fair trade, increasing production and consumption of

pulse crops worldwide. This established the predominant focus of the session, and emphasised that the cropped area currently used for pulse production must increase significantly to fulfil the anticipated elevated demand for fresh as well as dried grains for a wide variety of industries. The foresight on oil and protein demand by 2030, whatever the different scenarios, highlights a large increase of protein needs for feed and food, and to which pulses and fodder legumes will have to contribute.

Throughout the session, there was wide acceptance that pulses are multifunctional and offer tremendous opportunity to provide effective solutions to major global societal challenges: delivering more sustainable cropped systems under climate change scenarios, and highly nutritious commodities to help fight hunger and malnutrition. Most significantly, it was strongly communicated that any legume based solutions must be commercially competitive for the grower, and other businesses in the supply chain. To realise this, significant in-field challenges remain. For example, the development of Life Cycle Assessment based approaches should be adopted as decision aid tools to help inform growers and policy decision makers on the costs and benefits of legume based cropped systems. Decision aid tools will allow such actors to react with greater commercial efficacy in stochastic environmental and global-economic climates. The development of the LCA-informed tools was presented with special respect on how best to encourage the uptake of intercropping and/or mixed-cropping as key strategies for a more sustainable and environmental friendly agriculture. Inter- or mixed-cropping may be conceived as just one of several socio-technical innovations to be deployed as a means by which the supply chains may escape the dominant lock-ins, including that of mono-cropped cereal production and associated agrichemical



Figure 1. Innovation to improve the commercial potential of legumes is key. Here intercroops are being trialed (in Scotland) for brewing and distilling, the barley and peas too (©Pete Iannetta, James Hutton Institute)

dependencies. Related to this, a significant in-field challenge will be the development of effective integrated pest management based solutions for cropping systems which are heavily legume supported. However, the perception is that farmer uptake of legume base cropped systems will be determined and driven largely by market forces and the commercial value of pulses rests on the value of their protein component, and finding complementary and equally profitable markets for their carbohydrate component.

Success in this context will be determined by our capacity to exploit added value opportunities beyond the field boundary, such as capacities for dehulling, milling, fractionation and pre-treatment across scales whether at individual farm, regional and/or,

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national levels. The lack of processing and fractionation capacities in many countries pose a restriction which cascades up-the supply chain, limiting the range of materials for exploitation by food technologists wishing to develop advanced legume-based feed and food formulations. Thus, it seems the commercial competitiveness of pulses may be optimised only if their fractionated components are targeted towards premium markets such as the human food chains, and other tied lucrative opportunities such as aquaculture feed. Novel processing options also remain to be exploited on large commercial scales to serve major commercial product streams such as exist for fermentation based processes - such as beer, neutral spirit and pharmaceutical production.

Commercially effective legume based cropped systems will of course look different across the diverse array of biogeographical regions globally, and effective planning of such systems could conceivably realise EU self-sufficiency for food and feed. This speed at which this sufficiency can be attained could be accelerated with the continued

development of the EUs Common Agricultural Policy, which has evolved to ensure the uptake of more effective agri-environment measures, and payment of subsidies for increased legume cropping is likely to remain as a key component in future evolved forms of the CAP.

In a global context, it was also acknowledged that small producers deliver food security for many of the world's most impoverished regions and that the effectiveness of their legume-based approaches needs to be encouraged by easier access to capital for investment in cropping system and/or commodity processing ventures: and this should include improving awareness of, and therefore capacity to adapt to, market effective interventions. The need for greater investment was reiterated with respect to support for research to underpin our understanding of how best to optimise legume based cropping systems. The perception of legumes as an important eco-technological ('eco-tech'), and sociological tool remains to be recognised in terms of research funding support that should be


at least commensurate with levels of funding which are used pursuing bio-tech solutions to food system and nutritional security challenges.

What emerged from the session was therefore a need for concerted action among farmers, industrialists, academics and government policy-makers, and that such activity must be underpinned by a parallel investment in basic education to ensure that all public are accurately informed on legume function and potential. That is, consumer knowledge and perceptions of pulses should accurately reflect their environmental as well as nutritional qualities. Such priorities should ensure basic in-school learning objectives regarding sustainable cropping and food systems. This approach might extend to encouraging novel initiatives to ensure more effective public procurement strategies *e.g.* in government funded institutions such a local authorities, hospitals and schools. Such concerted action should aim to encourage positive behavioral changes, by ensuring that our understanding of biological nitrogen fixation is shared, and that legume protein



Figure 2. Pulses have been used in aquaculture feeds for many years. This feed industry offers a sustainable and high premium market for vegetable protein.

consumption may be more efficient and sustainable when it is better balanced with a reduction in the consumption of animal protein.

We can conclude from the session that the impact of 2016 as the FAOs IYoPs has been extraordinarily large. Going forward with proper institutional support, the pastoral activities of legume focused actors throughout the supply chain will remain effective. The IYoPs legacy therefore seems set to positively influence major cultural changes towards more sustainable legume supported food systems and associated shifts in dietary culture towards various levels of demitarianism. This will be achieved by harmonising the largely disparate, and even competing aspects of food supply-chains, or more correctly, *-networks*. 

Further Reading

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ILS2 Session 2 & 17 overview: Legumes and the environment

Chaired by Christine Watson¹, Marie-Hélène Jeuffroy², Diego Rubiales³ and Richard Thompson⁴

Abstract: The ability of legumes to fix atmospheric nitrogen gives them a crucial role in sustainable farming systems. Their presence can provide several other valuable ecological services. In Europe these assets have been neglected whilst developing high-input farming systems for maximizing yields. Diverse strategies are presented for maximizing ecological services provided by legumes in order to re-integrate them in cropping systems.

Key words: Inputs, intercrop, ecological services, mineralization, weeds, N-fixation, value chain

The second plenary session of the International Legume Society conference was dedicated to talks on “Legume and environment”, this was continued in parallel session 17, with a series of 10 minutes talks on the topic. This initiative aimed to highlight globally the environmental benefits of legumes in cropping systems.

Potential contribution of legumes to the farmed environment and how to benefit from it

The first talk of the session was delivered by Marie-Hélène Jeuffroy, (INRA-Grignon, France). Her presentation may be viewed on Youtube

(www.youtube.com/watch?v=tUbiWpDmmTs). Environmental benefits from legumes have been increasingly highlighted by scientists for the past 20 years.

They include:

- Suppression of fertilizer application on the legume and reduction on the following crop
- Yield increase of the following crop
- Decrease in greenhouse gas emissions (N₂O et CO₂) compared to fertilized crops, at field scale and at crop sequence scale (1-3)
- Decrease in fossil energy consumption: -50% compared to a fertilized crop, -11% compared to a 5-year rotation without legumes (4)
- Decrease of weeds and soil-borne pathogens in a crop sequence including a legume crop compared to cereal- and oilseed rape-based crop sequences, at field scale, allowing a reduction in pesticide use (5-8)
- Contribution to increasing crop biodiversity
- Decrease of insect pest populations with aerial dispersal on largely-grown arable crops (9,10)
- Key role in the associated biodiversity whether aerial (pollinators on faba beans, alfalfa, clovers; (11)) or in the soil microflora (12).

Despite this, there has been a steady decrease in legume cultivation over the past two decades.

Why therefore are legumes not more cultivated in Europe? Among several reasons, grain legumes have a lower productivity and a higher yield variability than major cereal crops, partly due to protein-rich seeds, to higher sensitivity to biotic stresses such as *Aphanomyces*. Legume performance is also directly influenced by environmental conditions and legumes are very sensitive to extreme weather events. Kevin McPhee (Univ. N Dakota, USA) presented an innovative way of examining this by using a flail harvester to simulate the action of hail illustrated with videos from the field.

Another reason is linked to the fact that there has been less breeding activity than for cereals or oilseed rape, and yield improvements are correspondingly lower. One consequence of variable performance is that in a rotation, the benefit for following crop can vary from 0 to 75%, and legume fertilization is not adjusted sufficiently to take into account the contribution from atmospheric N-fixation. In economic terms, the financial and environmental benefits for the following crop are not currently costed, but need to be to enable legumes find their rightful role in sustainable farming practice. Also, to promote legume cultivation, a territorial approach linking local cropping systems (CS) and value chains, is necessary. The objective should be to develop CS adapted to local conditions. Ideally coupled innovations should be designed, that is consistent innovations, both in agronomic practices and in food processes. For example, contract farming of a product for an added-value label is established, as in the production of organic IGP (locally sourced) lentils by intercropping with wheat in SW France. As the appropriate technological solutions depend on many factors, including local context, it will be important to maintain a diversity of approaches rather than trying to define a consensus.

Management of N-flux and greenhouse gas emissions by using legumes in CS

A great diversity of legume species is available, allowing their use in various situations, either as a companion crop, pure crop, intercrop, or cover crop. One way of increasing crop diversity in crop rotations is to include legumes as cover crops or as part of intercropping systems (Branco Cupina, U.

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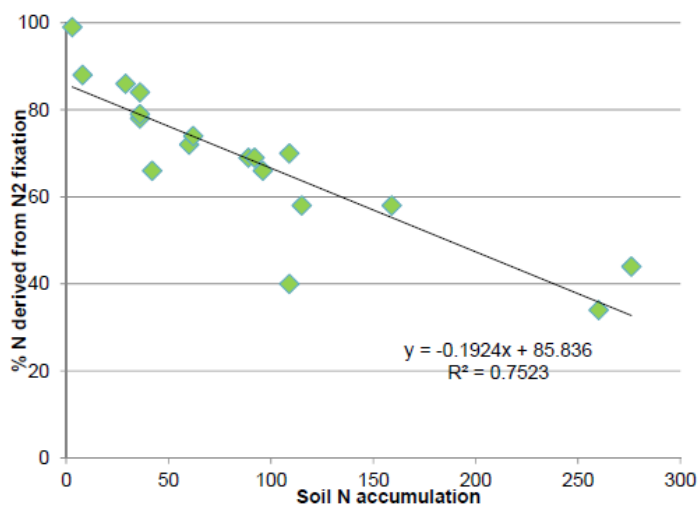
Novi Sad, Serbia). The cropping systems may involve long-term or short-term rotations. In long-term e.g. forage production, N from legumes is cycled to grass via soil and via grazing animals. In short-term: legumes are used as cover or companion crops, intercrops, cut or mulched. In these systems, N-decomposition rate depends on C:N ratio; legumes have a low C:N ratio and cause a fast release of N, whereas grasses have a high C:N ratio and display a slow release of N.

Legume cover crops have to release N in a time-frame that it can be efficiently used by the following crop, by exudation or decay and subsequently by mineralization. Failure to synchronize N-release from the legume with N-uptake by the following crop will result in a loss of efficiency of the system and risk of N-leaching into ground water. Thus, in European systems, after a legume crop, it is important to grow a crop with high capacity of N uptake, such as oilseed rape or a cover crop. In that case, the efficiency of N-recovery depends on the nature of the cover or catch crop used as well as that of the main crops in the rotation (13).

Similarly, in long-term crop rotation studies (Erik S. Jensen, SLU, Sweden) involving faba bean, the level of N-fertilizer supply to the non-legume crops is important. High fertilizer treatments will not affect N-concentration of the harvested crop, but will reduce the amount fixed by the legume, and result in N-accumulation in the soil (Figure 1). Therefore to benefit from legumes' ecological services, inputs need to be tailored to match the soil N-status and cropping regime.

The effects of organic and conventional fertilizer treatments on GHG (N₂O and CH₄) in cowpea-Broccoli rotations were examined (Sanchez-Navarro *et al.*, Univ. Cartagena, Spain). The conventional regime accumulated more CH₄ emissions whereas the organic regime did not follow this pattern. Organic management thus contributed to lowering CH₄ fluxes and also promoted increases in total soil N, beta-glucosidase and beta-glucosaminidase. Conventional and organic systems including legumes were also compared by D. Savvas *et al.* (Agric. Univ. Athens, Greece). The performance of 4 pea and 4 faba bean varieties under conventional and organic agriculture was evaluated in terms of GHG emissions and N₂ fixation efficiency. They found significant differences in cumulative N₂O fluxes and BNF efficiency between varieties in both organic and conventional systems.

Relationship between soil N accumulation and % N from N₂ fixation in fababeans



Data from 18 published studies with and without N fertilizers

Figure 1. Relationship between soil N accumulation and % N from N₂ fixation in faba bean (based on data from Jensen *et al.*, 2010 (18))

Assessing the legume's contribution to intercropping systems

Intercropping potentially offers a way of improving the efficiency of N management (use and fixation) from legume crops and cereal or other N-consuming crops. Legume-Oilseed rape intercropping, studied by Mediene *et al.* (INRA Grignon, France), had as objectives 1) to limit nitrogen inputs (14,15), and 2) to decrease oilseed rape sensitivity to weeds and insects (16,17). Currently, the legume is chosen solely on the basis of its suitability as a cover crop. However, all the services provided by the legume should be taken into account. To assess these, Mediene *et al.* applied a DEXI decision support system (Qualitative hierarchical multi-attribute model) to assess services potentially provided by legumes in the legume-oilseed rape IC. The input was plant Traits>> the resulting functions depended on agro-environmental conditions >> and the output was the services provided. To do this, data were acquired from expert knowledge and bibliography, and aggregated by the DEXI system. Among the services created were reduced weed pressure, reduced N-input, and reduced insect pressure. They found the DEXI tool could discriminate species in terms of these

services. These results now need to be validated with experimental data.

Verret *et al.* (INRA Grignon, France) carried out a meta-analysis of 34 studies on the effects of legume companion plants on weed control and cash crop yield. 3 types of IC were defined according to time of sowing of the companion plant: preceding ("living mulch"), simultaneous sowing, and late ("relay intercropping"). In most cases, companion plants had no significant effect on crop yield (although maize yield was improved), but reduced weed biomass by >50% compared to non-weeded controls and by 36% compared to weeded controls; there was also reduced weed density, although less data are available for this.

Long-term agricultural experiments can increase our understanding of how soil and climatic factors influence legume production. Watson, Walker and Topp (SRUC/SLU) gave a presentation on sustainable management of grass-white clover leys in ley-arable farming systems. In 1961 a long-term experiment addressing the impact of soil pH on the production of crops in ley/arable rotations was established at SRUC Woodlands Field, Aberdeen, UK (Latitude

N57:11:11; Longitude W2:12:55). Under Scottish climatic conditions, maintaining the pH of soils at 6 has been shown to give consistently good dry matter production (Figure 2) with ground cover of 40-50% white clover and high soil microbial biomass. As soil pH can vary widely within relatively short distances, precision approaches to applying liming materials can aid in the cost effective maintenance of grass/white clover swards.

Summary/Perspectives

Agronomy has reached a crossroads, where instead of pursuing higher productivity, sustainability and minimal environmental impact have become priorities. In order that legume-based cropping systems figure, much practical work needs to be done in order to exploit the range of crops and applications possible, to assess comprehensively the ecological services afforded, and communicate their value for society. To be adopted, future legume cultivation must also reflect the evolution of consumer demand towards organic, low-input, locally-produced crops, and develop value-chains that correspond.

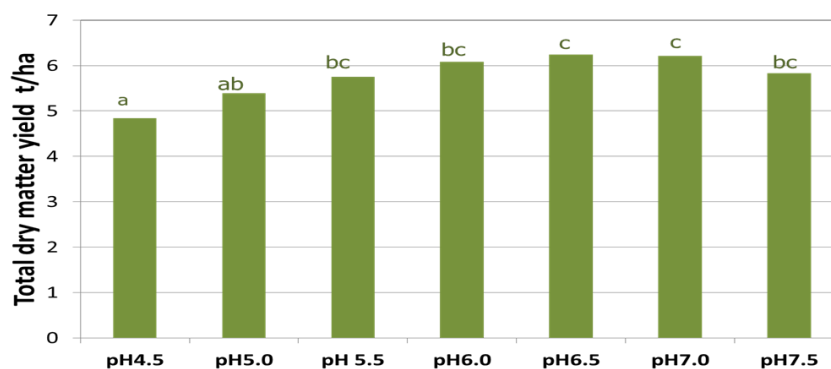


Figure 2. Dry matter yield of grass/white clover leys grown at different soil pH over the period 1969-2008 (C. Watson *et al.*, SRUC, UK)

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Cupina B., Krstic DJ, Antanasovic S, Mikic A, Eric P (2016) Environmental impact of introducing legumes into cropping system in temperate regions. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 57

Watson CA, Walker RL, Rees RM, Topp CFE (2016) Sustainable management of grass-white clover leys in ley-arable farming systems. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 58

Carlsson G, Ernfors M, Mårtensson LM, Jensen ES (2016) Soil nitrogen fertility and nitrogen acquisition in faba bean– long-term system effects. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 276

Savvas D, Pappa VA, Yfantopoulos D, Karkanis A, Travlos I, Bebeli PJ, Ntatsi G, Bilalis D (2016) Impact of organic practices on growth, yield, greenhouse gas emissions and biological nitrogen fixation by three local pea and faba bean landraces. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 277

McPhee K (2016) Effect of simulated hail treatment on yield loss in chickpea. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 278

Verret V (2016) Meta-analysis of the effects of legume companion plants on weed control and yield of the cash crop. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 279

Médiène S, Verret V, Felix J, Valantin-Morison M (2016) A tool integrating and sharing knowledge to select legume species for oilseed rape intercropping. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 280

Sánchez-Navarro V, Zornoza R, Faz A, Fernández JA (2016) Nitrous oxide and methane fluxes from a cowpea-broccoli crop rotation under conventional and organic management practices. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 281

ILS2 Session 3 overview: Beneficial legume-microbe interactions

Chaired by Carmen Bianco¹ and Pedro Fevereiro²

Abstract: Legumes play a pivotal role in developing new strategies to ensure the economic and ecological sustainability of agricultural productivity, without harming the environment.

The ability of many legumes to form associations with bacteria that fix atmospheric nitrogen (symbiotic associations) is a very interesting aspect not only for legumes themselves but also for any intercropped or succeeding crop, reducing or removing the need for nitrogen fertilization. However, there is not only a range of physiological and ecological situations that constrain the biological nitrogen fixation in legume system, but also a lack of knowledge of the molecular and biochemical mechanisms involved in the plant-microbe interactions. Then, to ensure legume crop at high level of productivity, well-adapted and efficient nitrogen fixing microorganisms should be selected considering the principle of specific legume-rhizobia association. Also a better knowledge of the mechanisms involved in legume-microbe interactions should be obtained. The ability of legumes to associate with other beneficial microorganisms is another level of legume-microbe interactions that should be taken into account. In doing this it is also essential to consider that the rhizosphere is one of the most complex ecosystems containing numerous organisms able to perceive even small changes in abiotic conditions, including environmental stress and perturbation.

Nitrogen fixation efficiency - Molecular Mechanisms and Specificities

Various microorganisms, which are constantly present in the environment, are able to affect the life cycle of plants, providing them with nutrient and additional defense mechanisms. Legume plants are able to establish symbioses with a broad range of beneficial soil microorganisms. The symbiotic association with nitrogen-fixing bacteria is one of the most ecologically important and well-known symbioses, requiring highly specific mutual recognition of partners (1).

What mechanisms are involved in plant-microorganism interaction? A complex plant receptor system is required for the accurate identification of the micro-symbiotic partner. The specificity of legume-rhizobia interactions is mainly manifested when rhizobia recognize the roots of specific host plants and colonize their surfaces. Flavonoids excreted by the plant roots are perceived by bacteria and activate the bacterial nodulation genes. These genes produce several related Nod factors, substituted lipooligosaccharides, which are excreted and serve as signals sent from the bacterium to the plant. The plant responds with the development of a root nodule. The plant-derived flavonoids and the rhizobial signal must have specific chemical structures to ensure that only matching partners are brought together (2).

More than 30 genes directly related to this interaction were identified by forward genetics and there seems to be a high level of conservation among them in legumes. Nod factor receptors are kinases that recognize microorganism-signalling molecules and are fundamental to the plant-microorganism interaction. The structure of Nod factor receptors is an important determinant of

bacterial-host specificity. Nod factor receptors recognize different signaling molecules, including lipochitin-oligosaccharides (3-5). Some of these signals are recognized but induce the formation of nodules that are non-viable.

How do plants distinguish between different types of chitin derivatives? There is a specificity that should be related to the existence of receptor families. In *Lotus japonicus*, LysM receptor kinases are one such family (6). As stated by the Key Lecturer of this ILS2 session, Jens Stougaard, it is important to recognize the existence of Nod factor receptors and their variability, but also to recognize the variability of microbial signalling molecules. In order to increase the efficiency of the interaction it is necessary to recognize this variability both on the side of the plants and on the interacting microorganisms.

In different legume species like in the case of *Lupinus* genus, specific symbiotic features have been observed, like absence of infection threads, differences in bacteria penetration and occurrence of symbiosis specificity (7). Keller and collaborators through the analysis of transcriptomes of three different species cultivated with two different strains identified a high number of differentially expressed genes. This suggests that in different genera/species of legumes, different mechanisms of interaction may occur.

The same perspective seems to arise when comparing the responses of 104 pea accessions to the choice among 5 strains of *R. leguminosarum* ssp. *viciae*. In this case changes in partner choice seems to be related with different pea genetic groups. Despite, the ability to compete does not correlated with the efficiency for nitrogen fixation. It has been documented that domesticated crop species tend to have fewer compatible symbionts (higher specificity) than their wild

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Figure 1. A- *Lotus japonicus* ; B- *Pisum sativum* ; C- Determinate Nodules; D- Indeterminate Nodules

counterparts. Knowledge of genetic control of symbiosis specificity will improve our ability to manipulate key genetic factors controlling the symbiotic interaction and allow the development of novel rhizobial strains able to improve the agronomic potential of the root nodule symbiosis (8).

Plant growth promoting microorganisms and production of sustainable agriculture

In an age of rapid population growth and climate change, alternative solutions are required to maintain and increase crop yields sustainably, without a concurrent increase in resource utilization. The manipulation and exploitation of beneficial plant-microbe interactions is one of the possible biological solutions (9-10). The knowledge acquired in plant-microorganism interactions should be used in the selection of strains to be associated with seeds to increase their productivity and adaptability to specific soil and climatic conditions.

In accordance to Bourion and collaborators, in the case of legumes it is possible that the interaction with N-fixing bacteria evolved as an adaptation to a limitation of nitrogen availability.

One of the strategies to develop this technology will be to select plant/rhizobia associations with greater efficiency to nitrogen fixation. The second is to find genetic determinants that allow the plant to compensate for a partial suppression of its symbiotic ability to N fixation due to abiotic stresses.

Vosatka and collaborators highlighted that the spectrum of plant-microbe interaction is highly complex and very often comprise different microbial species potentially acting as consortia. Consortia may involve tripartite interactions, for example between plant, fungi and bacteria. One of the few cases of mono-specific interaction is represented by the legume-rhizobia interaction. Plant growth-promoting microorganisms (PGPMs) could have a significant role in sustainable agriculture because their application helps not only to increase soil fertility, agricultural production, and food quality, but also to improve agro-ecosystems (11). PGPMs might act through direct mechanisms, such as the production of growth regulators (e.g., auxins, cytokinins, and gibberellins), the suppression of stress hormone production, such as ethylene, or improving the uptake of water and nutrients. Indirect mechanisms include the inhibition of pathogens through the production of antibiotics or cell wall lytic enzymes. PGPMs

may also limit the negative effects of drought, heat stress, salinity, and so on. Increase in crop yields following PGPMs applications in both greenhouse and field trials have been observed. It was also seen that plant growth promoting traits do not work independently but additively as it was suggested in the “additive hypothesis”, that multiple mechanisms are responsible for the growth promotion and increased yield (12). The delivery of selected microbial inoculants to seeds (rhizobia, rhizobacteria and micorrhizal fungi) is being explored to increase the ability of plants to support different environmental stresses. In the case of rhizobial inoculants, a short shelf life is being observed. Also there are concerns regarding the effectiveness of the establishment of the inoculated strains in field conditions. The lack of knowledge in the dynamics of the microbiome in the different soils is a concern and is hindering the development of more efficient solutions. A crucial aspect that needs to be controlled is the formulation of effective microbial strains. A microorganism functioning optimally under laboratory conditions might not be able to produce equivalent results under field conditions after its formulation. Lepetit and collaborators warned in their communication that it is necessary that the formulation maintain its activity during production, distribution, storage and field application. An efficient delivery system to vehicle microorganisms from the factory to the field in good physiological condition at right time is also very important (13).

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Stougaard J (2016) Receptor mediated signaling in legume symbiosis. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 33

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Vosatka M, Rozmos M, Latr A, Ma Y (2016) Beneficial microbes associated with legumes. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 74

Bourion V, de Larambergue H, Aubert V, Chabert-Martinello M, Siol M, Duc G, Burstin J (2016) Partner choice in a core collection of pea inoculated by a mix of five *Rhizobium leguminosarum* ssp. *viciae* strains. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 75

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ILS2 Session 4 overview: Genetic resources

Genetic Resources: From Mendel’s peas to underutilized legume species

Chaired by Rodomiro Ortiz^{1*} and Hari D. Upadhyaya²

Abstract: Plant domestication is evolution in a human-made environment. A diversity “bottleneck” changed the sample of genes passing from one generation to another. Today’s crops depend on humans for habitat and propagation because some of desired traits are often maladaptive in nature. Legume genetic resources (wild species, landraces, cultivars, breeding lines, segregating populations, genetic stocks and mutants) are most often used for studying genetic diversity, agro-morphological and nutritional quality traits, and host plant resistance to pathogens and insect pests. They also offer means for understanding plant domestication. Their diversity also shows a great potential for improving crops. Advances in omics are providing new knowledge for using this germplasm diversity in legume genetic enhancement.

Key words: chickpea, cowpea, DNA markers, lupin, mutants, pea, pulses

Legumes are the second most important family (*Fabaceae*) of crop plants after the grass family (*Poaceae*). As noted by the key lecture given by Prof. Noel Ellis (his presentation may be viewed on YouTube, https://www.youtube.com/watch?v=kQNh_mEBktho) in the Session on Genetics Resources at the Second International Legume Society Conference (ILS2, Tróia, Portugal, 2016.10.12) there are a variety of forms for various traits in legume germplasm (Figures 1a, 1b and 1c). They are broadly defined as either natural or bred populations. Segregating individuals or lines derived from crossing are included in the latter. Gregor Mendel (1866) was the first to show –using peas (*Pisum sativum*)– the value of genetic analysis to understand trait inheritance (6), while N.I. Vavilov (1920) began comparative genetics with the law of homologous series in variation (10), which established parallelism in the variability of organisms; i.e., a particular variation observed in a crop is also expected to be available in its related species.

mutations induced by X-rays (7) and ethyl methane sulfonate or EMS (1), respectively. Mutants are other source of useful allelic variation, and they also provide a powerful analytical tool for legume genetics. They are used to detect genes regulating a process (forward genetics) or to identify processes regulated by a gene (reverse genetics), while wild or bred populations allow finding genes that survived selection. Prof. Ellis provided three examples from research in which he was involved to illustrate their use. The characterization of mutants shows that novel filamentous leaf organs present in peas and their allies known as tendrils are modified leaflets inhibited from laminar development by the action of the *Tendril-less* gene, which encodes a Class I homeodomain leucine zipper transcription factor (4). Mendel used flower color to study inheritance in pea. The genome sequence of model legumes along with their synteny to the pea genome facilitated the finding of candidate genes determining anthocyanin pigmentation in pea (2). Likewise, the DNA marker analysis of genetic diversity of a large pea germplasm collection provided a means for understanding variation and evolution of this species as well as for defining a core subset of *Pisum* considering both the major distinct gene pools within this genus and their

Allelic diversity and genetic analysis

Herman Joseph Muller (1927) and C. Auerbach (1946) led the discovery of

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Figure 1a. Diversity for pod (left) and seed (center and right) traits in chickpea

geographical distribution (5).

Restriction site associated DNA (RAD) sequence data also enabled the study of the genetic determinant of the mutant *Stipules reduced* (St) (9) in peas, which was a transcription factor on chromosome 5 (3). These examples show both the different types of population that are available for further characterization of genetic determination of traits in legumes, and the role of genetic analysis in the high

throughput omics era.

Population bottlenecks in domestication

Eric von Wettberg (Florida International University) illustrated how he and co-workers used chickpea (*Cicer arietinum*) to elucidate loss of genetic diversity due to strong selection when domesticating annual crops. Their work included collecting wild relatives of chickpea in areas within the main

center of diversity (~ 60,000 km²) for about 2 months. This study shows that a thorough survey of source population for wild relatives along with their habitats may allow to infer the variation level that was available to early farmers as well as about the shifting environments resulting from the beginning of agriculture in the Fertile Crescent. Likewise, their research calls for having in depth collecting of crop wild relatives and landraces for making suitable conjectures about domestication.

A model for understanding plant domestication

Domestication offers an insightful model for understanding causes and consequences of evolution. Thousands of years took for taming a wild plant into a productive agricultural crop. Plants accumulated – through a gradual process– domestication traits that increase their usefulness to people. Fully documented, scientific age events are known for a 20th century domesticate such as narrow-leaved lupin, which was grown as a green manure and fodder crop in the Baltic States in the 19th Century and became a high-quality grain in the 20th century. Matthew N. Nelson (Royal Botanical Garden Kew, United Kingdom) told how he and research partners used 11690 DArTseq SNPs with unambiguous locations in the narrow-leaved lupin genome for genotyping 233 accessions (147 wild and 86 cultigenes) and studying both its recent and rapid domestication. Their research confirms that modern era domestication in narrow-leaved lupin reduced diversity, generated a differentiated gene pool and increased linkage disequilibrium. It appears that the founder populations of narrow-leaved lupin were of Iberian origin. The dominant mutation in the *Ku* locus removed the vernalisation requirement for flowering, thus allowing the adaptation of narrow-leaved lupin to cropping in southern Australia and northern Europe. *Ku* has been widely used in lupin breeding to confer early flowering and maturity (8). Reduced seed indehiscence and alkaloid content, flower color and removal of physical seed dormancy are other domestication traits in narrow-leaved lupin. This research highlights the impact of domestication on genomic-wide diversity in a modern legume crop.

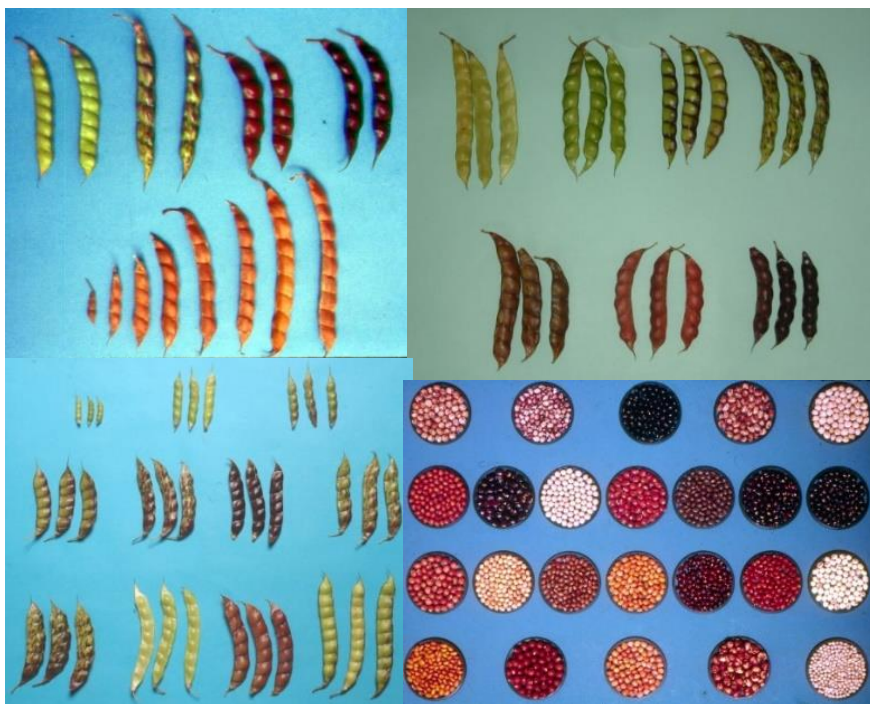


Figure 1b. Diversity for pod (top and bottom-left) and seed (bottom-right) traits in pigeonpea



Figure 1c. Diversity for pod (left) and seed (right) traits in groundnut (or peanut)

Characterizing cultigen pools with high density DNA markers

Single-nucleotide polymorphisms (SNPs) are utilized for studying genetic diversity in crop gene pools. Such an assessment allows an enhanced conservation genetic resources and their further use in plant breeding. Márcia Carvalho (Centro de Investigação e Tecnologias Agroambientais e Biológicas, Portugal) described her PhD research for characterizing the genetic diversity of 33 cowpea (*Vigna unguiculata*) landraces from Portugal and other 63 from elsewhere with the Illumina Cowpea iSelect Consortium Array that contains 51,128 SNPs. She found 44,054 high quality polymorphic SNPs useful for population genetics research using STRUCTURE v2.3.4 and principal component analysis with TASSEL v5.0. There were four subpopulations in these 99 cowpea accessions. The accessions belonging to the subspecies *sesquipedalis* were together in subpopulation 1, while the clusters of those belonging to subspecies *unguiculata* related to their geographical origin. There were two cowpea accessions from Portugal that were categorized as either admixed or belonging to other subpopulation (instead of being in the Mediterranean cluster). This study shows the value of using a high-density SNP array to characterize sources of genetic diversity, which is a requisite for the success of legume breeding.

Valorizing genetic variation

LupiBreed began in the Spring of 2015 with the goal of improving productivity and yield stability on both narrow-leafed sweet lupin (*Lupinus angustifolius*) and yellow sweet lupin (*L. luteus*). K. Fisher (Julius Kuhn Institute, Germany), on behalf of her public and private partners, provided an overview on how EMS-based mutagenesis allows broadening the genetic variability in advanced breeding materials of narrow-leafed lupin. Their research led to identifying and selecting novel growth types with high yield potential, which are undergoing multi-environmental testing. Preliminary results are very encouraging since some M lines had both higher yield and higher protein content than their “donor”. EMS mutagenesis provides a means for developing high yielding M lines showing great variability for growth type, maturity, pod number, seed number and weight, and protein yield.

Conference Session presentations:


Ellis N (2016) Where are we after 150 years of legume genetics? In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 35

von Wettberg E, Chang P, Singh V, Cordeiro M, Greenspan A, Alford B, Carrasquilla N, Dacosta-Calheiros E, Warschafsky E, Rouf Mir R, Bukun B, Kahraman A, Aydoğan A, Berger JD, Nuzhdin SV, Penmetza RV, Cook DR (2016) Using expanded collections of wild relatives of chickpea to understand domestication and improve climate resilience. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 88

Carvalho M, Castro I, Matos M, Lino-Neto T, Close T, Munoz-Amatriain M, Carnide V (2016) Characterizing the genetic diversity of cowpea accessions using a high-density SNP array. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 89

Fischer K, Roux S, Jansen G, Ulrich H-J, Dieterich R, Wehling P, Ruge-Wehling B (2016) LupiBreed - Valorisation of novel genetic variability in narrow-leafed lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 90

Nelson M, Mousavi M, Taylor C, Bayer P, Kamphuis L, Berger J, Clements J, Hane J, Edwards D, Erskine W, Cowling W (2016) Domestic bliss? Causes and consequences of a modern era domestication event in narrow-leafed lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 91

There were other 20 posters related to the ILS2 Session on Genetics Resources including the model legume species barrel medic (*Medicago truncatula*) and pulses such as broad bean (*Vicia faba*), chickpea, common and climbing beans (*Phaseolus vulgaris*), cowpea, grass pea (*Lathyrus sativus*), lentil (*Lens culinaris*), pea, tepary bean (*Phaseolus acutifolius*) and white lupin (*Lupinus albus*), among others. The research subjects vary from germplasm conservation, characterization, evaluation and regeneration, as well as gene identification, genetic diversity analysis, and a participatory approach involving farmers, the local government and scientists to identify sources of interesting traits for further pre-breeding. 

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- (7) Muller HJ (1927) The problem of genetic modification. Zeit ind Abst-und Verer Suppl 1:234–260
- (8) Nelson MN, Książkiewicz M, Rychel S, et al. (2016) The loss of vernalization requirement in narrow-leafed lupin is associated with a deletion in the promoter and de-repressed expression of a *Flowering Locus T* (FT) homologue. New Phytol doi: 10.1111/nph.14094
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ILS2 Session 6 overview: Root diseases

Chaired by Julie Pasche¹ and Nicolas Rispail²

Root diseases are among the most widespread and destructive diseases of legumes. Root rot, damping-off and vascular wilt are caused by a complex of soil-borne fungal and oomycete pathogens. Among the many wonderful presentations at the ILS2 Conference, the Root Diseases Section included demonstrations of diverse approaches to deepen understanding and advance the information known about three important host:pathosystems. The session began with a presentation entitled “Insights into the relative contribution of micro-evolution and phenotypic plasticity in the quantitative response of the model legume *M. truncatula* to *Verticillium* wilt”. *Verticillium* wilt is a very destructive disease of many legume hosts. The presented research focused on the importance of evaluating diverse pathogenic isolates as well as diverse host populations, supporting the idea that quantitative resistance encompasses interactions between host genotype and pathogen strain. In addition, Gentzbittel *et al.* demonstrated how evaluations in fluid environments, in this case temperature, can affect phenotypic response to the pathogen and what that might suggest for future climate changes.

Among the most important diseases of dry field pea worldwide are root rot caused by *Aphanomyces euteiches* and numerous *Fusarium* spp. including *F. solani* and *F. avenaceum*. The presentation entitled “Genetics of pea resistance to *Aphanomyces euteiches* in the genomics Era” outlined the research conducted over several years by Pilet-Nayel *et al.* Resistance to *A. euteiches* is quantitatively inherited. This presentation discussed the important and thorough research performed to find, validate and fine-map the genomic regions associated with resistance to this



Figure 1. Field pea with severe root rot infection. Severe reductions in emergence, stunting and yellowing are particularly evident in the lower areas of the field. Photo courtesy of Julie S. Pasche

devastating pathogen. These results contribute greatly to the efforts of incorporating resistance into dry field pea cultivars with adaptations to the varying environments in which this pathogen is an important economical restraint of growers.

The final two presentations of the session focused on very different approaches to the management of *Fusarium* root rot. The first, entitled “Progress on understanding genetic resistance to *Fusarium* root rot in pea” detailed the extensive effort undertaken over the past several years by Coyne *et al.* The approaches used in this research enabled the

detection of several QTL for resistance to *F. solani*, among which two were identified in different recombinant inbred line populations during both greenhouse and field phenotypic screening. The QTL identified will serve as an invaluable resource for improving genetic resistance to *F. solani* in field pea. The second of the presentations on *Fusarium* root rot entitled “Molecular quantification of pathogenic *Fusarium* spp. in soil to predict pea root rot risk in the field” was focused on the identification and quantification of pathogen populations in the soil to predict disease before sowing. This is

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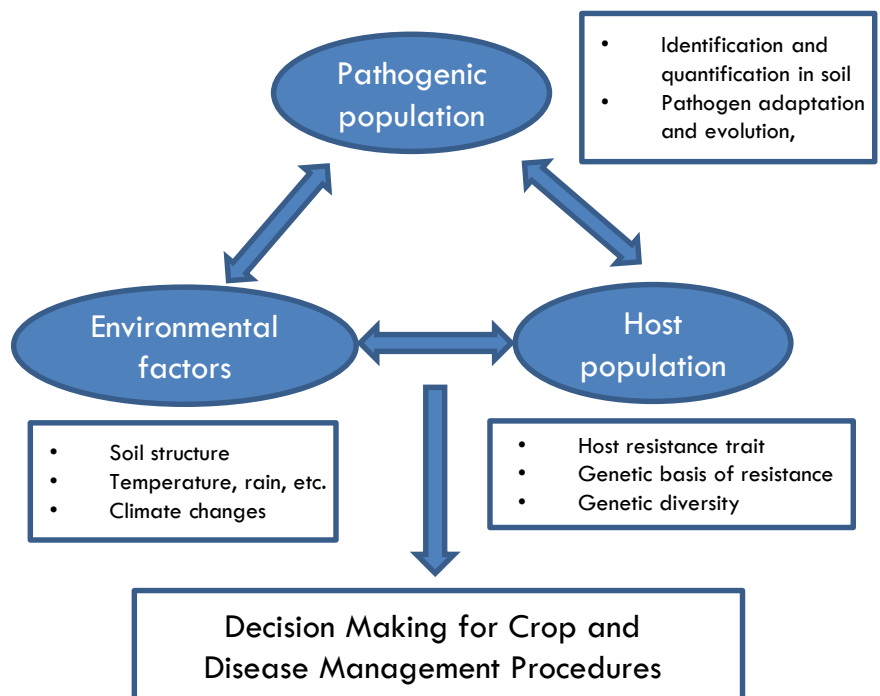
Figure 2. Below-ground symptoms of Fusarium root rot on field pea displaying blackened roots. Note that many of the tap roots are completely rotted. Photo courtesy of Jennifer Odom




Figure 3. Below-ground symptoms of Aphanomyces root rot on field pea displaying caramel discoloration and substantial reductions in root mass. Photo courtesy of Kimberly Zitnick-Anderson

instrumental to provide recommendation to growers and develop disease management practices based on disease avoidance. While it is not ideal to inform a grower that they should avoid planting field peas in a certain field or area, if given no other option, this can at least allow the grower to make an informed decision about crop placement. Additionally, this work also demonstrated that *F. avenaceum* was found at damaging levels in crop residue and not in soil, where *F. solani* was found inhabiting the soil. These results certainly support the basic biology of these two organisms, where *F. solani* produce long-lived chlamydospores and *F. avenaceum* does not and relies on survival in residue from season to season. These results not only move us closer to an assay for grower use, but also remind us that grower practices including tillage and crop rotation influence pathogens in varying ways.

Discussion of these excellent root diseases presentations focused on important concepts underlined by each presenter; principally, that none of these pathogens are occurring, and causing disease, in isolation. Each of the presenters highlighted, to some degree, the importance of environmental factors, pathogen variation, and possible synergisms with other pathogens. Certainly, these factors are imperative in our understanding of disease development and management; however, this is particularly important to



consider when soil-borne pathogens are being investigated. The soil environment provides many unique environmental considerations and challenges, the least of which are the number of pathogens involved in the root disease complex. This challenge beseeches us, as researchers varying in expertise, to work in concert, on cooperative efforts in the development of management strategies to ensure the economic viability of grain legume production. As outlined above, complete resistance to soil-borne pathogens has not been identified. Host resistance to root pathogens is strictly quantitative. While incorporating that resistance is crucial, integrating additional management strategies to decrease disease severity is equally important. As chairs, we thank these four tremendous researchers, and their collaborators, for their contributions to the success of this session and continued discussions about the challenges we face with soil-borne pathogens. 

Conference Session presentations:

Gentzbittel L, Ben C, Sbeiti A, Mazurier M, Toueni M, Negahi A, Sarrafi A, Benameur S, Tardin M-C, Gras M-C, Rickauer M (2016) Insights into the relative contribution of micro-evolution and phenotypic plasticity in the quantitative response of the model legume *M. truncatula* to *Verticillium* wilt. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 122

Pilet-Nayel M-L, Desgroux A, Lavaud C, Lesné A, Boutet G, Aubert G, McGee RJ, Coyne CJ, Bourion V, Burstin J, Baranger A (2016) Genetics of pea resistance to *Aphanomyces euteiches* in the genomics Era. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 123

Porter L, Boutet G, Pilet-Nayel M-L, Baranger A, McGee R, Ma Y, Coyne C (2016) Progress on understanding genetic resistance to *Fusarium* root rot in pea. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 124

Chatterton S, Heynen M, Safarieskandari S, Zitnick-Anderson K, Pasche JS (2016) Molecular quantification of pathogenic *Fusarium* spp. in soil to predict pea root rot risk in the field. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 125

ILS2 Sessions 7 & 9 overview: Legumes in food and feed and other alternative uses

Chaired by Maria Carlota Vaz Patto¹, Ambuj Bhushan Jha², Ruta Galoburda³ and Tom Warkentin⁴

Abstract: Grain legumes or pulses are an excellent source of proteins, carbohydrates, fibres, folates and many essential micronutrients and therefore considered as a nutritious complete food. Several different management and breeding approaches can be deployed to associate a good agronomic performance with an improved nutritional quality on legumes. Pulses are also helpful in maintaining good health as they can prevent several diseases including diabetes and cancer. Different human pathways and legume bioactive compounds are involved on these health benefits, being their bioavailability a constraint when characterizing germplasm collections nutritional potential. Innovation on food formulation and feed processing is a prerequisite to fully explore the nutritional potential of legume crops and indirectly increase their domestic cultivation and use by the processing industry with clear economic gains.

Recognising the importance of legumes, the organizing committee of the second International Legume Society Conference dedicated sessions 7 and 9 to the importance of legumes in food and feed and other alternative uses.

Different approaches for legume nutritional quality improvement have been discussed in these sessions from the introduction of changes in protein amino acid composition to the exploration of the protease inhibitors allelic variation and the identification of significantly associated molecular marker-bioactive compound content for marker-assisted selection. Preprocessing treatments were also shown to improve the nutritional value of legume feed.



Figure 1. Anne Flore Monnet (UMR Ingénierie Procédés Aliments, INRA/Université Paris-Saclay, France) during her presentation “Understanding the structuring of wheat-legume cakes to promote product innovation and to design new formulation tools for the industry” at ILS2 (photo courtesy of José Parreira)

The mechanisms associated with health beneficial effects of some pulse fractions have been analysed, as well as the importance of considering the different bioactive compounds solubility fractions when evaluating their nutritional potential. Innovative cereal-pulse food formulation tools as a way to promote their industrial processing and ultimately their domestic growing have been presented, as well as the economic gains arising from feeding these domestically grown grain legumes to poultry.

As a key speaker of session 7, Frédéric Marsolais (Agriculture and Agri-Food Canada), has delivered his talk on the topic “Using beans with novel protein compositions for nutritional improvement”. His studies showed an increased concentration of essential sulfur amino acids, methionine and cysteine in genetically related lines of common bean (*Phaseolus vulgaris*) integrating a progressive deficiency in 7S

globulin phaseolin and lectins proteins. Further, genomic studies identified polymorphisms which were responsible for the absence of specific storage proteins as well as differences in accumulation of storage protein.

Maria Bronze (ITQB NOVA, Portugal), and collaborators, presented their research on the extraction and characterization of the hidden phenolic content of faba beans (*Vicia faba* L.). Phenols present in pulses are beneficial for human health against various disorders such as cardiovascular diseases, type II diabetes and cancer. Phenols extracted from faba bean were characterized by LC-MS, whereas the antioxidant activity of fractions was estimated by ORAC method. Bio-availability and health benefits of these compounds were discussed in detail.

Jose C. Jimenez-Lopez (CSIC, Spain), and collaborators, presented on the topic “Use of narrow-leafed lupin b-conglutin proteins in

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human food to tackle diabetes through modulation of the insulin pathway". Lupin is an important pulse crop, which could be useful in prevention of cardiovascular diseases, obesity, and dyslipidemia. Research indicated up-regulation of b-conglutins with the increased synthesis of several genes involved in the insulin pathway. Down-regulatory effect by various b-conglutins in pro-inflammatory genes (IL-1b, iNOS) suggested possible use of narrow-leafed lupin in the prevention and treatment of type 2 diabetes.

The "Genetic diversity in pea and its impact on strategies for seed quality improvement" was presented by Claire Domoney (John Innes Centre, UK) and collaborators. Seed proteins of pea are important for human nutrition as well as animal feed. Domoney stressed the need to identify induced mutations and natural variation existing in genes using high-throughput screening methods that could be useful in breeding programmes for the improvement of visual traits and seed composition.

Anne Flore Monnet (UMR Ingénierie Procédés Aliments, INRA/Université Paris-Saclay, France) and collaborators, delivered a talk on "Understanding the structuring of wheat-legume cakes to promote product innovation and to design new formulation tools for the industry". Associations of cereal-legume are agronomical and nutritional beneficial for improving the sustainability of wheat and pea supply chains. With the aim to design new products and practical formulation tools for the processing industry, the partial replacement of wheat flour by pea flour on the structure properties of soft cakes was investigated. Results indicated that the honeycomb structure of pea-wheat mix flours (P-W) cakes was finer and more homogeneous than control (W) cakes. Further, the P-W cakes aerated cake crumb was softer than W cake. Results were compared with pea and wheat flours characteristics such as protein content and quality.

During ILS Session 9, Elsa Mecha (ITQB NOVA, Portugal) and collaborators demonstrated that protein quality varies between different Portuguese varieties of common bean (*Phaseolus vulgaris* L.). The study included 108 different varieties. Final data, treated by multivariate analysis, allowed systematization of overall characterization and bridged the gap of knowledge in protein

quality of common beans. This could be a tool for future breeding approaches.

E. Tormo (Terres Univia, France) and collaborators reported that the nutritive value of pea and faba bean can be improved by mechanical and heat treatments, such as grinding, dehulling and pelleting. They indicated that fine grinding improved starch digestibility and metabolisable energy content of pea and faba bean in non-pelleted diets, with a greater effect for broilers than for roosters. Dehulling improved metabolisable energy content for colored and white-flowered faba bean, but only for tannin-rich pea. Protein digestibility was improved for tannin-rich varieties of both grains. Liga Proskina and collaborators (Latvia University of Agriculture) presented their study on economic gains from feeding domestically produced faba beans and peas to broiler chickens. The research revealed that replacing imported soybeans with faba beans and peas in broiler chicken diets reduced feed costs, increased the production efficiency factor, as well as decreased the feed conversion ratio or the amount of feed consumed per kg live weight gain.

The studies presented in this session indicated that legumes are good sources of other biologically active substances important in human and animal diets. Thus, Carmo Serrano (INIAV, Portugal) and collaborators examined the variation of bioactive compounds like tocopherols and carotenoids in 86 chickpea accessions representing the chickpea germplasm diversity in use by the European breeders. The tocopherols were expressed in the fat fraction and mean concentration for γ -tocopherol was 626.59 $\mu\text{g/g}$, followed by α -tocopherol 139.11 $\mu\text{g/g}$ and δ -tocopherol 31.96 $\mu\text{g/g}$. The carotenoids were expressed in whole flours and the highest mean values were established for lutein, followed by zeaxanthin. Another research presented by Ambuj Jha and collaborators (University of Saskatchewan, Canada) dealt with evaluation of a pea genome wide association study panel for folate profiles by UPLC-MS/MS. The research is in progress to evaluate 177 pea accessions developed at the CDC, consisting of cultivars and land races from North America, western and Eastern Europe, and Australia. In further research using genome-wide association and candidate gene approaches, folate profiles will be associated with genotyping data to identify significant SNPs for marker-assisted

selection.

Alfonso Clemente (CSIC, Spain) and collaborators, reported results of their research on Bowman-Birk inhibitors (BBI) from legumes, such as soybean, pea, lentil, and chickpea and their effect on mammalian gut health. They have described a significant concentration- and time-dependent decrease in the proliferation of human colorectal adenocarcinoma cells, following treatment with BBI variants from pea, lentil and soybean. A major pea protease inhibitor, TI1, expressed in *Pichia pastoris*, and related engineered mutants having modified inhibitory activity, suggest that BBI proteins are taken up by colon cancer cells and exert their antiproliferative properties via protease inhibition. Serine proteases that become active in early stage of colorectal carcinogenesis are likely to represent a primary target of BBI.

There were other 24 posters related to the ILS Session "Legumes in food and feed and other alternative uses" covering soybean and pulses such as faba bean, pea, grass pea, common bean, chickpea, lupin, lentil, fenugreek or cowpea, forage legumes such as alfalfa, forage pea, vetches, clovers or sainfoin, and forest legumes such as black locust and carob. The research subjects vary from germplasm quality evaluation for food and feed uses, the study of the genetic control of bioactive compounds in pulses, agronomic management of feed quality and biomass production as sole crop or intercrop, the nutritional, processing and sensorial quality evaluation of pulse based innovative food products and food processing sanitation methods or feed products fungal contamination screening.



Conference Session presentations:

Marsolais F (2016) Using beans with novel protein compositions for nutritional improvement. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 36

Bento da Silva A, Cardoso C, Mecha E, Vaz Patto MC, Bronze MR (2016) The hidden phenolic content of faba beans. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 127

Lima-Cabello E, Alche V, Robles-Bolivar P, Alche JD, Jimenez-Lopez JC (2016) Use of narrow-leaved lupin b-conglutin proteins in human food to tackle diabetes through modulation of the insulin pathway. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 128

Domoney C, Rayner T, Moreau C, Ambrose M, Clemente A, Isaac PG (2016) Genetic diversity in pea and its impact on strategies for seed quality improvement. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 129

Monnet AF, Jeuffroy M-H, Michon C (2016) Understand the structuring of wheat-legume cakes to promote product innovation and to design new formulation tools for the industry. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 130

Tormo E, Heuzé V, Peyronnet C, Tran G, Chapoutot P (2016) A meta-analysis to assess the effect of fine grinding, dehulling and pelleting on the nutritive value of peas and faba beans for poultry. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 179

Mecha E, Figueira ME, Vaz Patto MC, Bronze MR (2016) Protein quality of different Portuguese varieties of common bean (*Phaseolus vulgaris* L.): the missing data for breeding. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 180

Proskina L, Sallija C, Konosonoka HI (2016) Economic factors of using the legumes in broiler chickens feeding. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 181

Serrano C, Carbas B, Vaz Patto MC, Brites C (2016) Tocopherols and carotenoids diversity in a chickpea germplasm collection exploited in chickpea breeding in Europe. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 182

Clemente A, Marín-Manzano MC, Arques MC, Olías R, Rubio LA, Domoney C (2016) Bowman-Birk inhibitors from legumes and mammalian gut health. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 183

Jha AB, Purves RW, Gali K, Tar'an B, Vandenberg A, Warkentin TD (2016) Evaluation of a pea genome wide association study panel for folate profiles by UPLC-MS/MS. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 184

ILS2 Session 8 & 10 overview: Frontiers in Legume Genetics and Genomics

Chaired by Bernadette Julier¹, Kevin McPhee², Roberto Papa³ and Marta Santalla⁴

The Frontiers in Legume Genetics and Genomics sessions (8 and 10) addressed a number of diverse topics related to development of new genomic tools for the legume community as well as application of new and some older technologies to address specific objectives. Several topics focused on legume quality with others addressing specific pathogen resistance, understanding photoperiod response in common bean, response to moisture stress in pea and application of genomic selection to improve lentil breeding and selection for forage yield in legume species.

Judith Burstin (INRA, France) presented progress on development of the full *Pisum* genomic sequence on behalf of the Pea Genome International Project. Several complementary strategies were used by partners in the Czech Republic, France, USA, Canada and Australia to produce the high quality map. Publication and availability of the sequence promises to open the door for many opportunities and greater understanding in pea genetics as well as syntenous species. Ping Wan (Beijing University of Agriculture, China) reported on development of a high quality draft sequence of adzuki bean (*Vigna angularis*). The assembled sequence represented 83% of the genome and the contigs were assigned to one of the 11 chromosomes using a single nucleotide polymorphism (SNP) map as reference. A total of 34,183 genes were predicted and functional analysis showed that differences in starch and fat content between adzuki bean and soybean were due to transcriptional changes.

G. Boutet (INRA, France) and collaborators reported on development of a SNP resource in pea using a whole genome genomic DNA genotype by sequencing approach. KASP assays for 1000 identified SNPs were designed and allowed greater refinement of map positions for QTLs associated with several traits of interest.

S. Lenka (Czech Republic) and collaborators reported on expanded characterization of a series of chromosome segment substitution lines between *P. sativum* and *P. fulvum* using 13,200 SNPs and DARTseq technologies. Detailed characterization of the introgression lines is expected to allow QTL and gene identification and their incorporation in desired genetic backgrounds.

Genetic control of seed quality of leguminous species has been studied

extensively due to the integral role these species play in the diets of many populations worldwide as well as their use in animal rations. Christine Le Signor (INRA, France) and collaborators used translational data derived from *Medicago truncatula* to identify eight protein quality loci (PQL) common between pea and *M. truncatula* which control legumin, vicilin and convicilins. The relationship between candidate transcription factor genes and seed protein composition was validated using tilling mutants for the transcription factor gene in pea. A set of candidate genes controlling protein accumulation in legume seeds was identified and through further analysis the nutritional value and technological properties of modified protein fractions will be determined.

Maria Carlota Vaz Patto (ITQB NOVA,



Figure 1. Judith Burstin (INRA, France) during her presentation “Towards the genome sequence of pea” at ILS2. (photo courtesy of José Parreira)

¹ INRA, France

² Montana State University, USA

³ University of La Marche, Italy

⁴ CSIC, Spain

Portugal) and collaborators reported on the application of genome wide association studies to study seed content of eleven phenolic compounds in 103 Portuguese common bean accessions. Ten significant marker-trait associations were detected for seven of the 12 traits measured on chromosome 6, 7 and 10. It was clear that GWAS identified several genes associated with the metabolic pathway for synthesis of phenolic compounds, but further work is needed to compile adaptive response to biotic and abiotic stress and clarify the hidden interactions involving the phenolic compounds.

Faba bean is a highly nutritious crop but contains anti-nutritional factors including vicine and convicine that affect digestibility and cause favism in humans. Ana Maria Torres (IFAPA, Spain) and collaborators reported on the genetic control of vicine and convicine. The gene for reduced accumulation is linked to white hilum color, however, this phenotype is maternally inherited and not expressed until one generation later, thus delaying breeding progress. Outcome from the three approaches discussed identified a single QTL for vicine-convicine content on the end of chromosome 1. Two markers, MTR2g008210 and MTR2g008225 had 97.1 and 89.5% efficiency in selecting genotypes

in the F₂. The next steps are to further saturate the genomic region with additional SNPs and develop diagnostic markers and validate these markers in other genetic backgrounds.


Marta Santalla (CSIC, Spain) and collaborators reported on analysis of GI (2) and FT (7) homologs in common bean responsible for photoperiod sensitivity. Phenotypic analysis of a RIL population identified days to flowering QTLs on chromosome 1 and 4. Experiments to further characterize individual action of genes underlying the QTL revealed greater detail on the control of photoperiodism in dry bean. This report is an example of how homology among species allowed greater understanding of the control of photoperiod responses in common bean to be achieved.

Ana Campa (SERIDA, Spain) and collaborators reported on the use of genotype by sequencing and analysis of near isogenic lines to delimiting physical positions of regions controlling resistance to anthracnose, caused by *Colletotricum lindemuthianum*, of common bean. Position of the single dominant resistance genes on Pv04 and Pv11 were confirmed and characterized in greater detail.

Paolo Annicchiarico (CREA, Italy) and collaborators reported on application and challenges of varied GBS protocols in alfalfa,

pea and white lupin. Genomic selection accuracy for forage yield in alfalfa and seed yield in pea were highlighted. Cultivar distinctness in alfalfa and genetic structure of lupin landrace germplasm were also presented.

L. Pembleton (Agribio, Australia) and collaborators reported on the application of genomic selection in the Australian lentil breeding program. Accuracy of the method for seed yield and seed weight were moderate to high and reflected the relative heritability of the two traits. The genomic estimated breeding values and prediction equations will be applied to future breeding cycles to optimize genetic gain.

Developments expanding the understanding of legume genomes have broadened the opportunities to study and improve many aspects of these crops. Complete genome sequences are available for some species while others are in various stages of development. The increased availability of many genomic tools will result in many new improvements in these important crops that will ultimately benefit populations worldwide. 

Conference Session presentations:

Madoui M-A, Labadie K, Kreplak J, Aubert G, d'Agata L, Capal P, Fournier C, Kougbéadjo A, Vrana J, Gali KK, Taran B, Belser C, Le Paslier M-C, McGee R, Edwards D, Batley J, Bendahmane A, Bergès H, Barbe V, Tayeh N, Klein A, Lichtenzweig J, Aury J-M, Coyne CJ, Warkentin T, Dolezel J, Wincker P, Burstin J (2016) Towards the genome sequence of pea. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 39

Wan P (2016) Genome sequencing of adzuki bean (*Vigna angularis*) provides insight into high starch and low fat accumulation and domestication. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 156

Annicchiarico P, Nazzicari N, Wei Y, Pecetti L, Brummer EC (2016) Genotyping-by-sequencing and its exploitation in forage and grain legume breeding. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 157

Pembleton L, Sudheesh S, Braich S, Rodda M, Slater T, Forster JW, Cogan NOI, Spangenberg G, Kaur S (2016) Application of historical data from Australian lentil breeding program to enable rapid implementation of genomic selection. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 158

Boutet G, Alves Carvalho S, Peterlongo P, Falque M, Lhuillier E, Bouchez O, Lavaud C, Uricaru R, Lesne A, Pilet-Nayel M-L, Rivière N, Baranger A (2016) Legume-based mixed cropping systems may have higher water use efficiency than mono crop systems WGGBS in pea without reference genome and data assembly: a successful strategy at low sequencing coverage for SNP discovery, genotyping and genetic mapping. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 159

Le Signor C, Aime D, Sanchez M, Young ND, Prosperi J-M, Thompson RD, Buitink J, Burstin J, Gallardo K (2016) A protein quantity loci approach combined with a genome-wide association study revealed regulators of protein accumulation in legume seeds. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 186

Vaz Patto MC, Alves ML, Mecha E, Bento da Silva A, Leitão ST, Almeida N, Gonçalves L, Rubiales D, Bronze MR (2016) Using genomics to decipher the grain legumes quality riddle. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 187

Gutiérrez N, Bakro F, Aienza S, Avila CM, Torres AM (2016) Strategies and advances to identify candidate genes controlling low vicine-convicine in faba bean (*Vicia faba* L.). In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 188

González AM, Yuste-Lisbona FJ, Fernández-Lozano A, Bretones S, Sussmilch FC, Weller J, Lozano R, Santalla M (2016) The homologues of Arabidopsis FLOWERING LOCUS T and GIGANTEA genes are involved in the control of photoperiod response of flowering in common bean. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 189

Lenka S, Gregoire A, Smykal P (2016) Wild pea *Pisum fulvum* and *Pisum elatius* chromosome segment substitution lines in cultivated *P. sativum* genetic background. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 190

Campa A, Murube E, Ferreira JJ (2016) Delimiting the physical positions of anthracnose resistance clusters Co-2 and Co-3 using GbS in a set of common bean near-isogenic lines. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 191

ILS2 Session 11 overview: Frontiers in plant and crop physiology

Chaired by Christophe Salon¹ and Luis Aguirrezabal²

In the context of the joint meeting of ILS and LEGATO/Abstress project, a workshop named “Frontiers in plant and crop physiology” was held the 13th of October 2016 and chaired by Christophe Salon and Luis Aguirrezabal. A key lecture from Dr. Phil Mullineaux (University of Essex, UK) highlighted novel results gained in the frame of the EU project Abstress (FP7-KBBE-2011-5:289562). His presentation may be viewed on YouTube, www.youtube.com/watch?v=w33YsN4gq9w. Legume plants are both ecologically and agronomically important due to their capability to establish symbiosis with soil bacteria (rhizobia) inside specialized structure (nodules) and fix atmospheric nitrogen. Biotic and abiotic stress negatively impact nodule formation and functioning, which can detrimentally reduce N acquisition, utilisation for developing seeds and in fine greatly reduce legume yield and its quality. Among stresses, drought has one of the most severe effect on crop yield and can modify root system architecture and the efficiency of biological nitrogen fixation (BNF). ABSTRESS applied combined, integrated systems biology and comparative genomics approaches to conduct a comprehensive study of the gene networks implicated in the interaction between drought stress and *Fusarium* infection in legumes and symbiotic organisms. It used *Medicago truncatula* as a model to rapidly identify characteristics for development in pea. Dr. Phil Mullineaux detailed the experimental protocol used to generate plant material subjected to combined stress. This was realized using a high throughput phenotyping platform equipped with imaging technologies. Bayesian models were applied to metabolomics and transcriptomics data derived from *M. truncatula* to identify genes

implicated in combined drought and disease response (1). In his speech, Dr. P. Mullineaux demonstrated how “hub” genes (whose identification represent a bottleneck even in *Arabidopsis*) controlling plant environment interactions were identified using highly replicated time series transcriptomics combined with dynamic modelling. This project trails the idea that hub genes identified as important in a model species (*Mt*) from a complex dual stress experiment can be fast tracked to a crop (pea) delivering faster novel germplasm to industry.

In his speech, Dr. J. Vorster (University of Pretoria, South Africa) provided us with new findings concerning drought induce transcriptome changes in soybean crown nodules. Dr. Vorster presented results regarding the identification in the nodule transcriptome of genes that were up-regulated over all drought treatments. Among the hundred gene that were induced by drought stress, cysteine proteases were

found to be associated and highly expressed with nodules senescence under drought stress conditions. The discussion with audience concerned the importance, relevance as to delay nodule senescence and the role of other determinants (carbon supply and its fate toward either nodule structure or function). Genericity of the findings concerning varieties more or less efficient in biological nitrogen fixation under drought conditions was also discussed. There is an increasing need for selection of improved drought tolerant soybean cultivars to address future food security. In this perspective, the work from Dr. Vorster provides new clues toward identifying candidate genes as major genetic regulators of root system architecture and BNF plasticity upon drought stress, a promising way to provide targets for future plant breeding and crop improvement.

Characterising plant phenotype both structurally and functionally is an important goal to precise the determinants of legume



Figure 1. Phil Mullineaux (University of Essex) during his Key Lecture presentation “The identification of novel genes controlling plant-environment interactions” at ILS2 (photo courtesy of José Parreira)

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sensitivity to drought. Besides genetic and genomic tools, which are being developed or currently used and faced to technical difficulties to physically access to plant organs such as roots or pods, various methods and platforms are being developed towards the aim to image dynamically and non destructively “hidden” plant compartments (2). In the context of the well recognized current phenotyping bottleneck, developing ways to monitor the development of legume roots, nodules and assess and how their relation with plant carbon supply is modulated under various abiotic and biotic stress is currently necessary. Moreover, *in vivo* non destructive measurements of the pod development and nutrient flow within pods (i.e. on seeds *in planta*) is a huge technical and scientific challenge. In his talk, Dr. R. Metzner (Forschungszentrum Jülich, Germany) illustrated how this can be achieved using quantitative non-invasive measurement of structural and functional development of plant organs. Dr. Metzner and colleagues developed non-invasive techniques based on nuclear magnetic resonance technologies. Using a portable devices (pNMR) they monitored dynamically pods dry matter and water content. Magnetic Resonance Imaging allowed studying the structural development of roots and nodules in soil filled pots. Lastly adding the technology of Positron Emission Tomography (PET) with the short-lived radiotracer ^{11}C allowed to follow the fate of photoassimilates among roots and nodules. The application of all techniques on pea and bean was presented. Interestingly, the transfer of these methods to similar investigation on other legume species was discussed. The potential of these tools to provide a direct view on the effects of genotype or rhizobial strain on plant performance under stress and on biological nitrogen fixation was also discussed. Pro and cons of the MRI methods were discussed. The throughput and spatial resolution of the MRI systems is lower than that of other morphometric designed methods using “simpler visible” imaging or fluorescence microscopy. As such, their application is rather devoted for deep phenotyping than for high throughput. However these can detect fine roots down to 250 μm diameter, which is sufficient to gain dynamically 3D representations of roots and nodules, allowing to extract a range of root system architecture geometric parameters (3). More-

over this can be done non-invasively inside soil, closer to natural root habitat than other “artificial substrates”.

Dr. G. Louarn (INRA, France) presented a common shoot development framework for perennial legume species, used in sown grasslands. Little is known about the elementary traits that make temperate forage legumes, in a wide range of growth habit, differ so substantially. Dr. Louarn in his studies compared the patterns of shoot organogenesis and organ growth of six contrasting forage species during their vegetative phase. Phytomer initiation and shoot branching appeared driven by temperature and highly deterministic in all the species in the absence of competition for light. While organ growth was highly related within a phytomer in all the species, independent of the position and axis order when expressed in phyllochronic time, organ dimensions at maturity were

dependent on phytomer position. Although a common framework of vegetative development was produced species differed in their architecture because of the values taken by component traits of morphogenesis. Environmental determinant and/or systemic (carbon availability and signalling) were discussed. Also, the genericity of the results in front of differential in light quality in field condition versus isolated plants was a subject of matter.

Other fruitful discussions concerned how to tackle rhizobium/plant interaction and their modulation by the environment. Audience acknowledged that this is a great challenge, and a potential lever for improving crop performance. As such translational genetics and genomics, the extrapolation of data from a species to another, even to orphan species was discussed. 

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Conference Session presentations:

Mullineaux P (2016) The identification of novel genes controlling plant - environment interactions. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 41

Vorster J, Cilliers M, van Wyk S, van Heerden R, Kunert K (2016) Drought-induced transcriptome changes in soybean (*Glycine Max*) crown nodules. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 193

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Faverjon L, Louarn G (2016) A common shoot developmental framework for perennial legume species with contrasting morphogenesis. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 195

ILS2 Session 12 overview: Integrated pest and disease management

Chaired by Jenny Davidson¹ and Christoph Le May²

How should IPM adapt/react to change in time and space?

Integrated pest management (IPM) is an intricate system made more complex with ongoing changes. New diseases can emerge, farming practices are constantly evolving which impacts on disease spectrums, and pathogen populations evolve with new virulence to cultivars and fungicides. Different climate also impacts on the pest and disease spectrum. In addition, it is recognized that IPM strategies would be different on small farms in comparison to large mechanical farms in response to social structures, and priorities of mixed farming in low economic regions such as North Africa (Seid Ahmed key lecture presentation may be viewed on YouTube, <https://www.youtube.com/watch?v=BL4tfCv-y2s>). The Integrated pest and disease management sessions of ILS2 had a variety of topics covering insect pests, foliar and soil borne diseases, incorporating cultivar resistance efficiency, impact of crop association on plant epidemics, agronomic practices such as sowing dates, fungicides and biological control, monitoring pathogens population change, and omics tools aimed to identify novel resistance sources.

Around the world, the main problems affecting pulse crops seem to be ascochyta blight and botrytis diseases, but there is now an increase of species like *Fusarium* spp, *Olipidium* spp, *Stemphylium botryosum* and *Botrytis fabiopsis* on faba bean. An expansion of insect pests such stem borer and pea weevil, as well as viruses in chickpea and parasitic weeds has been observed leading to an equilibrium change between pests and diseases. Disease incidence also varies



Figure 1. Seid Armed Kemal (ICARDA) during his Key Lecture presentation “Integrated disease and insect pest management in cool-season food legumes” at ILS2.

from region to region according to climate and farming conditions. Aphanomyces is an increasing problem in a number of countries as the pulse crops are expanding into new areas. This change underlines the importance of developing monitoring systems at local, regional, and international levels. For example, the PISOM program in France aims to develop knowledge and tools to describe, predict and manage the main pea and faba bean diseases. This survey evaluates the impact of climate and farming systems changes on diseases incidence and severity, with a view to anticipating new cultural systems for sustainable disease management.

It is recognized that monitoring for insect pests is relatively straightforward while fungal pathogens require more complex tools to identify the full spectrum of pathogenic species that are infecting the plants. For example, ascochyta blight on field pea can be caused by a complex of pathogens which may be present individually or in various combinations.

Monitoring is very important for pathogen populations as they evolve and adapt in response to farming systems. The Australian *Ascochyta rabiei* population was previously found to be genetically narrow with more than 64% of the population belonging to a

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single dominant pathotype. A recent isolate collection has been found to be highly aggressive on commonly grown cultivars that were previously resistant. The majority of these aggressive types belong to the common haplotype indicating their fitness to survive and replicate. This result underlines the importance to link epidemic surveys with population genetic studies as they provide information on the future risk that these populations present. In contrast, research on soil borne disease such as *Aphanomyces* suggest low genetic flow between populations in accordance with the biology of the pathogen. Population studies have also been applied to parasitic weed species such as *Orobancha crenata*, to monitor adaptation to host species. Similar to changes in aggressiveness, pathogens can evolve to commonly used fungicides. For example, Metalaxyl is an active ingredient in fungicides that have regularly been used to control plant diseases caused by oomycetes such as *Pythium* and *Phytophthora* diseases, but resistance has developed to this fungicide. Research has been undertaken to identify alternative fungicides to better control metalaxyl resistant *Pythium*, however adequate management system needs to be put in place to reduce the risk of resistant developing to these new fungicides.

Crop management impacts on plant disease incidence and severity. In the UK, delayed sowing can reduce damage by 50% caused by *Bruchus rufimanus* (bean seed beetle) on faba bean. It may allow growers to reduce insecticide applications in field beans during the critical flowering and pod set period. In France, studies on aphanomyces root rot found that autumn sown pea crop showed few symptoms and less yield loss than spring sown pea crops. This makes winter pea an alternative in moderately infested fields. However to secure yield and avoid pathogen multiplication, it is not advised to cultivate winter pea in heavily infested fields. Diversification at the field level can contribute to the durability of resistance and reduction in disease severity. Experiments have shown that mixing a resistant variety with a susceptible one can significantly reduce disease severity on the susceptible cultivar. This result is probably due to the dilution of inoculum and possibly physical barriers. Intercropping with spring cereal found that pea aphid colonies in field peas developed differently than in pea monocrops. Aphid populations were

significantly lower and encountered syrphid larvae attacks more frequently than in monocrops. Understanding fungicide efficacy is also important for industry to make correct management decisions while biological control is another management tool that is being investigated to control insect pests such as pea aphids and pea weevil.

This constant change in disease spectrum and disease management strategies requires an effective extension program. This will be achieved differently depending on social structures, and available technology. Farmers training centers, field schools and research groups are a common theme across most regions. Extension is now incorporating

warning services based on predictive models and decision support tools for fungicide strategies. Some of these are delivered through Mobile APPs for instant response.

To conclude, controlling pests and diseases is very important for food security. Pulse legumes are an important source of protein for a large amount of populations. The research in this area indicates that pest and disease control constantly needs to adapt to new threats as well as changes in pathogen populations. The IPM strategies must incorporate a number of pest and diseases within a crop, and the direct/indirect interaction between these pathogens occurring on the same host. 

Conference Session presentations:

Ahmed S (2016) Integrated disease and insect pest management in cool-season food legumes. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 43

Baranger A, Boutet G, Brier C, Le May C, Pilet-Nayel ML, Moussart A (2016) PISOM (Ideotypes, systems, surveys of pea and faba bean main diseases). In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 203

Mehmood Y, Sambasivam P, Ford R (2016) The Australian *Ascochyta rabiei* population structure and evidence of a highly adapted and evolving haplotype. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 204

Chen W, McGee R, Paulitz T, Porter L, Vandemark G, Guy S, Schroeder K (2016) Chickpea damping-off caused by metalaxyl resistant *Pythium* in the US Pacific Northwest. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 205

ILS2 Sessions 13 & 15 overview: Frontiers in legume breeding

Chaired by Annicchiarico Paolo¹, Duc Gérard² and Link Wolfgang³

Even if legume breeding programs entered the genomic era later than cereal or oilseed crops, large programs of genome sequencing and of markers development are presently in rapid progress on major species, offering breeders new tools to speed up their work and more precisely targeted polygenic determinisms and multi-trait objectives. From the relationship between DNA sequence and phenotypic variability identified within segregating populations or genetic resources, marker-assisted selection at specific loci and genomic selection based on genome wide sequence data are new strategies developed in numerous legume breeding programs. In addition, more recent works demonstrate that DNA can be methylated and histones can be subjected to variation responsible for heritable traits, therefore suggesting the use of epigenetic markers to assist breeding.

The level of epigenetic variation was explored by Scott Jackson *et al.* (U. of Georgia, Athens GA, USA) in breeding germplasm as well as association panels in soybean and common bean. Using sodium bisulfite DNA sequencing, Methyl C sequencing, they have sequenced over 200 soybean and common bean accessions including landmark cultivars from the past 80 years of soybean improvement in the US, parents of the public soybean NAM (nested association mapping) population, landraces, a common bean GWAS panel and undomesticated relatives. Using these data, including RNA-seq and small RNAs, they determined differentially methylated regions (DMR) for all three methylation contexts (CG, CHG and CHH). These DMRs were



Figure 1. Micro-sward cropping conditions (mixture with tall fescue and monoculture) by B. Julier *et al.* INRA Lusignan F.

then analyzed for their genomic context: exonic, UTRs, upstream, downstream, and intergenic or repeat (e.g. transposable elements). They could demonstrate that DMR variation within and between species, varieties, landraces and wild soybean made a great contribution to breeding/selection over the past 80 years, explaining a significant part of the variation.

New demands of society and farmers for agroecological practices reducing fertilizer and pesticide inputs support strategies based on the cultivation of mixtures of species. If breeding varieties for performance in sole crop is ordinary, the evaluation of varieties for their adaptation to mixtures is an important new question for breeders. In that direction, searching for ideotypes and for definition of traits for adaptation to intercrops, Bernadette Julier *et al.* (INRA Lusignan, France) have explored QTL for

forage biomass of alfalfa in mixture with a forage grass. A progeny of 200 F1 individuals obtained from two alfalfa parents contrasting by aerial morphology was evaluated for biomass and plant height under two micro-sward cropping conditions (mixture with tall fescue and monoculture). Phenotypic data showed a large variation among individuals for all traits in all cuts. The correlation between traits recorded in mixture and in monoculture was positive but the large variation around the correlation indicated that some genotypes were relatively more performant either in mixture or in monoculture. Most QTL were common to mixture and monoculture but some QTL were specific to the mixture condition suggesting that both common and specific QTL for biomass could be used in breeding alfalfa varieties adapted to monoculture and mixed cropping conditions.

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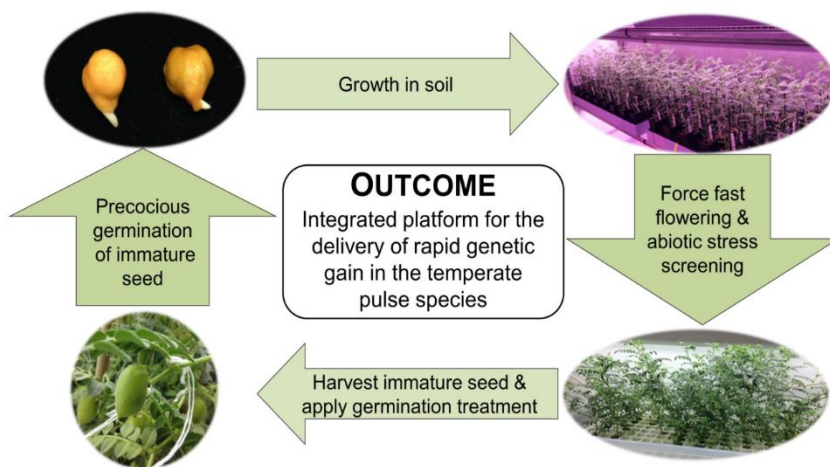


Figure 2. Accelerated-SSD (aSSD) technology enables up to 7 generations per year in the cool-season legumes – halving the time to homozygosity (Pazos-Navarro, Bennett, Edwards, Erskine, Munday, Ribalta, Tschirren, Wells, and Croser).

A further integrative perspective was presented by Adrian Charlton from Fera Science Ltd. (Sand Hutton, UK), who presented the most recent findings from the ABSTRESS project on resistance of legume crops to a combination of biotic and abiotic threats. Both modern phenomics and modern genomics were discussed. Integrated systems biology and comparative genomics were combined to study the gene networks and the metabolic pathways which are implicated in the interaction between drought stress and *Fusarium oxysporum* attack in *Medicago truncatula*. Stressed plants were phenotyped with a high-throughput platform, equipped with the latest imaging technology, used to monitor the performance of the plants without disturbing them. From network analysis of metabolomics and transcriptomics data, “hub” genes and corresponding key control points were identified with implications for the combination of drought and fungal disease attack. Genetic mapping identified 28 hub gene orthologs in pea (*Pisum sativum*). These showed a similar gene expression profile in *M. truncatula* and pea when exposed to combined stress, and correlated with the phenotypic response. Currently, new pea germplasm is produced via tilling and eco-tilling, and six very promising mutant genes were found. The germplasm will serve for validation in field trial and in breeding for

combined resistance.

Even when knowing the chromosomal location and the sequence of promising genes and their allelic variants, the speed of breeding progress is dictated by the time period needed to complete a generation – which can result in slow pace. The research by Maria Pazos-Navarro *et al.* from the University of Western Australia has resulted in a breakthrough technology to hasten the Single Seed Descent system in several legumes: chickpea, lentils, lupin, pea, and faba bean. The teams developed the so-called “Accelerated SSD”, which enables a turnover of 6-8 generations per calendar year, double as fast than the fastest traditional SSD. Key features of this Accelerated SSD are: plant growth under tightly-controlled environmental conditions (especially light quality and flurprimidol as one of the decisive elements) to rapidly initiate flowering; and a world-first system for precocious germination (without in vitro intervention). Some of the more subtle details were said to be subject to intellectual property. The team of Maria has designed a hydroponic selection screen for key abiotic constraints, such as salinity, aluminum and boron toxicity, and integrated it into their approach. The audience discussed the implications of these new proposals and findings for winter- and spring-crops and for epigenetically-modulated genes and traits.

Genotyping-by-sequencing (GBS), which can produce thousands of markers at a low cost, could make genomic selection economically feasible for improving yield and other polygenic traits of legume crops. Benyamin Tar’an and Amit Deokar (University of Saskatchewan, Canada) anticipated genomic selection results for several traits of chick pea assessed on a panel of 281 germplasm and elite lines that were evaluated in different environments. The genomic selection accuracy ranged from 0.20 for seed iron concentration, to 0.80 for 1000 seed weight (where 1.00 stands for perfect prediction of phenotypic variation). These results support the utilization of genomic selection for several of the studied traits.

Higher cross-fertilization rate can ensure higher grain yield and yield stability of faba bean synthetic varieties via greater heterosis. Knowledge on genotype’s cross-fertilization and parental success (as pollen donor) can be important for predicting inbreeding and heterosis of synthetics. Lisa Brünjes and Wolfgang Link (University of Göttingen, Germany) presented results on the extent of genetic variation for these traits, which were assessed by SNP markers on eight replicated genotypes subjected to polycrossing. Sizable variation was found for both traits. The degree of cross-fertilization varied between 28 and 63 %, and the paternal mating success between 8 and 19 %, confirming the importance of including this information for yield prediction of possible synthetic varieties.


Grazing-tolerant alfalfa can be important to sustainably intensify Mediterranean crop-livestock systems, but tolerant material tends to display marked cold-season dormancy and prostrate habit (typical of the subsp. *falcata* background) that make it unsuitable to its target region. It is unknown whether these traits are genetically correlated. Luciano Pecetti and Paolo Annicchiarico (CREA Lodi, Italy) assessed these correlations on 432 genotypes issued by four crosses between contrasting genotypes, reporting moderately high inverse genetic correlations of persistence under grazing with both erect plant habit and winter growth. These results highlight the need for screening large genotype numbers, a task that would be facilitated by the identification of molecular markers linked to each of these traits.

The development of pea varieties with

higher grain content of key micronutrients such as Fe, Zn and Se would enhance the pea nutritional value as a food or as a feed. Tom Warkentin (University of Saskatchewan, Canada) and collaborators summarized the results of a genome-wide association study, which revealed nine SNPs linked to Fe concentration, and 2 SNPs associated with Zn concentration. Some lines displayed a 60 % reduction in phytate-phosphorus concentration, owing to a single recessive gene mapped on pea chromosome 3. Some lines descending from crosses with low-phytate genotypes displayed up to 3-fold greater iron bioavailability relative to the conventional parent genotype. This material confirmed higher nutritional quality when used as a feed for broiler chicks.

Modified root system architectures could improve the resource use efficiency of crops. However, their detection and exploitation by breeding is currently limited by the time needed for manually- and image analysis-derived phenological evaluation of root traits. Boris Rewald (University of Vienna – BOKU, Austria) and collaborators reported on the potential contribution of machine learning approaches based on new algorithms aimed to identify the most discriminative root traits in three legume species, using random forest models and support vector machine analysis. The machine learning approach was able to accurately identify cultivars with root system architectures such as deep or shallow rooting in each species, indicating its reliability for breeding activities.

Mungbean is an important food legume in Asia. Ramakrishnan Madhavan-Nair (World Vegetable Center, South Asia) and collaborators summarized the germplasm evaluation and breeding activities of his institution on the crop, which include the conservation of the world's largest collection of *Vigna* species (11,591 accessions) and the development and the study of a mungbean core collection (1,481 accessions) and a further mini-core collection issued by SSR marker-based characterization. He described breeding work aimed at improving the tolerance to several major biotic stresses, as well as the nutritional quality and sprouting ability of mungbean. Finally, he described the planned activities of the International Mungbean Improvement Network.

The final discussion of the Frontiers in legume breeding sessions focused on the scientific innovations presented in the Conference (including also the other Sessions) that could actually be of practical interest for crop improvement. Various scientists committed to legume breeding expressed the conviction that just a subset of them will ultimately turn out to be of practical value for breeders, at least in the medium term. Genomic selection based on GBS-generated markers, more thorough exploration and exploitation of plant traits via relatively inexpensive phenotyping techniques, and the Accelerated SSD techniques, were cited as promising innovations in this context. 

Conference Session presentations:

Jackson S (2016) Genetic and Epigenetic variation in legume. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 54

Julier B (2016) QTL detection for forage biomass of alfalfa in mixture with a forage grass. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 219

Charlton AJ (2016) Improving the resistance of legume crops to combined abiotic and biotic stress (ABSTRESS). In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 220

Pazos-Navarro M, Ribalta F, Tschirren S, Munday C, Edwards K, Bennett R, Wells S, Erskine W, Croser J (2016) Integrated platform for rapid genetic gain in temperate grain legumes and wild *Cicer* species. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 221

Deokar A, Tar'an B (2016) Genomic based platform for chickpea breeding program at the University of Saskatchewan. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 262

Brünjes L, Link W (2016) Faba bean lines differ in their contribution as pollen donor to cross-fertilized seed. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 263

Pecetti L, Annicchiarico P (2016) Assessing and overcoming genetic trade-offs in breeding grazing-tolerant alfalfa. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 264

Warkentin TD, Arganosa GA, Bangar P, Liu X, Shunmugam A, Delgerjav T, Diapari M, Rehman A, Jha AB, Ashokkumar K, Purves RW, Thacker P, Tar'an B, Bett KE, Glahn RP (2016) Enhancing the nutritional quality of field pea. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 265

Zhao J, Gaonkar B, Bodner G, Rewald B (2016) Machine learning approaches allow for determination of important root traits and enhance differentiation of legumes cultivars. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 266

Madhavan-Nair R, Schafleitner R, Douglas C, Alam MdM, Garg AP, Shwe T, Huttner E (2016) An international network to improve mungbean breeding and production. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 267

ILS2 Sessions 14 & 16 overview: Frontiers in legume agronomy

Chaired by Erik S Jensen¹, Susana S Araújo², Fred L Stoddard³ and Claudio Porqueddu⁴

Abstract: Recent developments within legume agronomy were presented in two sessions. An important current focus in legume agronomy is on intercrops or crop mixtures of legumes and grasses/cereals, especially among European scientists. The quantification of legume services in cropping systems and participatory research approaches are other issues gaining interest.

Key words: agroecology, ecosystems services, intercropping, participatory research

Introduction

Enhancing yield stability in legumes, diversifying cropping systems for obtaining more ecosystem services, reducing climate change and improving vegetarian food protein supply, have prompted increased agronomic and agroecological research on legumes. The goals are linked, since adapting cropping systems to include more legumes to maximize their benefits, requires that farmers can expect stable legume yields.

Eight of the eleven papers in the two agronomy sessions involved intercropping of grain legumes (GL) and cereals. Four papers involved the services obtained from legumes in cropping systems and one paper had a research methodological character focusing on distinguishing between roots of legumes and other species in intercrops. Participatory research approaches involving stakeholders are gaining attention, as evidenced by four papers. We here present the highlights of the sessions.

Session highlights

Legume services

Legumes offer several ecosystem services in crop production systems and for human well-being. They produce protein-rich biomass and seeds for food and feed, they add N to agricultural systems via biologically fixed N₂, and by diversifying crop rotations, they reduce the requirements for fossil energy inputs and decrease pest, disease and weed pressures. Consequently, the integration of legumes in cropping systems is considered a key agroecological principle that can reduce requirements for external inputs.

Nevertheless, the effects of legumes on N and C cycle processes may lead to the risk of N losses via nitrate leaching and N₂O emissions, unless appropriate management interventions are made.

Justes *et al.* (INRA, France) framed the agronomy sessions by a keynote on a comprehensive field study of short crop rotations with varying amounts of GL crops with and without cover cropping. This presentation may be viewed on YouTube (<https://www.youtube.com/watch?v=OofTi2efV9o>). They found that pea and faba bean have positive effects as precrops for durum

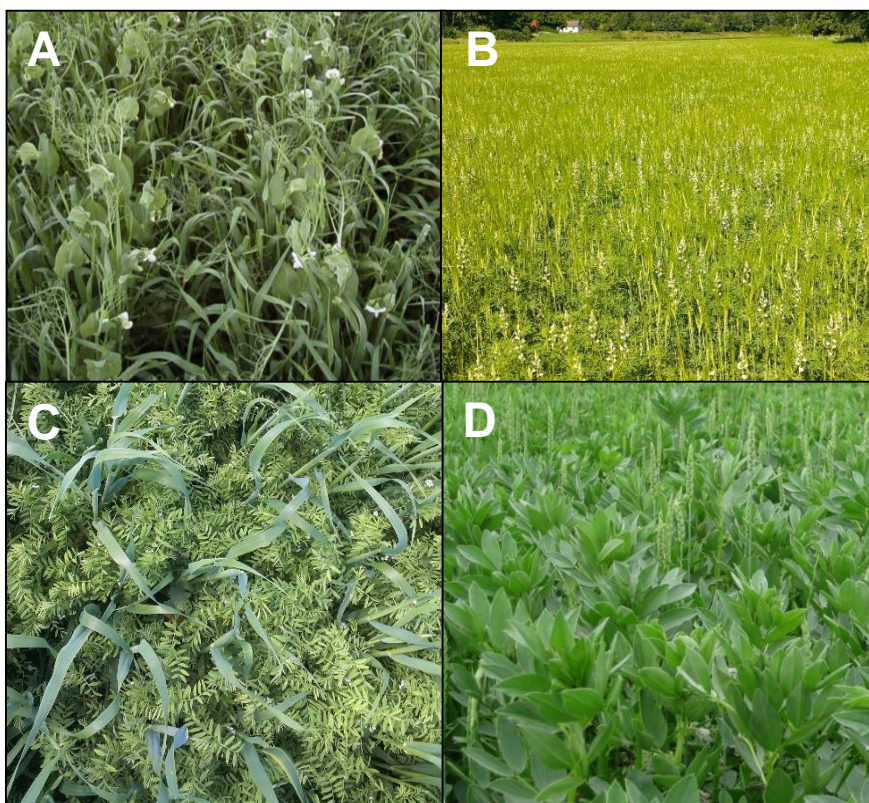


Figure 1. A - Pea/barley (Photo: ES Jensen); B - Lupin/barley (Photo: G Carlsson); C - Lentil/oat (Photo: G Carlsson); D - Faba bean/wheat (Photo: G Carlsson)

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Figure 2. Georg Carlsson (SLU) during his presentation “Participatory development of grain legume-cereal intercrops for enhanced productivity and reduced weed abundance in organic crop production” at ILS2.

wheat, whereas soybean did not. Their study showed that increasing the number of GL crops in a three-year rotation from zero to two increased the simulated nitrate leaching from 3.6 to 8.7 kg N/ha, but with cover crops included there was no significant difference between rotations, with only 2.5-3 kg N/ha being leached. Including several GL crops in the rotation reduced the simulated soil organic carbon and nitrogen in soil, but decline was eliminated by the use of cover crops in the rotation. Emissions of N₂O were low, but differed between crops and cropping systems.

Corre-Hellou (LEVA-ESA, France) and collaborators carried out research on the legume services: protein seed production, N₂ fixation and the residual N effect of the GL on the next crop. They showed that although significant amounts of N are fixed, it is the net balance between fixed N and N removed with seed that determines the residual N effect in the next crop. To optimize the grain legume N benefit for the next crop, it is necessary to know about the proportion of N in the crop derived from N₂ fixation and the N harvest index. Their results indicated that winter white lupin seems to be a promising crop in Western France. Similarly, Guinet (UMR Agroécologie, INRA, France)

and collaborators studied the N dynamics in grain legume-cereal sequences for similar services with ten GL species, and highlighted the importance of the GL residue quality for the N benefit in the subsequent crop. Winter pea was shown to have considerable potential as precrop for winter wheat by Guy (Washington State University, USA) and collaborators, in several USA states, and it is much higher yielding than spring pea.

Intercropping and mixed cropping of legumes and cereals

Intercropping research is increasing worldwide as a means of enhancing crop production per unit area by improved interception of light and nutrient resources, stabilizing yields of crops, reducing weed infestation, and enhancing the protein concentration of cereals in low input cropping systems. Construction of an effective intercrop is not easy: the components need to be complementary in canopy structure, root architecture, nutritional requirements and phenology, among others.

Corre-Hellou *et al.* confirmed in a participatory research project that lupin-triticale intercrops were more productive than sole crops and had less weeds than sole

lupins. Similar results were found in lentil-wheat intercrops, where the presence of the cereal reduced lodging of the lentil. Carton *et al.* (LEVA-ESA, France) analysed growth strategies of winter white lupin and triticale to determine how triticale can be optimized as a support plant for the lupin. Weed suppression was on average 65% compared to sole-crop lupin. The slight reduction in lupin yield in the intercrop was more than compensated by the triticale grain yield. Yield variability between years and sites was lower in the intercrop than in the sole-crop lupin.

Carlsson (SLU, Sweden) and collaborators highlighted that even if many benefits of intercropping are known, there is no Swedish market for intercropped GL and cereals. Consequently, most intercrops are used for feed on-farm. Ten organic Swedish farmers worked with SLU to overcome barriers and challenges in intercropping. The focus in participatory action research was on species selection, weed control and the proportions of species in intercrops. Viguiet (Qualisol/INRA, France) and collaborators found that lentil-wheat intercrop yields were similar to sole-crop wheat yields. Intercropped lentil yields were lower than sole-crop lentil for most genotypes, except the Beluga lentil. The proportion of wheat in the mixture should remain low; 17% wheat enhanced lentil intercrop yield over the corresponding sole-crop yield, while also reducing weeds and lodging. Justes *et al.* also presented a synthesis of ten years of research in Denmark and France on intercropping of cereals and GL, concluding that intercropping is an efficient way of increasing grain production in low-input and organic systems.

Mixed cropping is considered a method to improve robustness of cropping systems. Lingner (University of Goettingen, Germany) and collaborators found no differences between sole and mixed crops of faba bean/wheat and white clover/ryegrass with regard to water use efficiency during drought events. Porqueddu (CNR-ISPAAM, Italy) and collaborators studied legume-based forage crops consisting of pea, Narbon vetch or common vetch mixed with cereals in Italy, Morocco and Algeria. Combinations of either pea or common vetch with cereals resulted in high yielding forage crops, efficient weed control and high farmer acceptability scores. The results indicated that pea may also be useful in mixed forage crops in drought-prone environments.

To improve the understanding of competition/complementarity between


species for below ground resource use it is essential to develop methodology to study species root distribution. Streit *et al.* (University of Goettingen, Germany) showed that Fourier transform infrared (FTIR) spectroscopy could distinguish between faba bean and wheat roots in washed samples taken from mixed crops in the field.

Participatory research approaches

Participatory research approaches with farmers often leading to faster or enhanced innovation and are integrated in many Horizon 2020 projects. Pelzer (INRA, France) and collaborators used the multicriteria sustainability assessment tool MASC® to design legume-based, locally adapted cropping systems in three European regions, valuing the diversity of legume species and practices. The MASC® process involves both a design and sustainability assessment step with stakeholders. Carlsson *et al.* and Corre-Hellou *et al.* worked with farmer and collector groups to learn about challenges in real farm situations, to quantify legume services in farm fields and to design and test cropping systems based on specific farmer goals and improved legume services. Porqueddu *et al.* involved farmers in assessments of legume-based forage crops.

Discussion and future challenges

The discussion pointed to the need to establish more long-term field experiments to determine how to optimize legume yields of dry matter and protein and other services in crop production. Innovation in legume agronomy will be enhanced with increased use of participatory research approaches, where farmers and other stakeholders or actors are involved from the formulation of the project to the final dissemination.

The increasing interest in legume-cereal intercropping should also involve more long-term cropping-system experiments to determine how intercropping can be managed to avoid reducing the rotational effects of GL in cropping systems. There is little research on the optimal precrops for grain legumes. The role of grain legumes has for a long time been to be a “support” crop for cereals in cropping systems. Is a new era for grain legumes emerging with a global call for more food protein? 

Conference Session presentations:

Justes E, Plaza-Bonilla D, Bedoussac L, Gavaland A, Journet E-P, Léonard J, Mary B, Nolot JM, Perrin P, Peyrard C, Raffailac D, Tribouillois H (2016) Synthesis on the effects of grain legume insertion and cereal-grain legume intercrops in low input cropping systems in Southern France. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 47

Pelzer E, Bourlet C, Carlsson G, Lopez-Bellido RJ, Jensen ES, Journet EP, Justes E, Lecomte C, Voisin AS, Jeuffroy M-H (2016) Design and assessment of legume-based cropping systems with stakeholders in Europe. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 246

Porqueddu C, Melis RAM, Thami-Alami I, Abbas K, Pecetti L, Annicchiarico P (2016) Performance of legume-based annual forage crops in three Mediterranean regions. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 247

Lingner A, Dittert K, Senbayram M (2016) Legume-based mixed cropping systems may have higher water use efficiency than mono crop systems. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 248

Carlsson G, Modig P, Hunter E, Jensen ES (2016) Participatory development of grain legume-cereal intercrops for enhanced productivity and reduced weed abundance in organic crop production. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 249

Corre-Hellou G, Mauline M, Naudin C, Poret J, Sorin S, Pelzer E, Mediene S, Jeuffroy M-H, Bazot M, Journet E-P, Lecloux E, Justes E, Larribeau A, Garnaud D (2016) Ecosystem services provided by legumes and exploited by stakeholders in three French territories. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 269

Guinet M, Voisin A-S, Nicolardot B (2016) Quantification of nitrogen fluxes and explanatory plant traits during a two-year legume-cereal rotation. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 270

Carton N, Piva G, Naudin C, Corre-Hellou G (2016) Cereals as companion crops to improve weed control and overall productivity in cereal-grain legume intercrops: case of lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 271

Viguiet L, Bedoussac L, Journet EP, Justes E (2016) Intercropping lentil with spring wheat to improve productivity and income in organic farming. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 272

Guy SO, McGee RJ, Lauver MA (2016) Diversification and intensification of USA dryland cropping systems using autumn-sown winter pea. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 273

Streit J, Meinen C, Rauber R (2016) Quantitative analysis of the root distribution in a faba bean-wheat intercropping system by Fourier transform infrared (FTIR) spectroscopy. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 274

ILS2 Session 18 overview: Resistance to Biotic and Abiotic Stresses

Chaired by Weidong Chen¹ and Laurent Gentrabittel²

The ILS2 session 18 on “Resistance to biotic and abiotic stresses” featured six oral presentations, covering a wide range of subjects.

The first report by Mike Dickinson from Fera Science Ltd, York, UK, was on investigating metabolic changes in the model plant *Medicago truncatula* in response to exposure to combined drought and infection by the fungal pathogen *Fusarium oxysporum*. This work was part of the EU funded project Abstress. Modern and sophisticated techniques such as liquid chromatography high resolution mass spectrometry (LC-HRMS) were used to identify biochemical changes in lipid, flavonoid and alkaloid metabolism in a time-course study. These changes are being exploited to further our understanding of legumes responding to stresses.

The second talk by Dr. Toyoda from Okayama University, Japan, concerned the role of cell wall in interactions between plants and pathogens using pea and *Mycosphaerella pinodes* interaction as a model. The pathogen secretes two structurally related glycopeptide suppressins A and B that inhibit ectoATPase (apyrase) of pea plant cell wall. Exposure to suppressin A and B increased catalytic activity of extracellular peroxidase resulting in increase of ROS generation.

The third talk by Dr. Osorno from North Dakota State University, USA, dealt with flooding resistance/tolerance in dry bean (*Phaseolus vulgaris*). More than 500 genotypes of dry bean were evaluated for flooding tolerance under controlled conditions at two growth stages germination and seedling. Results suggested that different physiological mechanisms of flooding tolerance exist in the two gene pools (American gene pool and Andean gene pool). Significant differences were also detected among market classes within each of the two gene pools. Black and small red market classes appeared to be more tolerant to flooding in germination stage. Genome-wide association study (GWAS) indicated that more than 30 QTLs are related flooding tolerance, clearly a polygenic trait. Two of the QTLs that control root weight and germination rate under flooding were also reported to play similar roles in soybean.

The fourth talk was about GWAS of frost tolerance in pea (*Pisum sativum*) conducted in France. This association study, using 363 genotypes and 10739 loci, identified 8 QTLs distributed over different chromosomes comprising 61 SNPs significantly associated with frost tolerance, in a one-year experiment. The results confirmed three previously identified QTLs based on biparental populations and also identified three novel QTLs for frost tolerance. Additionally several candidate genes were found corresponding to the SNPs, and haplotypes with increased tolerance to frost accessions with favourite alleles were identified.

The fifth talk by Dr. Ruge-Wehling from Germany, dealt with marker-assisted breeding for resistance to anthracnose caused by *Colletotrichum lupini* in lupin. Anthracnose is the most important disease of lupin worldwide. Both the blue (sweet

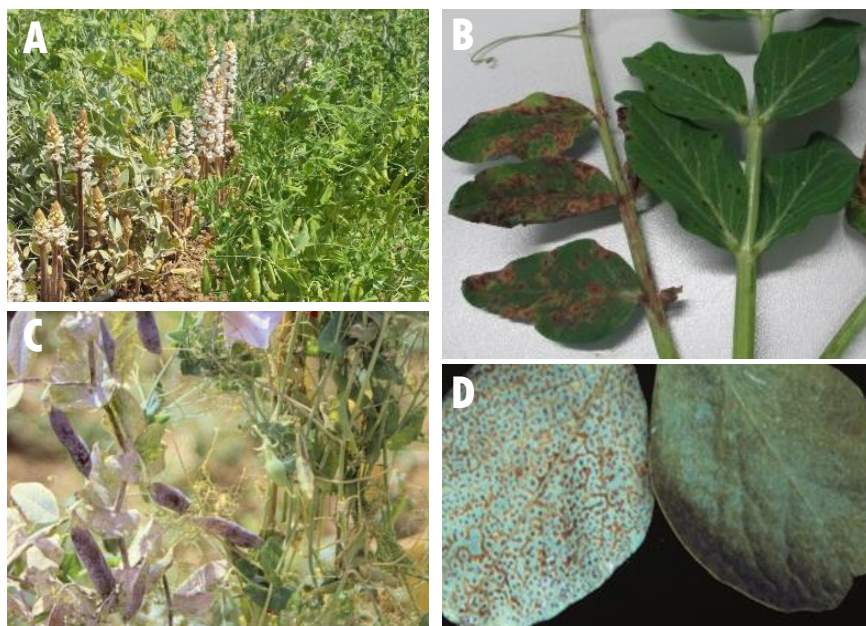



Figure 1. Susceptible (left) and resistant (right) pea accessions to (A) broomrape; (B) ascochyta blight; (C) powdery mildew; (D) rust

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² University of Toulouse, CNRS, INPT, UPS, France

narrow-leafed) lupin and the yellow lupin exhibited qualitative resistance to anthracnose, each governed by a dominant gene as revealed by genetic analyses of segregating F2 families and F3 progenies. The resistance level was high and stable, shown in multi-year field trials. The resistance gene (*LanrBo*) in the blue lupin was located in the linkage group NLL-11. Two markers flanking this gene with a joint recombination of <0.2% have already been used in breeding programs. Polymorphic SNPs are being used in mapping to locate the resistance gene (*Llur*) in yellow lupin.

The final talk of the session was by Dr. Rubiales from CSIC, Spain. He summarized many years of research on using wild relatives of pea in breeding for disease resistance. The germplasm collections of the cultivated pea have insufficient genetic resistance to a number of important pea diseases. It was imperative to exploit the genetic resources from wild relatives of pea for resistance to the important pea diseases like *Ascochyta* blight, powdery mildew, rust, fusarium wilt, parasitic weed broomrape, and even for resistance to insects like aphid and weevil. Research conducted at Institute of Sustainable Agriculture has identified valuable resources of resistance that have been incorporated into pea breeding programs, and resulting disease-resistant cultivars were developed and are being employed in production. The successful examples include a pea cultivar ('Eritreo') with *Er3* for resistance to powdery mildew and two cultivars with resistance to broomrape. Additionally, the resistance mechanisms of the introduced genes are characterized to provide better understanding of the resistance.

In addition to the featured six oral presentations, there were forty-five abstracts submitted to the session of Resistance to Biotic and Abiotic Stresses of the 2nd International Legume Society Conference. There were 24 abstracts dealing with biotic stresses and 14 abstracts dealing with abiotic stresses, two abstracts each on beneficial microorganisms, and yield potential, and one abstract each on insects, nutrient deficiency, and weed stress. These abstracts were submitted from 18 countries. 

Conference Session presentations:

Dickinson M, Rusilowicz M, Wilson J, Charlton A (2016) Exploring metabolic changes in legumes exposed to combined biotic and abiotic stress. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 283

Toyoda K, Miki M, Yamasaki S, Yao S, Matsui H, Noutoshi Y, Yamamoto M, Ichinose Y, Shiraiishi T (2016) The role of plant cell wall in resistance and susceptibility to pathogenic pathogen. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 284

Soltani A, Moghaddam SM, Walter K, Restrepo-Montoya D, Schroder S, Mamidi S, Lee R, McClean P, Osorno JM (2016) Detecting tolerant germplasm and QTLs associated with flooding stress in dry bean (*Phaseolus vulgaris* L.). In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 285

Beji S, Fontaine V, Devaux R, Negro S, Bahrman N, Aubert G, Delbreil B, Lejeune-Hénaut I (2016) Genome-wide association mapping of frost tolerance in *Pisum sativum*. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 286

Ruge-Wehling B, Fischer K, Dieterich R, Rotter B, Winter P, Wehling P (2016) Marker-assisted breeding strategies for anthracnose resistance in lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 287

Rubiales D, Barili E, Rispaíl N, Castillejo MA, Aznar-Fernández T, Fondevilla S (2016) Use of wild relatives in pea breeding for disease resistance. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 288

ILS Honorary Members Awards

The International Legume Society (ILS) was founded in 2011 after closure of former European Association for Grain Legume Research (AEP), with the mission to become the society of reference for ALL legumes WORLDWIDE. After five difficult years it is today a fact that ILS has consolidated with great success having established a solid and reliable series of triennial legume-devoted conferences. At the second conference, ILS established an Honorary Member Award with the aim to recognize both the scientific contribution and the commitment with the association of a number of well reputed legume experts. Such awards should be nominated by ILS committees and be given at each ILS conference. In this occasion, three well reputed colleagues were awarded “In commemoration of a career of extraordinary contributions to the community of legume researchers”.

These were Prof. Erik S. Jensen, formerly from Risø, DK, and presently at SLU, Sweeden, in recognition for his outstanding contribution to Agroecology and for serving as President of former AEP;

Dr. Noel Ellis, formerly from JII, UK and presently in New Zealand, in recognition for his long and outstanding contribution to legume genetics and for serving as President of former AEP; and

Dr. Gerard Duc, from INRA, France, in recognition for his long and outstanding contribution to faba bean and pea breeding and for serving in the Scientific Committee of former AEP and of ILS. 🌱

Diego Rubiales,

International Legume Society President 2011-2016



From top to bottom: Prof. Erik S. Jensen, Dr. Noel Ellis and Dr. Richard Thompson receiving Dr. Gerard Duc's ILS Honorary Members Awards

Other awards @ Second International Legume Society Conference

A special award was attributed by the ILS2 Organizing Committee to Prof. Diego Rubiales, from IAS-CSIC, Spain, for his outstanding contribution to the organization and success of the conference.

Thank you Diego!



Best poster and oral presentation awards

Awards were given to the best poster and to the best oral presentations at the conference as elected by a conference committee. These were:

Best Student Poster: H el ene Bobille with the poster entitled: "Effect of soil water on amino acid exudation in *Pisum sativum* roots"



Best Student Oral Presentation: Anne-Flore Monnet with the presentation entitled: " Understand the structuring of wheat -legume cakes to promote product innovation and to design new formulation tools for the industry".



Honorable mention for best answering: Annika Lingner with the presentation entitled: "Legume-based mix cropping systems may have higher water use efficiency than mono crop systems".



Third International Football Cup

Following an already long standing and healthy legume tradition, the Third International Football Cup (ILFC3) was held as an integral and inevitable part of the Second International Legume Society Conference. Four very reputable new teams were established specially for this edition according to legume research topics: FC GWASgow Centimorgans (for geneticists, omics and breeders) lead by Paolo Annicchiaro; FC Plantstresser Prohibited (for all those dealing with abiotic and biotic and any other kind of stresses) lead by Weidong Chen; FC Agrobarcelonomy (for agroecologists, agronomists, agroeconomists...) lead by Laurent Bedoussac and FC Qualitians Healista (for all those dealing with food and feed quality, nutrition and health benefits) lead by Alfonso Clemente.

The semifinal draw was kindly performed by a young Welsh-Serbo-Portuguese international ascending star, Mihangelinho, using a completely randomized design, with the following result:

FC GWASgow Centimorgans vs. FC Qualitians Healista
FC Agrobarcelonomy vs. FC Plantstresser Prohibited

Just like in the previous editions, the results of the matches were either impossible to follow or completely unimportant. Nevertheless, the flattering title of the ILFC3 winner was attributed to the **FC Gwasgow Centimorgan team, captained by Paolo Annicchiarico** that will be the trophy-owner during the next three years.

A mention must be made to this edition **super referee**: Frédéric Muel; the two **best players** in the fields: Lisa Brünjes and Svetlana Vujić, and finally, to the **best supporter**: Ana Margarida Sampaio.

In the name of FIFFA (Fédération Internationale de Fabaceaeuse Football Association) we would like to take this opportunity to acknowledge all the supporters and volunteers and in particular **Aleksandar Mikic** for all his enthusiasm and tremendous contribution for another very successful International Football Cup. 🌱



**Official emblems of the Third International Legume Football Cup
(ILFC3), Troia, Portugal, October 2016**



Selected moments of the Third International Legume Football Cup (ILFC3), Troia, Portugal, October 2016

Apulian pulse germplasm. A summary of SaVeGraINPuglia project

by Angela Rosa Piergiovanni^{1*}

Apulia, the most south-eastern region of Italy, is characterised by broad plains and low-lying hills and Mediterranean climate. Thanks to its relative flatness, Apulia has always been an agricultural region. This gave rise to the selection, by local farmers, of an unknown number of pulse landraces well adapted to the regional pedoclimatic environments. From 2014 Apulia region has promoted, in the frame of the European funding for “Projects of Rural Development”, five projects aimed to census, collection, characterisation, *ex situ* and *in situ* conservation of the autochthonous plant genetic resources. One of these projects, still in progress and named SaVeGraINPuglia (Recovery, characterization, safeguard and valorisation of pulses, cereals and forage species in Apulia), is focused on the regional herbaceous germplasm. The project partnership involves 21 partner comprising public research institutes, Universities, Parks, business owners, farmers and cultural associations operating in Apulia. The project is coordinated by the Istituto di Bioscienze and Bio-Risorse (IBBR-CNR, Bari).

The partnership activities have evidenced the *on farm* survival of several pulse landraces in the marginal areas of the region where intensive agricultural practices are still not applied. However, this germplasm is threatened of disappearance in short time since it is mainly cultivated on small surfaces by elderly farmers. Overall, about 200 accessions were gathered during the collecting missions carried out in the frame of the project. Eight legumes species are cultivated in the region but their diffusion is very different as testified by the percentage distribution within the collected accessions (Fig. 1). Moreover, the species are not homogeneously cultivated in the region. This is attributable to the existence of microenvironments with slight different climatic conditions, as well as to the preferences of local people.

In addition to the collection and multidisciplinary characterisation of collected germplasm, historical information have been acquired. The material was collected by consulting books and local publications conserved at regional libraries and archives. This allowed the recovery of knowledge about traditional agro-techniques, period of cultivation, ritual uses, traditional dishes and so on related to the most important landraces. The knowledges handed down only orally were recovered through interviews to elderly farmers.

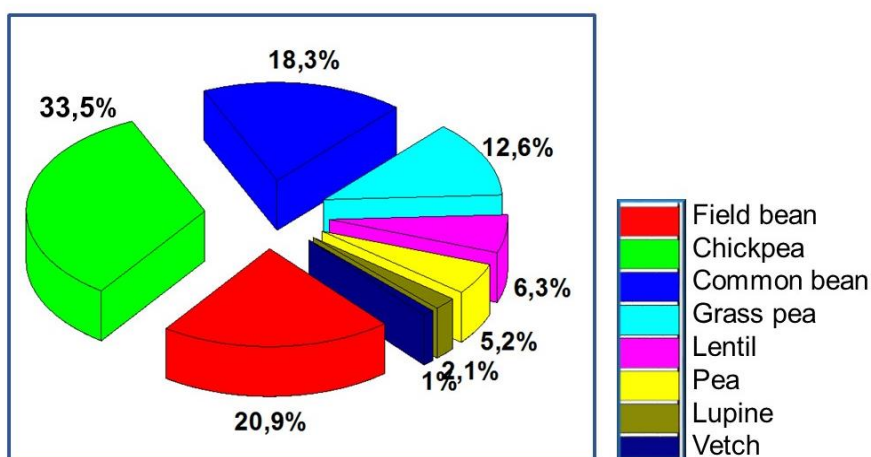



Figure 1. Percentage distribution of the collected accessions.

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Functional genomics of reproductive development in common bean

by Ana M. GONZÁLEZ¹, Fernando J. YUSTE-LISBONA², A. Paula RODIÑO¹, Antonia FERNÁNDEZ-LOZANO², Luis GODOY¹, María LORES¹, Dori POSE¹, Iria PORTAS¹, Antonio M. DE RON¹, Rafael LOZANO², Marta SANTALLA¹

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legume crops for human consumption in the world, as an essential source of protein, dietary fiber, minerals and vitamins. Globally, Spain is the seventh country in bean cultivated for consumption as fresh pod (vegetable) with 9900 ha and as dry beans (legume) with 6600 ha in 2012. The value of production in the domestic market was 245 (vegetable) and 19 (legume) € million in 2011 (www.faosta3t.fao.org, www.mapagrama.gob.es), and the crop has a deep-rooted tradition and prestige productive. Scientific experimentation is encouraged as it is a diploid species ($2n = 22$), with a short life cycle, high reproductive potential, a wide genetic variation, a small genome (587 Mbp), and with the genome sequence, recently available in both Andean and Mesoamerican genetic variation (1, 2). Despite these advantages, knowledge about the genetic control of agronomic interest processes, such as those regulating reproductive development, are very rare, which is a difficulty for the development of genetic improvement programs. In fact, most of what we know about the genetic control of reproductive development has been done in *Arabidopsis thaliana*, but found a large proportion of these genes in other legumes (3, 4).

The BAS-MBG-CSIC Group (www.bas-group.es) has developed, in collaboration with BITAL Group, common bean populations of Recombinant Inbred Lines and genetic linkage maps (5) for analysis of quantitative trait loci (QTL). Has identified QTLs associated with time to flowering in different photoperiod conditions, fruit development (pod and seed) and disease resistance (6, 7, 8), as a good starting point for characterizing new regulators of reproductive development candidate genes. In addition, a collection of 5,000 mutants of Ethyl Methane sulfonate (EMS), some affected in reproductive development which will allow the identification of novel allelic variants, is in progress. BAS is focussed on identifying new key genes in the regulation of reproductive development and to facilitate improvement strategies for adaptation, production and fruit quality in a crop of economic importance, responding well to the current demand for producers and consumers of well-adapted and good quality varieties. It will also contribute to the knowledge of the evolution of these traits along the domestication process. Multiple approaches are carried out to try to discover the genetic basis behind the reproductive development in common bean, using tools of functional genomics and massive sequencing technologies, combined with the generation of highly informative plant materials, and bioinformatic techniques. 

Acknowledgments

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Legume Perspectives is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on the most diverse aspects of various research topics and use of all kinds of legume plants and crops.

The scope of *Legume Perspectives* comprises a vast number of disciplines, including biodiversity, plant evolution, crop history, genetics, genomics, breeding, human nutrition, animal feeding, non-food uses, health, agroecology, beneficial legume-microorganism interactions, agronomy, abiotic and biotic stresses, agroecology, sociology, scientometrics and networking.

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