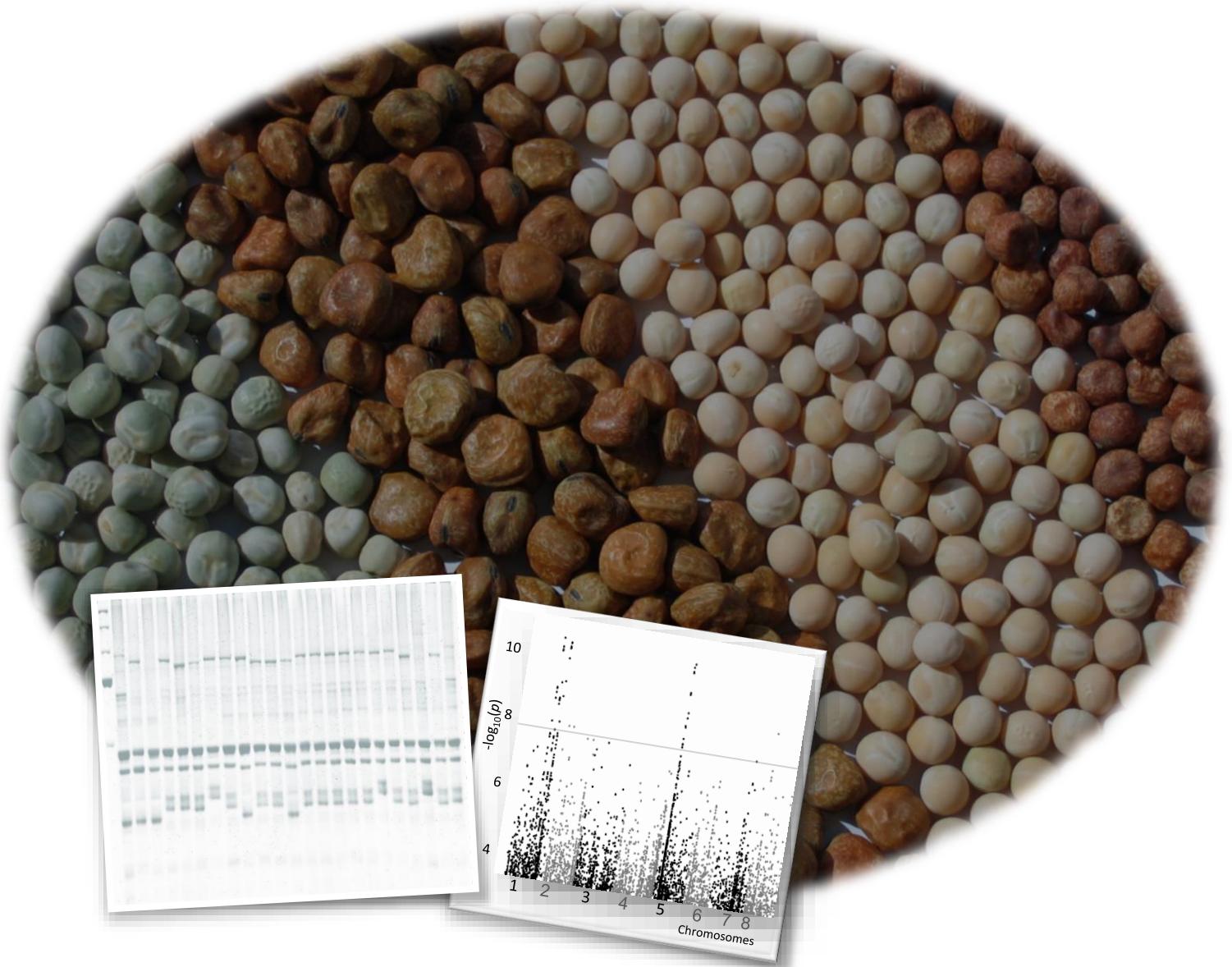




LEGUME PERSPECTIVES



Joint LEGATO-EUROLEGUME Conference

Advances in grain legume breeding, cultivation and uses for a more competitive value-chain

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Front cover photos:

Pea seed diversity (Legato-Eurolegumes) under *Medicago truncatula* seed protein composition (Lesignor & Gallardo, INRA) (Left) seed proteins 1-D polyacrylamide gel; (Right) GWAS for protein abundance

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Under the FP7 Framework (2014-2017) the European Commission has funded two complementary projects, the EUROLEGUME and LEGATO (<http://legato-fp7.eu/> ; <http://www.eurolegume.eu/>) for a four-year period, which involved 47 partners from 18 European member states. From the early stages of the scientific activities, both coordinators have identified several complementarities which have taken science in legumes much further, contributing to reinforce the European position on this thematic area. To mark the culmination of the four years of research and to improve the dissemination of the major achievements of both projects, the coordinators jointly organized an international EUROLEGUME-LEGATO conference, which was held in Novi Sad, Republic of Serbia, on the 27th and 28th of September, 2017. Aiming to reinforce the links with scientists worldwide, and to create a bigger impact on several areas of research, distinguished speakers were invited to complement the programme by giving a number of wider perspectives not covered within the two projects.

This issue of the journal *Legume Perspectives* summarizes the presentations held at the joint LEGATO-EUROLEGUME conference in a series of overviews of each session. For further information, the conference abstracts are cited. These may be consulted on line at : <http://www.legato-fp7.eu/FinalConference/>. To complete the volume, Gérard Duc has kindly agreed to contribute this issue's « Carte Blanche » in which he gives his perspective on the current status of legume research and future needs. As project coordinators, we are grateful to the editorial committee of *Legume Perspectives* for giving us the opportunity to bring the proceedings of our joint conference to a wider audience, and to our colleagues and guest contributors for their efforts in making this, we hope, an interesting issue for all those concerned by legume breeding, cultivation and processing.

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Gérard Duc
INRA UMR Agroécologie
INRA Dijon F.

Breeding grain legumes: a must to develop agroecology, but also a need for further investments after LEGATO and EUROLEGUME projects

In order to promote sustainability and to alleviate new climatic constraints and major biotic stresses that European agricultures have to face while at the same time lowering chemical inputs, grain legumes represent a key lever. Indeed these species, which do not require nitrogen fertilizers, and which bring diversification into cropping systems, have the potential for producing seeds of high nutritional quality for foods and feeds. However, in Europe these crops are cultivated on a very limited area while imports of soybean products are still very high, resulting in a low autonomy in plant proteins.

The LEGATO and EUROLEGUME projects were aimed at boosting and accelerating breeding of the most important grain legume species for diverse zones of Europe (i.e. pea, faba bean, lupin, lathyrus, cowpea). They assembled collections of genetic resources, developed molecular markers for traits of interest to support breeders, and addressed the question of choice of varieties adapted to diverse European environments including biotic stresses and the symbiotic rhizobium context. The projects also evaluated the genetic diversity available for adapting the seed quality for diverse food or feed uses and various non-destructive methodologies for measuring this. Very important results and tools have been acquired in both projects, but considering the diversity of requirements in terms of species and traits, even if certain results are transferable to several species (particularly in genomics), much further research is still required, with a great need for bridging disciplines. Indeed, the varietal progress made has to be integrated in overall strategies of protection, production, and commercial exploitation for various agrosystems.

While the unique ability of grain legumes to fix atmospheric nitrogen through a symbiotic association with *Rhizobium* justifies more research for a better efficiency of this physiological pathway, it should not be forgotten that N-fixation has a C cost to the plant, which should be considered when the seed yield and prices of these crops are compared to those of cereals. This symbiotic mechanism, susceptible to stresses such as heat, drought or *Sitona* larvae, adds complexity to breeding legumes compared to non-legume crops.

Varieties adapted to alternative insect pest management, to new cropping systems such as intercropping, evaluating benefits of legumes in human diets and developing labelling are new and urgent demands which are added to major requirements for disease and pest resistances (*Aphanomyces*, *Ascochytes*, *Bruchus*, *Sitona*...) and tolerance to climatic stresses (freezing, drought, heat). LEGATO and EUROLEGUME have brought significant lines, data and tools to breed in these directions, but further research work is required. Because of the limited crop area sown, and its value in comparison with cereals or oil seed crops, breeding programs on grain legumes excepted soybean, are young, small and few. However, such breeding actions and conducting local variety trials will be keys to exploiting the research outputs. In situations where development is limited by short-term economic considerations, policy support is needed to help long-term orientations. Among the very good signs are the evolution of social demands for sustainable food and agriculture and the rapid research progress in various programs at international level, which can promote these evolutions. In the long term, beyond biotechnical progress, the organization and development of value chains will be synergistic to a joint political push-and-pull effort. 🌱

Session 1 overview: Genetics & Breeding

Chaired by Paolo Annicchiarico¹ and Juan Fernández²

Abstract: The projects LEGATO and EUROLEGUME were largely complementary for crop improvement research on target species, target traits and scientific methods. Reports relative to LEGATO activities focused on yield regulation genes of pea and faba bean, anti-nutritional factors of faba bean and grass pea, drought tolerance of pea, genomic selection of pea and white lupin, new linkage maps of white lupin and faba bean, the exploration of the global white lupin biodiversity, and the development of pea genomic resources. Reports relative to EUROLEGUME activities focused on cowpea biodiversity in a world germplasm collection and on cowpea's metabolism as controlled by the plant circadian clock.

Key words: drought tolerance, genetic diversity, genomic selection, grain quality, linkage map, *Lupinus albus*, *Pisum sativum*, yield, *Vicia faba*, *Vigna unguiculata*

The projects LEGATO and EUROLEGUME faced complementary scientific challenges with respect to target species and crop improvement issues. There were five full-length or flash-presentation talks issued by LEGATO that concerned pea, faba bean, white lupin or grass pea, and two talks issued by EUROLEGUME that were relative to cowpea. (N.B. The conference abstracts are cited as C1-12).

The first talk issued by LEGATO, presented by F. Madueño (CSIC) and A. Torres (IFAPA), focused on yield regulation genes. In pea, the work aimed to identify new allelic variation for MORE FLOWERING NODES (MFN) genes (C1). There are various genes controlling the pea flowering pattern (2). Earlier

unpublished work identified from a mutant population the alleles *mfn1* and *mfn2*, which caused a remarkable increase of fertile nodes and a distinct seed production increase in the single and the double mutant (Figure 1). New polymorphisms investigated within LEGATO were found for both genes in wild and modern germplasm, but were unrelated to the number of flowering nodes. Thus, *mfn1* and *mfn2* alleles (which were absent in wild or modern material) probably are the main genes at hand for improving the node fertility. These alleles, which did not affect grain nutritional quality, have been introgressed into elite varieties, producing new genotypes that will be evaluated for yield increase in the future. The work on faba bean (C2) aimed to locate traits and genes associated with self-fertility, as a means

to select either pure lines or synthetic varieties with maximized heterosis (via obliged cross-pollination). Fine mapping analyses revealed two quantitative trait loci (QTLs) for the number of flowers per node and a few QTLs for the transformation of ovules into seeds, all of them stable across years and evaluation conditions. Putative QTLs were also found for the numbers of pods per node and pods per flower. Self-sterile material exhibited production of largely abnormal pollen and narrower style-ovary angle than self-fertile one.

A talk offered by C. Vaz Pato (ITQB) and A. Torres (IFAPA) explored marker-assisted selection (MAS) for reduced anti-nutritional content in faba bean and grass pea (C3-C6). Vicine and convicine (v-c) are faba bean compounds with anti-nutritional effects

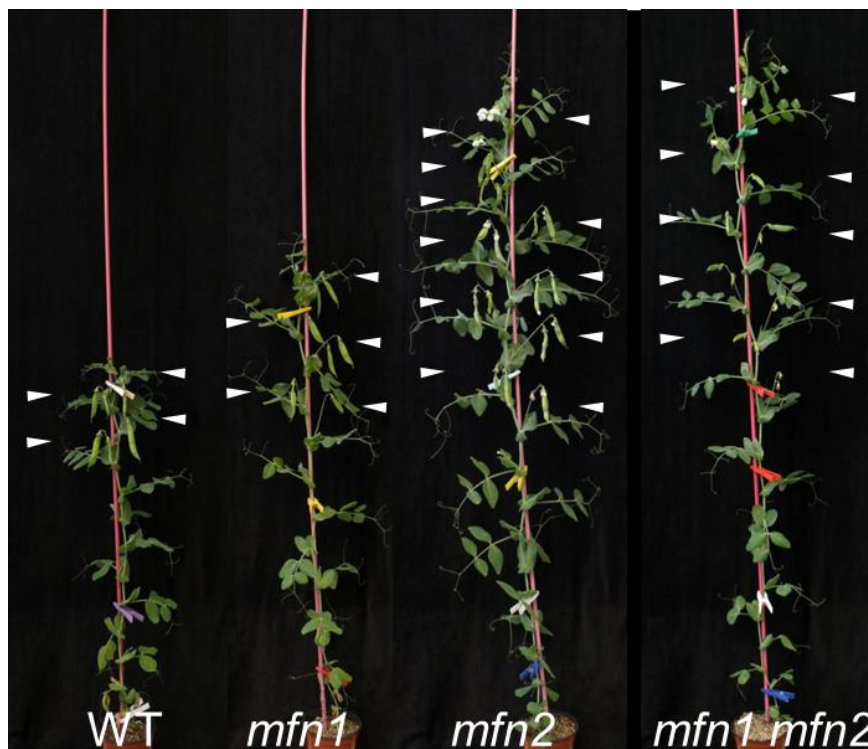


Figure 1. Effect on the pea number of flowering genes of the single and the double mutant of the alleles *mfn1* and *mfn2* compared with the wild type (WT). Arrowheads mark flowering nodes. Photo by Irene Martínez-Fernández, Cristina Ferrándiz and Francisco Madueño

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in monogastric animals and potential toxicity in humans, which are controlled by one gene (vc-) responsible for a 10-20 fold reduction (4). A genetic map including more than 4000 markers was generated using KASPar and DarTSeq markers. Fine-mapping work identified two markers closely linked to the gene, which are exploitable for MAS and offer opportunities for future research on candidate genes. β -ODAP (β -N-oxalyl-L- α,β -diaminopropionic acid) is the main anti-nutritional compound of grass pea, inducing the neuron degeneration syndrome known as lathyrism in humans and animals that eat its grains frequently. Trait phenotyping in two locations and the molecular characterization by about 3500 polymorphic DarTseq markers of 115 accessions representing the worldwide species distribution allowed to identify six markers that could assist in the selection for low β -ODAP.

Genomic selection (GS) for pea and white lupin grain yield was the object of a communication presented by P. Annicchiarico (CREA) (C7). For pea, a genomic selection model was defined using thousands of genotyping-by-sequencing (GBS)-generated SNP markers along with phenotyping data under managed severe terminal drought in Lodi and rain-fed field conditions in Morocco of 315 recombinant inbred lines (RILs) (1). Also, results from a genome-wide association study (GWAS) were exploited to develop a MAS criterion for intrinsic drought tolerance (i.e., tolerance not depending on drought escape via an early flowering) based on allelic variation at six independent loci. Genomic selection and MAS approaches, assessed on the basis of yield responses in a managed-stress experiment of different sets of independent lines (Figure 2), were able to produce remarkable genetic progress, resulting about as efficient as phenotypic selection in terms of yield gain per selection cycle. For white lupin, a world collection including 83 landraces and 8 French varieties was characterized by GBS. Genomic selection applied to allele frequencies of over 6500 SNP markers exhibited high predictive ability (as correlation between true and predicted phenotypes in a cross-validation procedure > 0.50) for grain yield in a sub-continental climate site and a Mediterranean site of Italy.

One flash presentation authored by M. Hybl and P. Smykal (Univ. Palackeho v Olomouci) (C8) reported on the development of chromosome segment substitution lines (CSSL) from the cross of a wild pea (*Pisum fulvum*) accession and a

cultivated pea variety. Some 145 lines were genotyped by genome-wide DarTseq technology. Fifty lines underwent extensive evaluation for morphological, agronomic and grain quality traits. The thorough phenotyping of this introgression will provide means for QTL and gene identification and subsequent incorporation into desired commercial genotypes.

A flash presentation by M. Książkiewicz summarized the work by Poznań's Institute of Plant Genetics and CREA on landrace genetic diversity and the construction of a new high-density consensus linkage map in white lupin based on GBS-generated markers (C9, C10). Landraces originating from several European, African and Near East regions displayed mainly a latitudinal pattern of variation along with outstanding variation among material from Near East. The linkage map was exploited to map onset of flowering, low alkaloid content of seeds, and resistance to anthracnose and to *Phomopsis* stem blight, obtaining markers that can be used for MAS. QTLs for these traits did not match those located in narrow-leaved lupin, despite the large sequence collinearity observed between white lupin and narrow-leaved lupin genomes (5).

The first talk issued by EUROLEGUME, presented by M. Egea-Cortines (Univ. Pol. Cartagena) (C11), investigated to which

extent cowpea's primary metabolism is controlled by the plant circadian clock. The main findings were that the cowpea circadian clock undergoes transcriptional reprogramming during the transition from early to late pod development and seed filling. Furthermore, while protein deposition during seed filling is a linear process, the expression pattern of the mRNAs coding for storage proteins is circadian. This gated expression may involve a better energy usage, and hints towards a circadian coordination of storage protein and grain filling (6). The phylogenetic tree of the clock gene *ELF3* related predicted proteins is shown in Figure 3.

A flash presentation by M. Carvalho (CITAB) (C4) reported on the characterization by the Illumina Cowpea iSelect Consortium Array (containing 51,128 SNPs) of 43 Iberian landraces and cultivars and 53 landraces collected worldwide. Most Iberian Peninsula accessions clustered together with those from other southern European and northern African countries. A lower genetic diversity level was found in the Iberian Peninsula accessions compared to worldwide cowpeas. This study brought some insights into worldwide genetic diversity and structure and possible dispersion routes of cultivated cowpea (3).



Figure 2. One of CREA's phenotypic platforms used for studies of pea drought tolerance. Photo by Paolo Annicchiarico

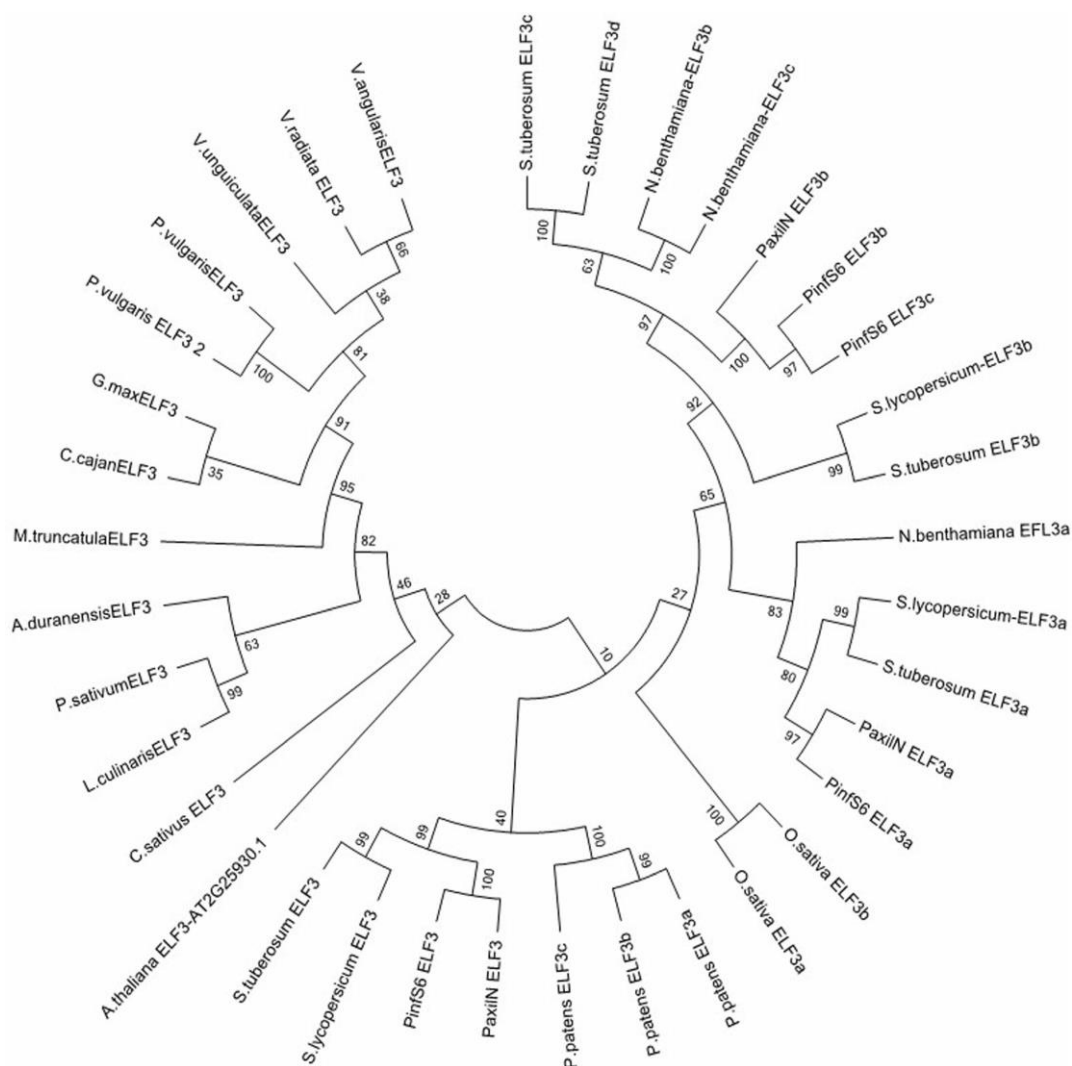


Figure 3. Phylogenetic tree of ELF3 related predicted proteins of legume species and other species. The analysis involved 36 amino acid sequences. Reprinted from Weiss et al. (6); original figure, of which BioMed Central is the original publisher

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- (C1) Martínez-Fernández I *et al.* (2017) Increased production of pods and seeds in pea through mutation in the MFN genes. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 34
- (C2) Casimiro-Soriguer I *et al.* (2017) Unravelling autofertility in faba bean (*Vicia faba* L.). Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 35
- (C3) Alves M *et al.* (2017) Grass pea association analysis of bioactive compounds for quality precision breeding tools development. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 22
- (C4) Pereira P *et al.* (2017) genetic diversity of a worldwide collection of *Lathyrus sativus* assessed by cross-species amplifiable microsatellite molecular markers. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 23
- (C5) Gutierrez N *et al.* (2017) Identifying positional candidate genes controlling low vicine-convicine in faba bean (*Vicia faba* L.). Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 30
- (C6) Kiss G *et al.* (2017) Generation and use in MAS of linked markers for low vicine-convicine (lvc) trait. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 20
- (C7) Annicchiarico P *et al.* (2017) Genomic selection for production traits in white lupin and pea. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 32
- (C8) Hybl W *et al.* (2017) Evaluation of *Pisum sativum* subsp. *elatius* and *Medicago truncatula* seed dormancy as adaptation to environment. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 31
- (C9) Ksiazkiewicz M *et al.* (2017) A new high-density consensus linkage map of white lupin. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 10
- (C10) Annicchiarico P *et al.* (2017) Genetic diversity in a world collection of white lupin. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 24
- (C11) Weiss J *et al.* (2017) Clock resetting during cowpea seed development. Implications on storage protein. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 11
- (C12) Carvalho M *et al.* (2017) Illumina Cowpea iSelect Consortium Array used to evaluate the genetic diversity and population structure of Iberian Peninsula cowpeas. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 21

Cowpea: a Warm Season Legume and its Genome

Timothy J. Close¹

Abstract: An annotated genome sequence of an elite African variety of cowpea (*Vigna unguiculata* L. Walp, IT97K-499-35) is now available. Synteny between cowpea and common bean (*Phaseolus vulgaris* L.) supports a new chromosome numbering convention for cowpea. Marker-trait associations have been identified using biparental and multiparent recombinant inbred line (RIL) populations, a minicore of diverse cowpea germplasm and a set of lines compiled from several West African breeding teams. QTLs and candidate genes for traits now include flowering time, pod shattering, seed coat patterns and texture, leaf shape, seed size, resistance to several pests and pathogens, and others.

Key words: Cowpea, *Vigna unguiculata*, genome sequence, synteny, QTL

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a diploid warm-season legume (tribe Phaseoleae) with an estimated genome size of 641 Mb. Among major crop legumes, cowpea is the most resilient to heat and sparse rainfall. Cowpea is presently the main source of protein in the human diet in sub-Saharan Africa, where it is grown for food and livestock forage, and it is a substantial crop in the USA, Asia and parts of Europe. A common cowpea food product in Africa is akara, which is prepared by making a paste from cowpea seeds, adding salt and spices, and then deep frying (Figure 1).

Cowpea seems to have originated in Southeastern Africa and been domesticated at least 3600 years ago, where it was present in early settlements of migrants to West Africa. Cowpea was also a crop of ancient Greece and Rome, and was introduced into South and Central America, the Caribbean Region and Southern USA by colonials and



Figure 1. Making akara. A paste is made from pulverized cowpea grains mixed with salt and some spices, then deep fried to make a protein-rich snack. Photo credit: TJ Close, along the road outside of Ibadan, Nigeria, August 2017.

slave traders. Cowpea seems to have been introduced into Northern Mexico by the Italian Jesuit missionary Eusebio Francisco Kino in 1685, spreading from there to California, where landraces typical of modern-day Iberian Peninsula cowpeas (2) took hold and persisted into the 20th century. Cowpea grows well in the Central Valley of California and in the hot summer and fall climates of other Southwestern USA regions, and in the US South. It was a major crop of the California Central Valley until the 1980s when it was displaced by urban development and more profitable, water-thirsty crops. Cowpea was also the most highly cultivated legume in the USA until the 1930s, when it was surpassed by soybean.

Breeding and Genetics

There are several active cowpea breeding programs in Africa, particularly West Africa, and a few in the USA, China and elsewhere, but genetic resources and tools that typically streamline and accelerate breeding in other annual crops have only recently begun to become very advanced for cowpea. Genotyping using single nucleotide polymorphisms (SNPs) using a commercially available fixed platform began with an assay of about 1500 SNPs, which was applied to several biparental recombinant inbred line (RIL) populations to develop a consensus genetic linkage map (7). Following from this, steady progress was made to genetically map

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several major trait determinants, guide parent and progeny selection, and in some cases support marker-assisted introgression of major genes into elite backgrounds (1). More recently, an assay of about 50,000 SNPs and a more detailed genetic linkage map were developed, along with additional genomic sequences from the African elite accession IT97K-499-35 (8). This higher-density SNP assay was applied to a collection of West African breeding accessions to reveal the level of diversity and relatedness between programs in Burkina Faso, Ghana, Nigeria and Senegal, and to guide the establishment of a standard set of “partner favorites” for future use in multilocation and multiyear field trials (8).

Even more recently, cowpea genome resources have been upgraded by the development of a first-class genome assembly (pseudomolecules) of IT97K-499-35. This genome assembly is based on single molecule real-time sequencing (91x coverage; Pacific Biosciences) together with two optical maps (BioNano Genomics) and ten genetic linkage maps containing a total of 44,003 SNPs. The v1.0 pseudomolecules contain 519 Mb of sequence, derived from superscaffold sequences with N50 = 16.4 Mb and L50 = 12. A total of 29,773 gene models were annotated using a combination of *ab initio* and transcript (RNA-Seq and Sanger EST) evidence (7, 9, 10), providing a measure of 95.9% plant completeness using BUSCO v2. The annotated genome was made publicly available July 31, 2017 through Phytozome (phytozome.jgi.doe.gov), where it can be browsed and downloaded. The Legume Information System (legumeinfo.org) also provides an interface with additional tools to browse this genome, especially in the context of comparisons between legumes. One benefit of the cowpea genome sequence is a view of synteny between cowpea and other legumes. For example, six of the eleven chromosomes (pseudomolecules) of cowpea are essentially completely collinear with common bean chromosomes, while the other five each are composed of two parts that are syntenic with two common bean chromosomes (Table 1). Since the common bean genome sequence has already been published, the cowpea chromosome numbering has been revised to optimally match common bean. The shared numbering system is a simple way to increase the synergy between research on cowpea, common bean and other legumes because it is expected that often a gene controlling a

trait in one Phaseoleae also has been coopted to control the same trait in another Phaseoleae.

Several genetic populations of cowpea now exist that are of general use to establish associations between traits and genetic markers, leading also to candidate genes. A population of 305 RILs derived from eight elite parents that carry variability for numerous traits was recently developed and used to map several major loci including days to flowering, photoperiod sensitivity, growth habit, seed size and days to maturity (5). A minicore composed of 384 diverse accessions has been used to map major loci for flowering under short days and other traits using genome-wide association (GWA), and to define six sub-populations, one of which includes accessions from California, Iberian Peninsula (2) and NE Africa (https://plan.core-apps.com/pag_2018/abstract/fff6e3855de940b33de03bc4eff0b62d). As noted above, another specialized population is the


“partner favorites”, about 170 genetically defined accessions that may be amenable to breeding by “genomic selection” to improve varieties for West Africa. In addition to opportunities specifically with these genetic populations, the new genome sequence and genotyping resources enable research synergy with sets of biparental RILs, near isogenic lines (NILs) and gene expression data (10) to improve the precision of marker-trait associations. Some recent examples include the narrowing of a root knot nematode resistance locus to a few TIR-NBS-LRR family resistance genes (9), and the identification of QTL and a list of candidate genes for domestication-related traits including pod-shattering, seed size and flowering time (6). Finally, genes controlling black seed coat and purple pod tip (Figure 2), which were some of the first traits ever to be studied genetically in cowpea or in any plant (3), have been narrowed to a cluster of genes encoding MYB transcription factors (4). 

Table 1. Synteny. Six cowpea chromosomes are collinear with a single common bean chromosome; five have two parts, each syntenic with a different common bean chromosome. Previous numbering from references 7 and 8.

Revised Cowpea LG	Common Bean LG	Previous Cowpea LG
Vu01	1,5	4
Vu02	2,3	7
Vu03	3,2	3
Vu04	4	11
Vu05	5,8	1
Vu06	6	6
Vu07	7	2
Vu08	8,1	5
Vu09	9	8
Vu10	10	10
Vu11	11	9

Summary Cowpea and its genome information constitute important resources that are amenable to further studies on the biology, history of domestication and development of new varieties of this strategically important food and nutritional security plant. An accumulating list of QTLs and candidate genes for traits provides opportunities to improve marker-trait associations, guide breeding and germplasm management operations, and develop mechanistic studies to precisely understand the basis of trait variation.

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Figure 2. Purple tips of young cowpea pods, one of the first traits studied genetically in cowpea (3). Photo credit: TJ Close, Coachella Valley Agricultural Research Station, Thermal, California, September 2016.

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Session 2 overview: Biotic stress resistances

Chaired by Diego Rubiales¹ and Graça Pereira²

Key words: pest, disease, ascochyta, broomrape, chocolate spot, fusarium wilt, powdery mildew, rust, aphid, weevil

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The session took place the morning of 27th September 2017 and consisted on two key presentations followed by four oral presentations. None of these presentations corresponded to specific posters or abstracts, as speakers were asked to compile comprehensively the information submitted by the different labs participating in the project, whose contributions were individually presented in a number of excellent posters, some of them given as flash presentations that can be visited in the program. Here we will focus on the content of such presentations given in session 2.

The first full length talk was presented by

D. Rubiales (CSIC), summarizing major results on phenotyping novel resistances to fungal diseases in legumes achieved by the various partners contributing to Task 2.1 of WP2 of LEGATO project (C4). Results presented outlined fruitful cooperation between CSIC, IFAPA, UNINA and/or ITQB on identification of resistances to rusts, powdery mildews, ascochyta blights, fusarium wilts and chocolate spot in faba bean, pea, lentil and grasspea, summarizing information provided by posters of Rubiales *et al.* (C16), Rispail *et al.* (C13), Castillejo *et al.* (C6), Barilli *et al.* (C3) and Santos *et al.* (C18) (Figure 1).



Figure 1. Symptoms of main diseases on susceptible (left) and resistant (right) accessions: (A) broomrape on faba bean; (B) broomrape on pea; (C) pea powdery mildew; (D) pea ascochyta blight; (E) faba bean rust; (F) pea rust; (G) pea fusarium wilt; (H) pea aphid; (I) pea weevil


The second full length talk was presented by S. Fondevilla (CSIC), showing the progress made by various partners (CSIC, GenXPro) in Task 2.2 of WP2 of LEGATO project. This summarized major achievements on identification of genes and proteins involved on resistance to *Didymella pinodes* in pea presented in posters Castillejo *et al.* (C5) and Fondevilla *et al.* (C8). By using a proteomic approach, Castillejo *et al.* (C5) suggested a number of peptides that could be used as markers for resistance. Fondevilla *et al.* (C8) used MACE technique to identify genes differentially expressed in resistance.

M. Seidenglanz (AGRITEC) issued a presentation of resistance to aphids and weevils in peas and faba beans, summarizing achievements resulting from screenings made during several years in Czech Republic and Spain by AGRITEC, CSIC and IFAPA. This talk nicely compiled information from Aznar-Fernández and Rubiales (C2) and Aznar-Fernández *et al.* (C1) identifying and

characterizing resistance in pea to aphid and weevil, respectively; of Magnin-Robert *et al.* (C12) describing little resistance to Sitona weevil in faba bean; of Seidenglanz *et al.* (C19) reporting screenings to bruchid weevil in faba bean; and of Sillero *et al.* (C20) reporting resistance to black aphid in faba bean (Figure 1).

J. Sillero (IFAPA) showed achievements on identification of resistance to broomrape in pea and faba bean performed by CSIC-IFAPA cooperation, presenting information provided by the poster of Rubiales *et al.* (C15) on detailed info on the various mechanisms of resistance including low induction of broomrape seed germination. This was complemented with information of the development and recent release of resistant cultivars (Figure 1). A. Torres (IFAPA) (C9) and C. Vaz Patto (ITQB) (C18) presented the results of IFAPA, ITQB and ULE on task 2.3.1 of LEGATO on orthologs of pea ascochyta resistance genes

in faba bean, grasspea and lentil, complemented with IFAPA own studies on identification of genes in faba bean, nicely compiling information provided in the poster of Gutiérrez *et al.* (C9).

C. Polanco (ULE) (C17) presented the outcomes of CSIC, IFAPA, ITQB and ULE on task 2.3.2 of LEGATO on the search of MLO homologous genes in legume crops. Loss-of-function mutations in one MLO protein causes an efficient papilla-based penetration resistance. This analysis revealed several evolutionary differences between tropical and temperate legume crops. Interestingly, legume genomes were shown to encode several clade IV and V MLO members that are potential candidates to confer resistance to powdery mildew in these species. This talk nicely compiled information provided in the posters of Rispaíl *et al.* (C14), Gutiérrez *et al.* (2017) (C9) and Sáenz de Miera *et al.* (C17). 

Conference Session Contributions:

(C1) Aznar-Fernandez T, Carrillo-Perdomo E, Rubiales D (2017) Identification and characterisation of resistance to *Bruchus pisorum* in *Pisum* germplasm. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 49

(C2) Aznar-Fernandez T, Rubiales D (2017) Antixenosis and antibiosis to pea aphid in *Pisum* spp. germplasm. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 48

(C3) Barilli E, Cobos MJ, Kilian A, Rubiales D (2017) Construction of a high-density integrated DArTseq SNP-based genetic map and identification of genomic regions controlling rust resistance in *Pisum fulvum*. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 44

(C4) Barilli E, González-Bernal MJ, Cimmino A, Masi M, Rubiales D, Evidente A (2017) Potential application of fungal and plant metabolites in rust and powdery mildew disease management Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 43

(C5) Castillejo MA, Fondevilla S, Rubiales D (2017) A targeted proteomic approach to identify protein markers for *Didymella pinodes* resistance in pea. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 51

(C6) Castillejo MA, Villegas-Fernández AM, Rubiales D (2017) Comparative analysis of proteome changes induced by *Botrytis fabae* in faba bean (*Vicia faba*). Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 42

(C7) Cimmino A, Aznar-Fernández T, Reveglia P, Evidente A, Rubiales D (2017) Identification of constitutive metabolites in pea responsible for antixenosis and antibiosis against *Bruchus pisorum*. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 54

(C8) Fondevilla S, Winter P, Rubiales D (2017) Identification of candidate genes for resistance to ascochyta blight in pea by combining transcriptomics and high-throughput mapping of differentially expressed genes. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 58

Conference Session Contributions (cont.):

(C9) Gutiérrez N, Fernández MD, Torres AM (2017) Search for resistance gene orthologs in *Vicia faba*. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 52

(C10) Koblai S (2017) Influence of climatic conditions on the adaptive capacity of different morphotype cultivars of peas. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 47

(C11) Lesne A, Boutet G, Cartelier K, Desgroux A, Pilet-Nayel ML, Baranger A (2017) Identification and validation of positional candidate genes for partial resistance to *Didymella pinodes* and *Aphanomyces euteiches* in pea. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 45

(C12) Magnin-Robert JB, Deulvot C, Duc G, Marget P (2017) Screening a *Vicia faba* L. collection for *Sitona lineatus* resistance. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 59

(C13) Rispaïl N, Bani M, Cimmino A, Pérez-de-Luque A, Evidente A, Rubiales D (2017) Characterization of pea defense mechanisms against *Fusarium oxysporum*. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 41

(C14) Rispaïl N, Rubiales D (2017) Identification and characterization of the MLO family in legume genomes. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 50

(C15) Rubiales D, Fernández-Aparicio M, Villegas-Fernández AM, Sillero JC (2017) Mechanism of resistance in faba bean (*Vicia faba*) against broomrape species. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 56

(C16) Rubiales D, Fondevilla S, Fernández-Aparicio M, Villegas-Fernández AM, Barilli E, Sillero JC (2017) Prospects for durability of available resistances to fungal diseases and parasitic weeds in pea and faba bean. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 40

(C17) Sáenz de Miera LE, Pérez de la Vega M, Polanco C (2017) Characterization and expression analysis of MLO gene family members in cultivated and wild species of lentil. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 57

(C18) Santos C, Almeida NF, Alves ML, Rubiales D, Vaz Pato MC (2017) QTLs and eQTLs detection for rust resistance in *Lathyrus cicer*. Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 53

(C19) Seidenglanz M, Huňady I, Sillero JC (2017) Testing of *Vicia faba* accessions on resistance to bruchids (*Bruchus rufimanus*). Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 55

(C20) Sillero JC, Avila CM, Rubiales D (2017) Screening faba bean (*Vicia faba*) for resistance to aphids (*Aphis fabae*). Book of abstracts of international conference “Advances in grain legume breeding, cultivation and uses for a more competitive value chain” Novi Sad, 27-28 September 2017, pp. 46

Session 3a overview: Enhancing legume quality – novel food

Chaired by M. Carlota Vaz Patto¹ and Ruta Galoburda²

Key words: novel food formulations, processing approaches, phenolic diversity, spectroscopic selection tools

The first part of Session 3 from the LEGATO-EUROLEGUME International Conference was focused on “Enhancing legume quality” from a food perspective. Besides the invited talk concerning food ingredients that might be obtained from legumes by S. Mittermaier (see Mittermaier & Eisner (C1), for more detailed information), there were three full-length presentations on legume based food formulations covering bread (Figure 1), cheese and snacks or spreads (Figure 2), and two other full-length presentations on the quality characterization of different legume

species germplasm and development of high-throughput quality selection tools. Additional to these were two flash-presentations dedicated to different legume processing approaches.

The first full length talk issued by LEGATO, presented by C. Brites (INIAV) (C2) and resulting from a collaboration between INIAV, the University of Porto and the SMEs Patrimvs and Sense Test, was dedicated to the formulation improvement of a maize bread fortification with legume flours based on consumer preferences. Maize bread legume fortification aimed to increase the total protein content of a traditional cereal bread (“broa”). Flour of several maize varieties (white, yellow, regional or industrial) were blended with pea, chickpea, faba bean and lentil flour through different formulations, each consisting of a different legume species. Based on the overall liking evaluated by a panel of 60 consumers (naïve tasters), significant differences were identified between the different maize flours,

but no significant effect was found for the legume flour incorporated. This multidisciplinary approach, with the consumer engagement, created an array of fortified “broa” formulations, qualifying for a high protein content claim and well accepted by consumers.

M. Bronze and A. Bento da Silva (ITQB NOVA) (C3) issued the second full length talk by LEGATO, resulting from a collaboration between ITQB NOVA, iBET, and FFULisboa. This presentation focused on the characterization and comparison of five of the most important European food legume species (pea, faba bean, chickpea, lentil, and grass pea) in what concerns their phenolic content and antioxidant capacity, with the subsequent development of a high throughput prediction spectroscopic tool for grass pea, as a case study. The total soluble-free phenolic content and the oxygen radical absorbance capacity, as a measure of the antioxidant activity, was determined in a collection of around 100 samples of each of

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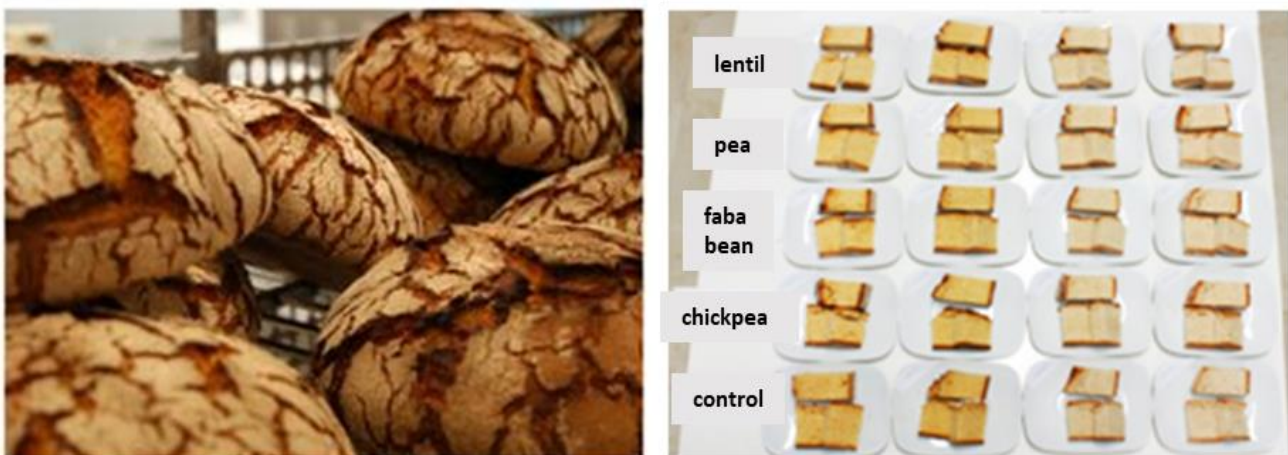


Figure 1. Fortified maize bread with different legume flours, uncut bread and slices (lentil, pea, faba bean chickpea vs. control not fortified) developed within the LEGATO project. Photo by Carla Brites.

the five legume species, with a total of 509 accessions. The objectives were to quantify the existing variability and to identify outstanding accessions as sources of the adequate health beneficial compounds contents for breeding programs and genetic studies. Both traits were highly correlated and significant variability was found among and also within the five legume species. At species level, faba beans showed the highest total phenolic content and the highest antioxidant activity, followed by lentils. Peas, grass peas, and chickpeas presented similar phenolic contents and antioxidant activity values. The variability detected also within these species provide good opportunities for quality improvement through conventional legume breeding. However to facilitate the routinely implementation of these otherwise time consuming evaluations in the quality breeding programs, rapid, cost efficient analytical methods are still needed. Using only the grass pea accessions as a case study, Fourier Transform InfraRed - Attenuated Total Reflectance (FTIR-ATR) spectra of seed flour were collected and used to predict grass pea phenolic content and antioxidant activity (C4). A multivariate analysis was performed to compare all the samples and to develop a prediction model that may be used to get rough estimates for phenolic content and antioxidant capacities in quality screenings of this legume species.

The third full length talk by S. Muizniece-Brasava (LLU) (C5) from EUROLEGUME presented new legume based products which are alternative to traditional animal-derived products, having all nutritious components of legumes: quality protein, complex carbohydrates, dietary fiber, minerals, and vitamins. After screening of available pulses, dry seeds of maple peas (*Pisum sativum* var. *arvense* L. cv. Bruno), faba beans (cv. Barteks), and cowpeas (*Vigna unguiculata* (L.) Walp. cv. Fradel) were selected for further studies. Three types of new products were developed at the Latvia University of Agriculture – extruded snacks; pulse spreads; and protein-rich snack bars. Evaluation of physical, chemical and sensory qualities allowed selection of the best products for further studies. Various packaging materials and technologies allowed selection of the most suitable for extending the shelf life of non-flavoured extruded snacks up to 24 months, but for flavoured products, it should not exceed 12 months. Pulse spreads proved to be highly perishable products, therefore sous vide and high pressure

processing were applied to provide microbiological quality of pulse spreads during 62-day refrigerated storage.

F. Fogelberg (RISE) (C6) described a faba bean based blue mould cheese – an alternative to products based on soya beans. It aims to satisfy the needs of vegans and vegetarians. In EUROLEGUME, the Swedish partners from RISE have been able to develop a “cheese” bases entirely on faba beans with a creamy texture and taste of blue mould culture. The basic production steps include bean soaking and milling, obtaining a milky slurry. In further step, milk is separated from the slurry and boiled. The addition of curdling agents and mould culture results in curd, which then is pressed and matured. Researchers look forward the commercialization step.

The next full-length talk of I. Karapanos (AUA) (C7) covered research completed by collaboration of three EUROLEGUME partners – Agricultural University of Athens, Universidad Politecnica de Cartagena, and University of Tras-os-Montes and Alto Douro. The evaluation of quality and dietary characteristics of 37 cowpea landraces grown in southern Europe (Greece, Spain, and Portugal) revealed that fresh pods could be introduced to the market and serve as an alternative legume vegetable. Pods from the *sesquipedalis* subspecies were heavier and larger, and reached commercial maturity two days later than those from the *unguiculata* subspecies.

One of flash talks of the session by EUROLEGUME focused on the development and characterization of new foods in order to promote legume

consumption. P. Gomez (IBV) (C8) addressed quality changes of fresh and then microwaved minimally processed faba seeds. Seeds treated with UV-C maintained their sensory attributes above the limit of acceptability after 10 days, while those treated with NaOCl were acceptable until day 7. The antimicrobial effect of sanitizers after application is very important, but the maintenance of their antimicrobial effect during storage is also essential. In this study, aerobic microflora, psychrophiles, moulds and yeasts were below 3 log CFU/g at the end of cold storage, independently of the sanitizer used. That microbial load can be considered as low and safe for human consumption. However, more research is needed to study the effect of these treatments on other quality parameters.


The last flash talk of the session given by L. Strauta (LLU) (C9) focused on the nutritional value of legume based extruded products, indicating that legume cooking process can be rather long and time consuming. Therefore, the extrusion-cooking was applied as the possible type of treatment to obtain ready to eat legume products. Extrusion processing gelatinizes the starch and partially eliminates anti-nutritional factors that are present in many legumes. Flour obtained from peas, faba beans and cowpeas were extruded through the twin-screw extruder. The extruded snack base made from grey peas contains 26.73 g protein and 0.66 g fat per 100 grams of dry weight, on average. The energy value of 100 grams of extruded snacks didn't exceed 300 kcal. 

Figure 2. Different legume based innovative food formulations covering faba bean cheese (in the back of the photo), pea pesto and spreads (in the middle) or extruded snacks (in the front) developed within EUROLEGUME project. Photo by Ruta Galoburda during the LEGATO-EUROLEGUME International Conference at Novi Sad, where the new products were presented for tasting.



Conference Session Contributions:

(C1) Mittermaier S, Eisner P (2017) Food ingredients from lupins. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 6

(C2) Cunha LM, Fonseca SC, Lima RC, Loureiro J, Pinto AS, Brites C (2017) Improvement of maize bread formulation and fortification with legumes based on consumer liking. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 71

(C3) Bento da Silva A, Brito E, Pereira AB, Cardoso C, Mecha E, Vaz Patto MC, Bronze MR (2017) Characterization of phenolic content and antioxidant activity of legume accessions from five different species. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 79

(C4) Silva A, Pereira A, Brito E, Ferreira A, Silva S, Patto M, Bronze M (2017) Prediction of grass pea (*Lathyrus sativus*) phenolic composition and antioxidant activity by FTIR-ATR. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 80

(C5) Muižniece-Brasava S, Kirse A, Strauta L, Galoburda R, Straumite E, Puiskina E (2017) Innovative legumes based food products. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 78

(C6) Fogelberg F (2017) Bean Blue – a faba bean based blue mould cheese. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 65

(C7) Karapanos I, Papandreou A, Skouloudi M, Makrogianni D, Fernandez JA, Rosa E, Ntatsi G, Bebeli P, Savvas D (2017) Cowpea fresh pods, a new legume for market. Assessing the quality and dietary characteristics of 37 cowpea landraces grown in southern Europe. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 63

(C8) Collado E, Venzke Klug T, Artes-Hernandez F, Aguayo E, Martinez-Sanchez A, Artes F, Fernandez J, Gomez PA (2017) Quality changes of fresh and then microwaved minimally processed faba seeds. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 68

(C9) Strauta L, Muižniece-Brasava S (2017) The nutritional value of legume based extruded products. Book of Abstracts Legato-Eurolegume International Conference Advances in grain legume breeding, cultivation and uses for a more competitive value-chain, Novi Sad, Serbia, 27-28 September 2017, pp. 82

Session 3b overview: Enhancing legume quality – novel feed

Chaired by Miguel Rodrigues¹

Abstract: The potential utilization of legumes in animal feeding has been confirmed with results showing that cowpea stover can be ensiled together with other accessible agro-industry wastes and used as ruminant feed. It was also found that its treatment with white-rot fungi can enhance its nutritive value leading to high levels of incorporation in commercial rabbits' feeds with positive effects in animal performance. The supply of pea straw as a replacement of barley straw to dairy goats can successfully be achieved with an improvement in the milk fatty acid composition. Pea grains can be used as ingredients in broiler diets at levels of 20%, and peas and beans can also be included in dairy cow diets up to 12%, reducing the inclusion of soybeans and thus, enhancing the sustainability and competitiveness of the agro-food sector. Pea and field beans can also be used in ruminant nutrition as long as mild treatments are applied during grain processing in the animal feed industries with clear advantages in terms of protein digestibility.

Key words: Legume grains, nutritional valorization, residues, animal feeds

Global demand of food sources has registered a constant increase and are due to meet the use of less available natural resources. This scenario has led to the implementation of policies that enhance the production of legumes for human food within European agriculture. These products have high nutritive value and have been given increased relevance based on its adaptability to different environmental conditions. Thus, it is expected a global intensification of its production. Besides the

agronomic, environmental and economic advantages of including legumes in cropping systems, the production of grains can also be used and valorized as animal feeds. This potential was evaluated by the EUROLEGUME project aiming to develop nutritional added value feeds.

The amount of residues resulting from legume grain production may cause serious environmental problems due to its disposal without any type of treatment. Therefore, its utilization as animal feeds might be foreseen has a possible approach. For this purpose cowpea stovers were collected to evaluate the possibility of using them through two different methodologies. The first one involved its ensiling with discarded apple and the second one aimed to upgrade its nutritive value by solid state fermentation using white-rot fungi. Through ensiling, it was possible to clearly outline the most promising mixture of substrates being selected the mixture containing 15% and 85% of cowpea stover and discarded apples in a fresh weight basis, respectively (1). White-rot fungi treatments showed that *Pleurotus citrinopileatus* strain was the most effective in promoting higher delignification (46%), higher increase in the digestibility values of the treated cowpea stover (30%) and a net crude protein increase of 13% (2). This strain was selected to treat sufficient amount of cowpea stover in order to allow its inclusion, in a commercial rabbit growth compound feed, at 10% level. No differences were observed for the rabbits' performance (daily weight gain, daily feed intake and feed conversion rate) when comparing feed containing treated cowpea stover and a normal commercial diet. Furthermore, no differences were obtained for meat quality, carcass traits characteristics, digestive tract histology, as well as for blood haematology and serum biochemistry. Pea straw was also evaluated as a potential raw matter in dairy goat's diets and results point out that it could

replace barley straw without modifying gross composition and hygienic quality of the milk (C1).

Legume grains are considered to be an alternative vegetable protein resource in animal nutrition, due to the lower crude protein contents when compared to soybean. Nevertheless, pea grains inclusion in dairy goat's diets (C1) was able to increase Omega 3 fatty acids percentage. Moreover, short and medium chain fatty acids tended to decrease ($P < 0.06$) and monounsaturated ($P < 0.09$) and Cis fatty acids to increase ($P < 0.07$), presenting clear advantages for the nutritional health of consumers. Furthermore, the economic assessment of the inclusion of peas and fava beans as ingredients in dairy cow and broilers diets has also shown its potential in decreasing the levels of soybean incorporation. Results indicate that the feed cost per kg milk produced for dairy cows (C2) decreased between 2.3-4.5% for inclusion levels of 10-12% of peas and fava beans. In addition, dairy cows diets containing up to 24% inclusion of var. 'Lielplatones' fava bean could increase milk yield (C2). This gives an opportunity to decrease the amount of imported soya in the diet, as well as to lower feed costs thus increasing the income for farmers. For broilers, when peas were included up to 20% in the diets its live weight and the production efficiency factor increased by 5.5 and 5.2%, respectively (C2).

The inclusion of legume grains in animal feeds could be further enhanced if its digestibility could be improved. In the EUROLEGUME project feed value of field peas and field beans for ruminants through processing was also evaluated. The processes examined were steam pelleting, expander pelleting, extruder processing and roasting. The effect of the various processing methods was examined through analyses of main nutrients (protein, starch, neutral detergent fibre, ash and fat), analyses of

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amino acids and rumen nylon bag incubations (*in sacco*) measuring rumen digestion of protein and starch. The conclusion is that nutritive value of field peas and field beans for ruminants can be improved by processing, but the effect is depending on processing conditions (C3). Processing at mild conditions increased rumen digestion of protein. By the contrary, processing at higher intensity and higher

temperatures reduced rumen digestion of protein. With respect to starch, heat processing increased rumen digestion. Thus, since there is a positive correlation between rumen digestion and small intestine digestion of starch in ruminants, heat processing can be used to improve energy value of field beans and field peas for ruminants. Roasting at high temperatures in combination with extended treatment time increased the


concentrations of fibre. In addition, the concentrations of total amino acids and in particular lysine were reduced. This indicates a possible overtreatment of protein and care should be taken when roasting. No indications for overtreatment of protein were observed for steam pelleting, expander pelleting or extruder processing. 



Figure 1. Grazing on cowpea

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Grains and Claims: Consumer Perceptions and Intention to Buy

Moira Dean¹

Abstract: This talk presents an overview of the research on health claims, including consumers' perceptions of such claims and their intention to buy products that carry health-related claims. Results show that adding health claims to products does increase their perceived healthiness. Claim structure impacts on perceptions, but its influence depends on the level of relevance, familiarity and individuals' need for information. Further, the type of health benefit proposed and the base product used affect perceptions of healthiness. I will conclude outlining factors that should be considered in public nutrition communications relating to health claims.

Key words: Health claims, perceptions, intention to buy

Introduction

Different forms of nutrition communication include campaigns, leaflets/booklets as well as labels on food products. The nutrition information communicated on the food label usually takes three forms: the back of pack information, the front of pack label, and nutrition/health claims.

Nutrition claims imply that a food has particular beneficial nutritional properties due to the nutrients that it does, or does not, contain e.g. 'low in fat', 'high in fibre' or 'no added sugar'. In contrast, health claims propose that if a particular product is eaten it will improve a specific physiological function or reduce the risk of a certain disease. A health claim can be made up of three components: an active ingredient, an effective function and a health benefit. For

example, 'contains bioactive peptides (ingredient) that help to maintain normal blood pressure (function) which reduces risk of heart disease (benefit)' is a health claim that contains all three components. However, a health claim can address only one or a combination of any of the three parts (2).

Health claims convey a positive health message to consumers, highlighting which products should be consumed, so that they may positively enhance their bodily functions and reduce the risk of diseases. While some effects of consumption of products with claims such as 'lowering blood cholesterol level' can be easily measured, others such as 'improving immune defence' are difficult to demonstrate, because for many of the claimed functions there are no biomarkers that could be used as indicators to verify the promised effects. Thus, in order to gauge what information health claims are communicating to the public, it is important to investigate the public's perceptions of health claims and how the public intend to use the claims when they make purchase decisions (2).

Consumer perceptions of health claims

Studies have measured perceptions of health claims in different ways, which include perceptions of the healthiness of the product, the benefits gained, the credibility of the claim and understanding of the claim. People's intentions to buy products with health claims have been obtained by asking consumers to state their willingness to buy these products.

A review of the studies (see Dean *et al.* (2) for details) shows that the base product used, together with the ingredient added and the way the food is produced, affects consumer perceptions of products with health claims.

However, these perceptions may be country specific and depend on how familiar the claims are to the public. Further, studies suggest that consumers prefer health claims that address physiological issues, especially in the area of cardiovascular risks. In terms of claim architecture, there is no evidence for clear consumer preference. As different studies used multiple designs and addressed different research questions, the results are difficult to compare and sometimes contradictory (6).

Intention to pay for products with health claims

The effect of health claims on intention to buy is generally lower than perceptions of healthiness (2). Even when people perceived the product with a health claim to be healthy, this did not automatically lead to an intention to buy the product. The reward people thought they would get for using products with health claims, how necessary they thought these products were, and factors such as the brand, price and the perceived attractiveness of the product are the best predictors of the consumption of products with health claims (10).

Who is influenced by health claims?

In general, women are more positive than men about products with health claims. However, as the relevance of the health benefit is gender related, perceptions may be product specific, and so need to be assessed on a product-by-product basis (2).

Use of products with health claims was found to be higher among the older age groups; even when different age groups perceived the products to be equally healthy, the older age group are more willing to buy

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the product compared to the younger group. However, not all studies found this to be the case (9). Further, consumer perceptions of claim vary from country to country and are argued to be linked with familiarity of the claims (8). In addition, a person's own belief in their self-efficacy and their belief in the product's effectiveness are strong motivators for intending to choose products with health claims (1). Personal relevance is also shown to affect perceptions and willingness to buy (2).

Nordic and Healthgrain study results

Results from a Nordic study (6) and Grunert *et al.* (4) which investigated health claim perceptions in Denmark, Finland, Iceland, Norway and Sweden and the Healthgrain study (7) which investigated the impact of personal factors on consumers' perceptions of healthiness and likelihood of buying cereal-based products in Finland, Germany, Italy and the UK, have added to our understanding of health claim communication.

These studies show (2) that the familiarity of the ingredient added to the carrier interact with the claim architecture to affect public perception. When the ingredient is a familiar one, then, in general, people prefer shorter claims, although there may be individual

differences. However, when the added ingredient is not known, then a full claim is preferred. It could be that until the product becomes familiar, people like to see a full claim that details the ingredient, its function and the proposed health benefit.


In addition, these studies (2) showed that people's preferences for different claim architecture and whether they prefer a positive or negative frame depend on the type of benefit proposed. This may explain some of the contradictory results of earlier research.

Personal relevance and people's perceived need for information affect perceptions of health claims with different architecture (2). The link between people's reaction to different claim architecture and their need for information (rather than their processing capacity) was demonstrated in the Healthgrain study (7). This shows the influence of different personal characteristics on perceptions related to different types of health claims.

Finally, people's intention to buy food with health claims is not totally dependent on their perceptions of healthiness of the products. Unless the health claim is related to a disease risk that is personally relevant, people are influenced by factors such as the taste, attractiveness and the method of production of the product. This evidence adds to previous work that showed that brand, price, credibility and uniqueness also

affect willingness to buy (2).

Lessons for legumes

Studies have shown that adding health claims to products does increase their perceived healthiness. Thus, it could be argued that health claims are communicating nutrition information to the public and can be used as a way of communicating the benefits of legumes. Further, although claim structure does make a difference to perceptions its influence depends on the level of relevance, familiarity and individuals' need for information. This needs to be considered when claims are added to legume products. The type of health benefit proposed and the base product (type of legume) used will also affect perceptions of healthiness. In addition, healthiness perceptions relating to products with health claims may vary between men and women, older and younger and between countries. However, the main factor influencing perceived healthiness and intention to buy a product with a health claim is personal relevance and this needs to be considered when promoting legumes. 

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Session 4 overview: Defining ideotypes, root phenotyping and microbial interactions

Unraveling the hidden half of legumes: root phenotyping and microbial interactions

Chaired by Boris Rewald¹ and Christophe Salon²

Abstract: Improving plant resource acquisition is decisive to counter suboptimal growing conditions and towards sustainable agriculture. Key levers are interactions between plant roots and soil biota, including symbionts. To assess why certain legume varieties are tolerant or resilient to biotic and abiotic single or multiple stresses, and to better understand their yield potential, additional information on root phenotypes and desirable root characteristics, taking interactions with symbionts into account, is pivotal. Consequently, several of the oral presentation within this session (studies conducted in both LEGATO and EUROLEGUME projects) addressed recent knowledge gains on legume root systems, and the diversity of rhizobia and mycorrhizal strains and their influence on legume performance.

Key words: Arbuscular mycorrhizal fungi, Rhizobia, Root phenotyping

Improving plant nutrient and water acquisition is a key issue in the occurrence of abiotic and biotic stresses and towards sustainable agriculture. Key levers are interactions between plant roots and soil biota, including microbial symbionts. To assess why certain varieties are tolerant or resilient to biotic and abiotic single or multiple stresses, an ongoing challenge for plant breeding is the limited ability to phenotype and select for desirable root characteristics – being the “hidden half”. For most crops, and among them legumes, both the technical difficulties to visualize the roots, their high diversity among species and

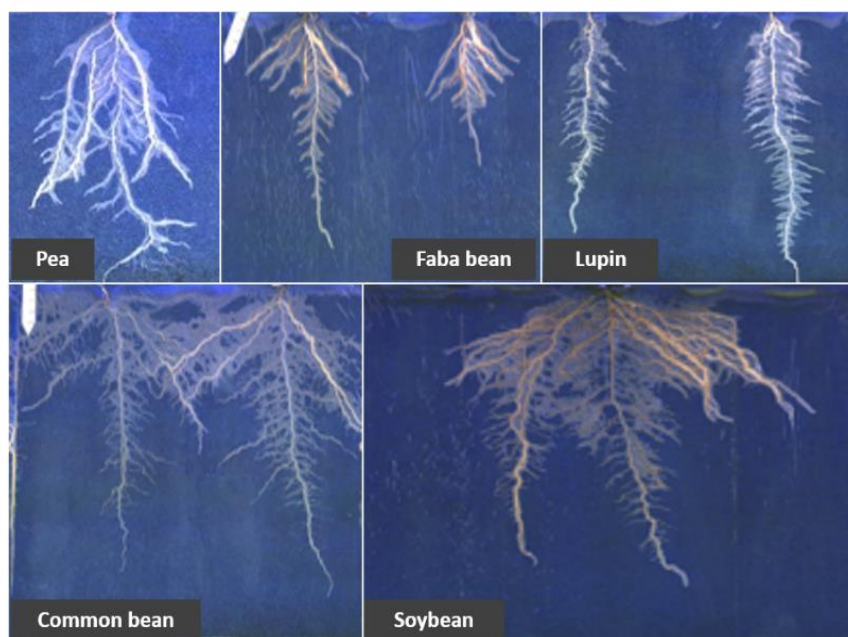


Figure 1. Contrasting root phenotypes of legume seedlings (Pea, Faba bean, Lupin, Common bean and Soybean) in growing pouches. © Christoph Salon

their plasticity, modulated by the environment, explains why information on root phenotypes is especially scarce (Figure 1). Until recently, phenotyping efforts were focused on shoot traits. New knowledge concerning the mechanisms involved in both root development and growth, up to now neglected, will certainly feed breeding programs and can result in novel, more stress-tolerant crops and increased yield by enhancing the capacity of the plant for soil exploration and, thus, effective water and nutrient acquisition. To precise the determinants of legume sensitivity to stress, characterizing plant phenotypes both structurally and functionally is an important goal. Various methods and platforms have been developed (e.g. Jeudy *et al.* (4)) towards the aim to image dynamically and

non-destructively “hidden” plant compartments in various substrates, from natural field soil to hydroponics, using different enclosures and imaging methods (3).

As outlined by Bodner *et al.* (9) the inference from seedling root phenotypes to mature root systems is challenging. However, as high throughput methodologies are applicable on seedlings, methodologies are needed to predict the architecture of mature root systems from this data. In his presentation, Bodner *et al.* (9) suggested to use the root system architecture (RSA) model “RootBox” to predict mature root traits and overcome the inference problem. Among the 16 studied pea genotype, seedling plant tap and lateral root elongation rates, (scaled) interbranch distances, and time

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of flowering were sufficient model parameters to predict genotype ranking among mature plants. The authors thus presented convincing evidence that RSA models should be included in the phenotyping pipeline to provide reliable information on mature root systems to breeding research (8).


Under low soil mineral nitrogen (N) availability, legumes root systems form nodules in symbiosis with N₂-fixing rhizobia and, in addition, symbioses with arbuscular mycorrhizal fungi (AMF). It is currently not well understood how plants integrates internal, environmental and soil microbial signals to optimize the formation of lateral roots and nodules on the whole root system (2), posing a significant challenge for legume root system phenotyping. While soil mineral N nutrition and atmospheric N₂ symbiotic fixation of legumes complement for their optimal N nutrition, these two regimes are antagonists for C utilization (7) which can impact, as outlined by Metzner *et al.* (C4) legume performance and yield. These authors thus reported on two non-invasive techniques to monitor C partitioning *in situ*:

i) Positron Emission Tomography (PET)

with the short-lived radiotracer ¹¹C to monitor the partitioning of photo-assimilates and its dynamics among roots and nodules directly at the scale of hours, and ii) Magnetic Resonance Imaging (MRI) to study the structural development of roots and nodules in pots over the course of several weeks (Figure 2) (1). The potential of these advanced techniques for functional, quantitative, high throughput and non-invasive phenotyping of legume roots and nodules (under stress conditions) was discussed.

The overall benefits of Rhizobia and AMF symbioses on yield and its components (under suboptimal conditions) are generally well established. However, current research in the field is focusing on the host specificity, efficiency and competitiveness of different strains. Consequently, several of the oral presentations within this session, in link with works conducted in both the LEGATO and EUROLEGUME projects, addressed recent knowledge gains on specific rhizobia and mycorrhizal strains on legume performance. Lepse *et al.* (C3) showed that Latvian faba bean cultivars responses varied with different associated rhizobia strains. However, while their data evidenced significantly increased yield by (some) rhizobial inoculation,

concurrent AMF inoculation only had limited effects. No influence of either inoculate type on faba bean protein content was found. Similarly, Šenberga *et al.* (C6) outlined that the efficiency of Rhizobia and AMF inoculants for pea and faba bean production in Latvia varied not only with plant developmental stage and cultivar but also with environmental factors such as soil type, temperature and rainfall. Both Lepse (C3) and Šenberga (C6) concluded that, due to the determined host specificity of Rhizobia strains, mixtures of inoculants might be most efficient to improve yield across a broader spectrum of genotypes and environmental conditions in Latvia. In accordance, the presentations of Pereira *et al.* (C5) and Tampakaki *et al.* (C7) outlined that the efficiency of rhizobial strains concerning improving cowpea yield can be highly variable in different geographic regions in Portugal and Greece, respectively. They also found that the phylogenetic multilocus sequence analysis (MLSA) based on the newly described marker genes *SMt00019*, *truA*, and *thrA* was superior compared to the conventional ones (16S rDNA, *recA*, *glnII*, *atpD*, etc.). Moreover, Tampakaki *et al.* demonstrated that the cowpea-nodulating rhizobia in Greece were slow- and fast-growing rhizobia depending on the soil type (i.e. alkaline, acid or neutral soils) and may constitute putative novel species and symbiovars within the genera *Bradyrhizobium* and *Ensifer* (Figure 3) (5, 6). Similarly, using rhizobox system to phenotype root and nodule structures and function, Heulin-Gotty *et al.* (C2) addressed the benefits of Rhizobia for drought resistance of pea and its variation with the nodulating *Rhizobium* strain. The authors used the developed experimental system to screen for *Rhizobium* strains able to promote rapid compensatory responses to water stress.

In summary, the interventions and discussions highlighted the crucial need of accurate tools and methods (phenotyping, models, molecular) for assessing the determinants of legume performance under abiotic stress, significantly driven by root systems and their interactions with soil symbiotic microbes. 

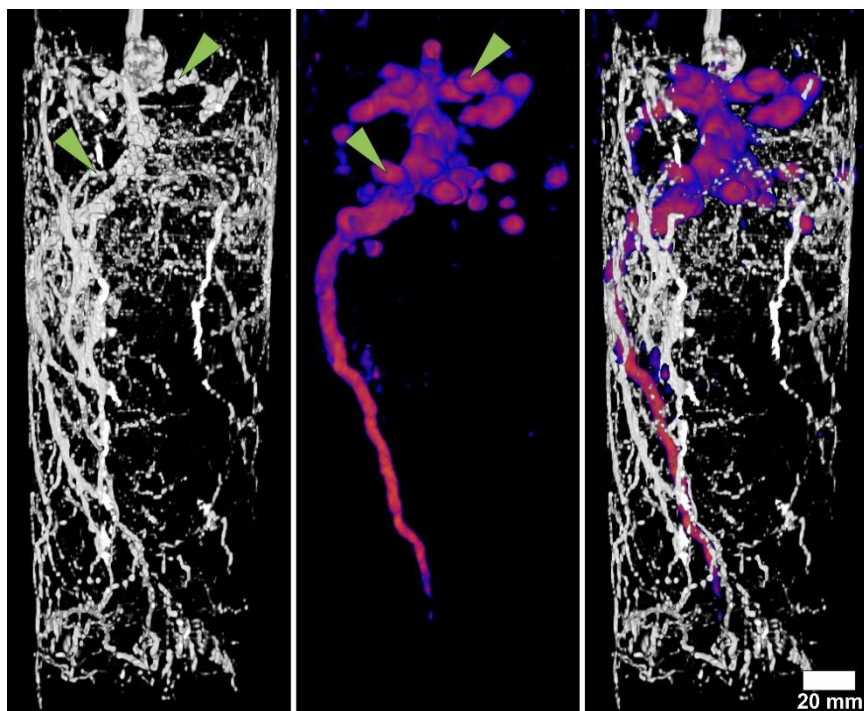


Figure 2. Root system of a 29 days old, nodulated pea plant (greyscale) growing in soil visualized non-invasively by Magnetic resonance imaging (MRI) and allocation of short lived (¹¹C) carbon tracer applied to the shoot as CO₂ (false color) visualized by Positron Emission Tomography (PET). Arrowheads highlight the same nodules as imaged by both modalities. © Ralf Metzner (C4)

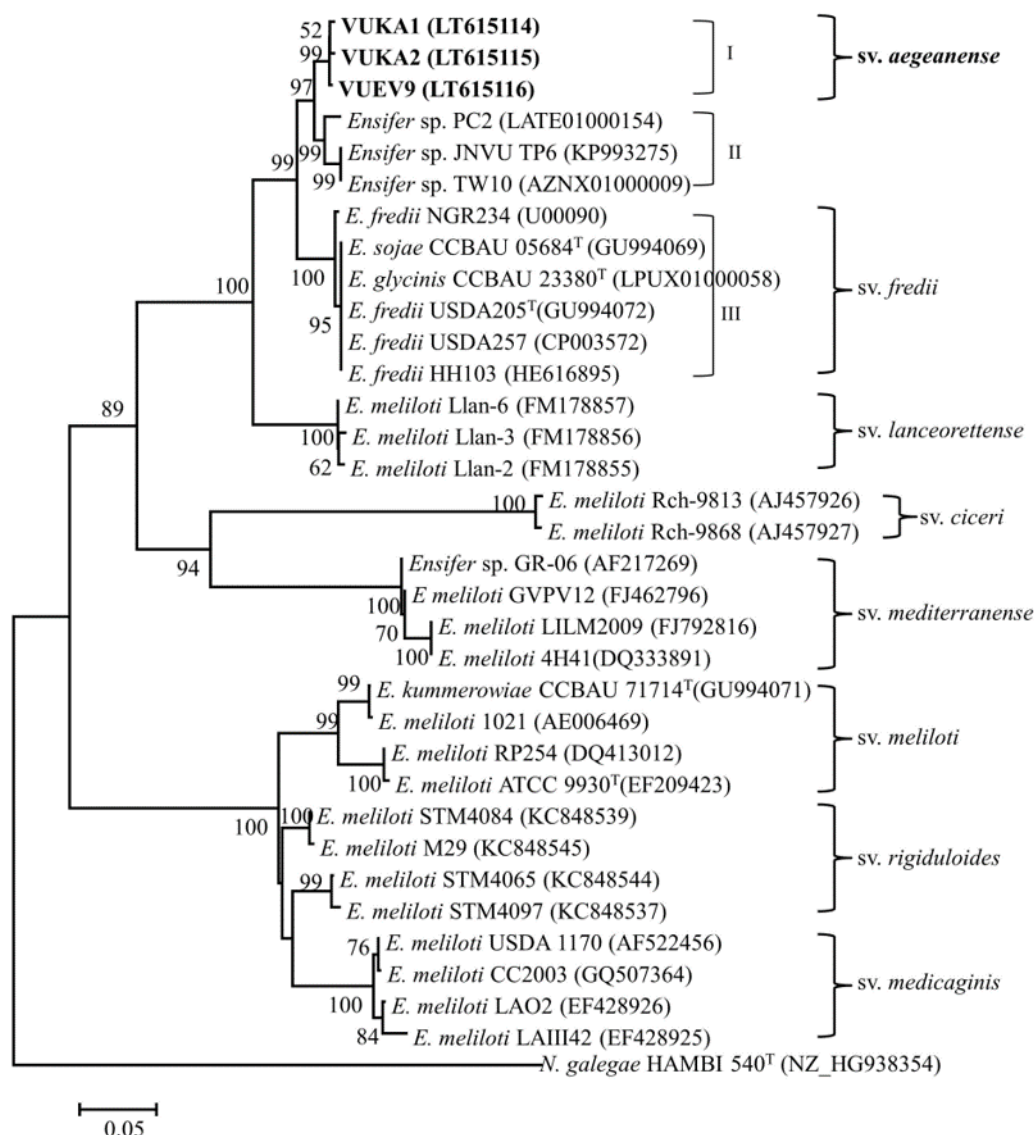


Figure 3. Rhizobial isolates, *VUKA1*, *VUKA2* and *VUEV9*, constitute a novel symbiovar within *Ensifer* for which the name '*aegeanense*' was proposed. Maximum likelihood phylogenetic tree based on *nodC* nucleotide sequences of *Ensifer* isolates. Strains isolated in Tampakaki *et al.* (5) are shown in bold and type strains are indicated by superscript "T". GenBank accession numbers of the sequences are indicated within parentheses. Bootstrap values (greater than 50%) were calculated for 1000 replications and are shown at the nodes. The scale bar shows the number of nucleotide substitutions per site. Phylogenetic analysis was conducted in MEGA 6 using the Tamura 3-parameter model plus Gamma rate distribution (T92+G). Figure adapted from Tampakaki *et al.* (5).

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Session 5 overview: Legumes in cropping systems – advantages and perspectives

Chaired by Liga Lepse¹ and Erik Steen Jensen²

Abstract: Legume re-integration in cropping systems and crop rotations has increased during recent years. Intercropping is found as one solution to introduce legumes in cropping systems. It was found that a pea variety mixture can combine the beneficial traits of the single varieties with maintained pea grain yield relative to the highest-yielding variety. It was also found that the use of legumes in intercropping with strawberries, needs further research to determine moisture influence on crop performance. Crop rotation trials showed that the soil NO₃-N concentration was increased by pea or faba bean as preceding crop in comparison to cabbage.

Key words: intercropping, variety mixtures, fababean, pea, crop rotation, sustainability assessment

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During recent years legume reintegration in cropping systems and crop rotations have increased in comparison to several decades ago. This is caused by the fact that European agriculture needs to meet both environmental and food production challenges. Agronomic and environmental benefits of legume-based cropping systems (CS) have been evaluated from different perspectives – with studies of a broad range of legume species on the soil and environment in various cropping systems (monocrop, cover crop, intercrop, agroecological service crop, etc.) not only in Europe but across the world, including Australia (CA4).

Introduction of legumes in broader scale in CSs is strongly dependent on the interest and knowledge of stakeholders. The nitrogen (N) contributed by legumes is an important component of the N supplied to subsequent crops. However, most Australian grain-growers have little idea about the potential

inputs of fixed N provided by their pulse crops, and few routinely monitor soil mineral N before applying N fertiliser to crops grown after a legume. Peoples (CA4) presented a study on the role of legumes in cereal-dominated cropping sequences gained from 16 dryland (rainfed) experiments conducted in eastern Australia between 1989–2016, and from on-farm measurements of the symbiotic performance of 50 commercial pulse crops undertaken since 2001. The data collated from these studies were examined to identify the main factors regulating inputs of fixed N, and to explore the possibility of developing simple predictive relationships which farmers' could use to benchmark (i) likely inputs of fixed N, (ii) the expected availability of soil mineral N after legumes, and (iii) the relative value of legume N to a following wheat crop.

The understanding and knowledge by stakeholders regarding legumes value for design of cropping systems was investigated



in the LEGATO project (C3). The aim of investigation was to evaluate the sustainability and feasibility of legume-based locally-adapted CS, in three European regions (Scania, Sweden; Paris Basin, France; Andalusia, Spain). First – innovative CSs were designed in three steps: (i) an initial diagnosis in each region, i.e., description of the most frequent CSs and their advantages and limits, and definition of local improvement targets; (ii) the design of innovative CSs including legumes in a workshop (iii) multicriteria assessment of the CS with the MASC© tool. Second – stakeholder meetings were organized in each region, to (i) present and discuss innovative CSs including legumes and their assessment results compared to the reference CS, (ii) discuss the feasibility of the innovative CSs with stakeholders, to see if some of them could be implemented in farmers' fields, and (iii) gather the preferences of stakeholders on ranking of performance criteria (weight sets) of MASC©, reflecting their point of view on sustainability. Weight sets were synthesized into four MASC© trees and each innovative CS was re-assessed according to those trees. The results showed that it was possible to identify feasible CSs with grain legumes for each region, that were assessed sustainable according to four MASC© trees (C3).


One of the promising arguments *pro* legumes is their ability to improve soil properties and fix nitrogen. Thus, their incorporation in existing cropping systems would be promising for sustainable cropping practices. Crop diversification in space and time is a key component in the design of resource-efficient cropping systems with reduced dependency on synthetic inputs. A well-known example of crop diversification is the practice of intercropping, i.e. growing two or more crops together in the same field. Intercropping grain legumes and cereals in low-input systems makes use of complementary acquisition of light, water and nutrients to enhance the crop's resource use efficiency and competitiveness against weeds. Within-species diversification in variety mixtures may also increase yield stability, via e.g. complementary tolerance to abiotic or biotic stress. As part of the LEGATO project, a study has investigated whether a mixture of pea (*Pisum sativum* L.) varieties with two types of leaf morphology can increase the legume crop's standing ability and competitiveness against weeds. The pea varieties 'Dukat' (normal-leafed) and 'Partner' (semi-leafless) were grown as

single varieties and variety mixtures with and without a wheat intercrop in field experiments at four locations in Europe. The results showed that a variety mixture of pea can combine the beneficial traits of the single varieties with maintained legume grain yield relative to the highest-yielding variety. However, the benefits in terms of weed reduction and standing ability were not as strong in variety mixtures as in pea-wheat intercrops, which remained the most efficient mixtures (C1).

Intercropping is assumed also as a powerful tool to develop sustainable cropping systems in horticulture. In order to match sustainable and optimal land use in strawberry plantation and to increase biological diversity a trial was established at Püre Horticultural Research Centre in Latvia, in the frame of the EUROLEGUME project (C2). Three species of legumes (faba bean, pea and alsike clover) were sown as intercrop components in the strawberry fields to evaluate strawberry – legume intercropping efficiency. Intercropping influence on the soil biological activity was determined by detecting soil respiration rate, activity of dehydrogenase and cellulase. Strawberry yield and quality was determined in order to evaluate legume influence on the strawberry productivity. As the years 2015 and 2016 were uncommonly dry during vegetation season, the lack of moisture in the soil negatively influenced plant performance and soil microbiological activity. In the intercropping variant with faba beans soil was significantly dryer than in other treatments. Beans and peas had no notable influence on disease spreading in strawberries, but clover intercropping promoted strawberry disease spreading. In 2015 there were nonsignificant differences in yield quality and quantity between treatments with legumes intercropping and control treatment with nitrogen application. In the second yielding year (2016) with extreme lack of precipitation and no irrigation, treatments with legumes intercropping showed significant decrease in strawberry yield. It was assumed that pea and faba bean intercropping in strawberry plantations could be promising both for strawberry production and soil improvement under irrigation possibilities, but further investigations are necessary to clarify the plant performance in intercropping systems under different moisture regimes (C2).

Grain legumes do not compete strongly for soil N, leaving more soil N to be used by

intercropped or successive species. It was demonstrated also in the trials performed in Greece (Agricultural University of Athens) in the frame of the EUROLEGUME project. To quantitatively assess the benefits of cabbage from rotation with legumes, a field experiment was carried out during 2014–2017. In the trial cabbage was grown either after pea, or after faba bean, or after cabbage, by applying either conventional or organic farming practices. It was found that the soil NO₃-N concentration was higher in plots with pea or faba bean as preceding crop than in those with cabbage as preceding crop. Pea as preceding crop increased significantly the yield of cabbage compared with faba bean, while cabbage after cabbage resulted in the lowest yield when grown according to organic farming practices. However, in the conventional cropping system, the highest yield of cabbage was obtained when the preceding crop was also cabbage. The quantification of biological N₂ fixation by measuring the natural abundance of ¹⁵N in the tested legume species and reference plants at anthesis revealed that faba beans were capable of fixing from 119 to 194 kg N ha⁻¹ (1), while the total amounts of biologically-fixed N₂ (BNF) by peas ranged from 45 to 125 kg N ha⁻¹ (2).

Simultaneously with the introduction of legumes in crop rotations or intercropping also reduced tillage or no-till techniques are used in sustainable farming approach. In order to clarify some aspects of using non-legume cover crops, weed suppression, yield formation and N accumulation of organic peas grown in organic system were tested. Pea plants were sown after reduced tillage and no-tillage system by the use of cross slot no-tillage seeder. Early sown niger (*Guizotia abyssinica*) and millet (*Panicum miliaceum*) led to high shoot biomass accumulation of the cover crop and low weed growth in reduced tillage as well as in no-tillage pea plants. What is more important, after specific cover crops like niger and millet the grain yield of organic pea plants were higher by the use of reduced tillage than in no-tillage system. The study clearly show the importance of non-legume cover cropping for weed management and improved crop growth in organic pea production (C5). 

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Session 6 overview: Climate changes, stress adaptation & mitigation measures.

Chaired by Steve Belcher¹ and Eduardo Rosa²

Abstract: Europe is a diverse community in terms of geographic regions and climate and the species of legumes grown predominantly in each region varies accordingly. Regional changes in weather patterns, heat, cold, and rainfall may require changes to cropping practices or mitigation measures taken to ensure successful cropping. Grain legumes are indispensable elements of sustainable farming systems and future human nutrition. The benefits legumes give to diverse cropping systems are well documented and the plenary talk given by Thomas Nemecek highlighted key environmental impacts of their use (C1), that justify increasing their cultivation.

Key words: Gene x environment, cropping systems, drought, greenhouse gas emissions, nitrogen, symbiosis

J. Poslusna and V. Biarnes presented papers on a two-year study of gene x environment interactions in a European wide network of grain legumes trials (C2). Trials were conducted at 15 locations across Europe divided into Maritime, Mediterranean and Continental climatic zones. The species tested were pea, faba bean, chickpea, grass pea and lupin. A total of 106 cultivars were selected based on breeder's input. Cropping methods were according to local practice. Data was analysed using ANOVA, Joint regression and Diag Var (pea only). Diag Var is a novel approach for analyzing GXE interactions and identifying cultivar behaviour according


to limiting factors. Resistance to drought stress is often quoted as a desirable character, but in the case of pea an excess of water during flowering was seen as a factor limiting yield at some locations.

Understanding responses to too little or too much water may be a key factor to crop production in the future. Drought stress can have a severe impact on plant growth and productivity. The next two papers investigated effects of drought stress on pea and cowpea.

C. Henriët presented a paper on the response of pea to drought stress during seed filling (C3). Drought stress occurring in the late reproductive stage can greatly affect seed yield and quality. Drought stressed plants of cv Cameor were subjected to transcriptomic profiling of leaf tissue which revealed metabolic and regulatory pathways affected by drought. Candidate genes were selected and one of those, RAMOSUS1, encodes a carotenoid cleavage dioxygenase involved in strigolactone biosynthesis and controlling aerial branching. Sulphur nutrition has also been suggested to play a role in stress tolerance, and this was investigated using sulphate-deprived plants during drought stress. The combined drought and sulphur starvation stresses strongly affected yield components. A network of co-relations derived from the responses at the transcript, protein and metabolite level is under construction that will be enriched with phenotyping and physiological data.

M. Carvalho reported on the effects of drought stress on cowpea (C4). Cowpea is one of the most tolerant crops to drought stress and is therefore considered an ideal model to study the molecular mechanisms of drought tolerance. Two Portuguese cultivars of cowpea and two controls were subjected to two watering regimes post-flowering. Leaves, pods and seeds at different stages of development were collected and RNA was extracted. The gene expression levels of

eighteen genes related to drought, oxidative stress and important storage proteins were evaluated by semi-quantitative PCR. Differences between genotypes and water regimes were observed. A total of six genes were chosen to perform a real-time quantitative PCR and consequently to help understanding the effects of drought stress in reproductive stage.

The potential role of legume cultivation in reducing Greenhouse gas emissions (GHG) is often a debated topic and H. Trindade presented a paper on GHG emissions from legume-cereal rotations in N. Portugal (C5). Extra inputs of nitrogen and carbon to cropping systems provided by legumes related to their ability to fix atmospheric N through symbiosis with rhizobia may enhance GHG emissions due to increased soil microbial activity. Cowpea was grown in short rotation with triticale (succession cropping) for forage production. Experiments included treatments to evaluate the effect of removal of legume residues or their incorporation into the soil after harvest. Triticale crops grown as monoculture not fertilized or fertilised with 80 kg mineral N ha⁻¹ were used for comparison. Incorporation of cowpea residues, instead of their removal at harvest, represented an extra input of N into the soil ranging between 15.9 and 60.9 kg ha⁻¹. The highest annual emission of N₂O was measured in the monoculture of triticale fertilized with 80 kg mineral N ha⁻¹. The soil acted as a sink for CH₄ in all treatments, but the highest sink effect was observed when cowpea residues were incorporated, which represented about four times more soil CH₄ absorption than when cowpea residues were removed. However, there were no differences in the soil CH₄ absorption between the treatments of cowpea-triticale and the treatments of cereal monocultures. The results showed that the introduction of the cowpea crop did not produce any harmful effect in terms of GHG emissions. 

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Environmental impacts of the production and use of grain legumes

Thomas Nemecek¹

Abstract: Through their ability to fix nitrogen and to diversify cropping systems, grain legumes offer the possibility to reduce environmental impacts. However, the concrete effects depend on the context: the highest mitigation potentials by introducing grain legumes are observed in cereal-rich crop rotations with high N fertilization. Replacing imported soybean meal in animal feed formulas can reduce environmental burdens related to soybean cropping, provided that the substituting feed components have lower environmental impacts. Grain legumes would play a more important role in an environmentally optimized human diet, compared to current nutritional patterns.

Key words: Grain legumes, life cycle assessment, environmental impacts, food, feed

Grain legumes have specific characteristics with consequences for the environment. During the last years, several life cycle assessment (LCA) studies analysed the environmental impacts of the introduction of legumes into cropping systems (for an overview (1)). LCA is a method of environmental assessment to quantify the environmental impacts of products or production systems. It is characterised by the inclusion of all relevant environmental impacts and the consideration of the whole life cycle ("from cradle to grave"). This allows in a comprehensive environmental assessment to show and to avoid burden shifts from one environmental impacts to another, and at the same time burden shifts between phases of the life cycle. It is crucial that grain legumes are not analysed as individual crops only, but that their manifold interactions with other crops within a cropping system are assessed.

Hence, the effects of the inclusion of grain legumes are studied best at the level of a crop rotation or a cropping system.

The main effects of the introduction of grain legumes in cropping systems are:

1) Reduction of N fertiliser use, due to the absence of N fertilisation in the legume crops and reduced fertilisation of the following crop. Mineral N fertiliser manufacturing is associated with a high consumption of fossil fuels and greenhouse gas (GHG) emissions, mainly N₂O and CO₂, and needs for transporting and spreading the fertiliser. Furthermore, the application of N fertilisers leads to various N losses, of which nitrous oxide (N₂O), ammonia (NH₃) volatilisation, nitrate (NO₃) leaching and nitrogen oxides (NO_x) are of environmental relevance. Nemecek *et al.* (2) showed that N fertiliser strongly correlated with non-renewable energy demand, global warming potential, ozone formation, acidification, and eutrophication potentials in intensive cropping systems, and the reduction of N levels, e.g. by the introduction of legumes or moderated N fertilisation, is a way to reduce these impacts.

2) N leaching is often increased after the harvest of legume crops, as higher residual N is often observed. Growing catch crops or winter legumes instead help to reduce N leaching. However, if a longer period of several years is considered, N leaching is not necessarily increased, since the lower N fertilisation level in crop rotations with legumes partly compensate for the effect of the legumes.

3) Positive pre-crop effects of legumes generally result in a higher yield of the following crop at similar N fertiliser levels, same yields at lower N fertilisation levels or a combination of both effects. This improves the environmental performance of the whole system (3). Such effects need to be taken into account in the analysis, which is done

best by extending the system boundaries to the entire crop rotation or at least to a crop sequence.

4) Diversification of the crop rotation results in breaking pathogen cycles, and has the potential to increase yields and to reduce pesticide inputs. The effects of diversification are highest in intensive cereal-based crop rotations, while in diversified crop rotations or low-input cropping systems these effects are weaker or absent (4). LCA studies show contradicting results regarding the effects on ecotoxicity; some grain legume crops also require intensive treatments with pesticides (1), and therefore ecotoxicity is not always reduced. Introducing additional crops into the rotation may also increase associated biodiversity. Diversification is also possible in the spatial dimension by inter-cropping legumes and cereals, which reduces environmental impacts (5).

5) Legume crops produce higher protein yields at the expense of the yield of digestible energy compared to cereals. Symbiotic N fixation has a cost in terms of energy (renewable energy in this case). Legumes provide an important source of protein for human nutrition and animal feed (see below).

6) Legumes are feeding sources for pollinators and have therefore the potential to maintain or increase their populations and to have a positive effect on biodiversity.

7) Flowering legumes increase the aesthetic value of the landscape; most people consider them as beautiful.

Grain legumes can replace soybean meal in animal feed rations. Soybean production has rapidly expanded during the last decades, often at the expense of former forest areas. Soybean produced on clear-cut land result in very high GHG emissions (6) and is detrimental for biodiversity due to habitat loss. Europe has become very dependent on soybean imports, in particular to satisfy the demand of the animal production sector.

LCA studies investigated the substitution

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of imported soybean meal by European grain legumes for cattle, pig and poultry nutrition in Europe (e.g. (7-9)). The main effects were reduced transport distances, avoided deforestation impacts and rotational benefits. Since soybean is also a legume, the advantages of symbiotic N fixation are present in both systems. However, replacing soybean meal in a feed ration requires adapting the whole feed ration and the resulting environmental impacts can therefore be lower, similar or higher, depending of the feed components selected. Best results are achieved, if an optimisation algorithm defines the feed formulas and environmental indicators are included into the goal function.

Only a few LCA studies also investigated the environmental impacts of using grain legumes in human nutrition (10). Replacing animal protein sources, in particular meat, by grain legumes can generally reduce environmental burdens, since animal protein sources are associated with relatively high impacts. Again the concrete outcome

depends on the complete nutritional pattern and the production methods. Intensive processing methods can reverse some of the environmental advantages of plant-based food (10). A nutritional optimisation study for the Swiss population showed that environmental impacts of the diet could be reduced by over 50 % in the optimized scenarios, mainly by reducing feed imports, food imports and animal production impacts (11). The composition of the average diet changed significantly: drop in the proportion of meat (-70 %) and larger proportions of grains or potatoes (+35 %) as well as legumes including tofu and peanuts (20 % of protein supply), whilst milk consumption levels remained constant. Legumes would play a more important role for covering the protein requirements in the environmentally optimized diet (Figure 1).


Grain legumes are key elements of sustainable farming systems and future human nutrition; their potential is currently underused. 



Figure 1. Legumes would play a more important role for covering the protein requirements in an environmentally optimized diet. Photo by Carole Parodi, Agroscope.

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*Kevin McPhee, on behalf of the Scientific Committee and
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