



# **LEGATO**

## **LEGumes for the Agriculture of TOMorrow**

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agriculture as well as protein supply for food and feed]**

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## **Stakeholder topic meeting report n°3**

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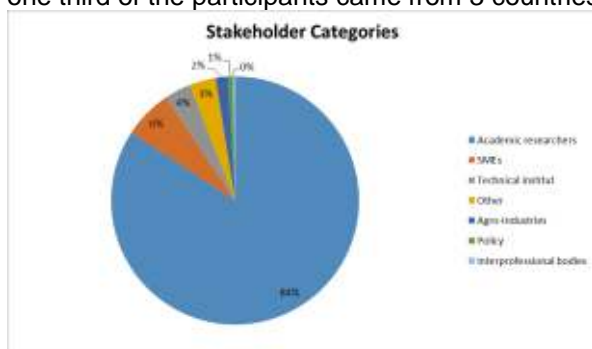
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## 1. Focus

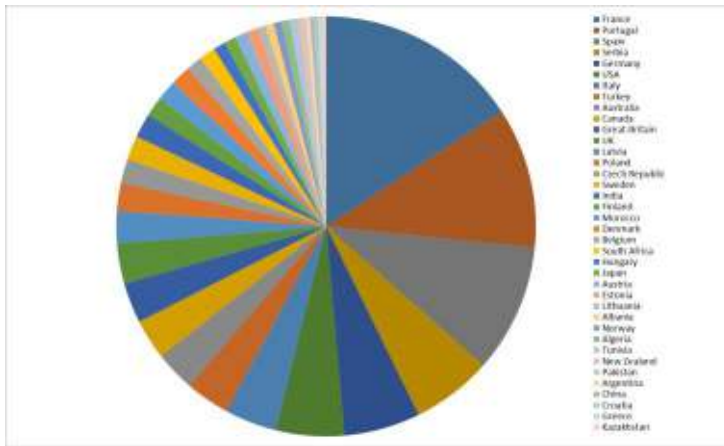
The LEGATO stakeholder topic meetings are related to the task 6.2 - sharing expertise to orientate and evaluate possible levers of improvement offered by the project results.

The annual meeting of the LEGATO project which took place on the 11<sup>th</sup> October 2016 was synchronized with the Second International Legume Society (2ILS) Conference which took place from 12<sup>th</sup> October 2016 to 14<sup>th</sup> October 2016 at Troia in Portugal. ILS is a stakeholder group run as a not-for-profit association and spanning a wide range of participants from academic through commercial, sharing an interest in legume cultivation and uses. The biennial meeting is a unique opportunity to exchange with the international community that ILS represents. Several EU projects including LEGATO therefore decided to join this event. Several results from the workpackages of the LEGATO project were presented during the 2ILS Conference, which we are considering as the third stakeholder meeting.

The program and the list of participants are given respectively in annex 1 and annex 2. The meeting attracted 317 participants, including 84% academic researchers, 6% from SME, 4% from agro-industries, and under 1% from interprofessional bodies (See figure 1 below). The participants came from different countries at international level: 38 nationalities were represented (figure 2). More than one third of the participants came from 3 countries: France, Portugal and Spain.



**Figure 1: Stakeholder categories**



**Figure 2: Origin of the participants**

## 2. Objectives (what was asked of the speakers, why this topic)

The content of the stakeholder meetings was defined in the DOW. Three topic meetings of stakeholders was initially planned: 1 for science, techniques and economy of production; 1 for science, Techniques, and economy of uses; 1 for interfacing production x uses and analyzing the limits.

One final general meeting in year 4: on the basis of the input of results from the different WPs and of the agro-economic context, definition of adapted ideotypes (genotypes x cropping management systems) for different European regions, with seed product quality fitting user requirements, and estimations of their potential development in areas and volumes.

The first stakeholder meeting at Alnarp, Sweden, on the 26<sup>th</sup> February 2015 was dedicated to the analysis of the brakes and levers to grain legumes production.

The content of the second meeting at Cordoba, on the 1<sup>st</sup> December 2015, as planned initially, was focused on the use of legumes as pasture and forage in Mediterranean Region and in animal feeding in different European countries (Spain, France and UK) from an economic point of view and by underlining techniques which can improve the economic value of legume seeds. It thus responded to priorities expressed by the Andalusian stakeholders.

The third stakeholder meeting at Troia, on the 12<sup>th</sup> to 14<sup>th</sup> October 2016, covered various themes studied in the different work packages of the LEGATO project and was a platform for presenting the first results of the project. Several LEGATO partners were selected for oral presentations and poster presentations.

## 3. Short summaries of the presentations

The presentations are available on the 2ILS web site at the following link: <http://www.itqb.unl.pt/meetings-and-courses/legumes-for-a-sustainable-world>

The table in annex 3 presents the list of the oral presentations and posters grouped by session.

- **Session 1, plenary: Legumes value chain: market requirements and economic impact**

Two partners of the LEGATO project were involved in this session.

First, Frédéric Muel (Terres Inovia) made an oral presentation on a prospective study which aimed to guide the reflections of the French professionals of vegetable oils and proteins. The thinking was organized in the form of four different scenarios for 2030, which illustrate different logical evolutions of

the context and related key issues, under the pressure of demographic, economic and socio-political constraints. It shows that, whatever the scenario, the demand for vegetable protein will increase.

A poster in this session also presented results from the LEGATO project. It was presented by A. Seabra Pinto, C. Brites (INIAV) and C. Vaz Pato, L. Cunha (ITQB). Under the research work in experimental economics considered in LEGATO, an experimental procedure was used to elicit consumers' willingness to pay with regard to legume-fortified maize bread "Broa". This product is specific Portuguese maize bread, very well-known and eaten separately during the meals. A sample of Portuguese consumers participated in an experimental auction and they evaluated five different breads in three different treatments. The incentive compatible elicitation mechanism used was the original Surplus Comparison Mechanism (SCM) that takes into account the influence of the diversity of products actually available on the market and their comparison. This study contributes to a better understanding of consumers' perceptions and their behavior regarding new uses of legumes.

- **Session 2, plenary: Legumes and environment**

Two LEGATO project partners participated in this session.

Marie-Hélène Jeuffroy (INRA, France) introduced the subject by a key lecture: despite several benefits of legumes (decrease in greenhouse gas emissions, decrease in fossil energy consumption...) a major reduction in legume-sown areas in Europe is observed. There is a great diversity of available species but a low use in cropping systems. Lower breeding activity has led to fewer registered varieties. This is due to different factors. First, legume benefits vary greatly according to the situation (for example, the yield increase on the following crop: from 0 to 75% (Cernay et al., 2016). Then, farmers do not adapt enough the rate of N fertilizer application, and pea has the lowest gross margin among arable crops. Socio-technical systems are mainly organized within value chains thus concealing some legume benefits. Finally, it is difficult to take farmer's requirements and to identify and take the diversity of targets into account.

The example of lentil-wheat intercrops (conducted in the south of France with Qualisol) shows that it can be a solution to increase crop area. It is important to associate researchers with economic structures. The solution is to choose the actor with whom the researchers can work, a time-consuming task requiring an interdisciplinary approach to recognize which skills are needed.

Branko Cupina (Univ Novi Sad, Serbia) presented the example of introducing legumes into cropping systems in Serbia. The use of legumes in cropping system is one measure that has been taken in agricultural production to increase environmental protection and to encourage sustainable use of natural resources. Legumes offer many benefits to sustainable agriculture and the integration of legumes into cropping systems brings costs and benefits, both internal and external to the farm. For ecological reasons, legumes are gaining in importance. Legumes can be easily included in a crop rotation and, in contrast to grasses and crucifers, contribute additional N to the nutrient cycle by symbiosis. Some of this N can be used later as animal feed in the form of protein in forage while the rest of the accumulated N can be taken up by subsequent crops. On the basis of experiments conducted in Serbia it can be concluded that the management decision concerning the use of legumes as cover crops should be based on the balance between farm profitability and environmental sustainability.

The effect of legumes on cash crop yield and quality is mainly positive. N mineralization should be regulated in accordance with the N demands of the subsequent crop. In animal production areas, legumes can be an important source of quality forage or can be used for mulching. In such cases and in rotation with corn or Sudan grass, legumes should fulfil the following requirements: low-cost production, yield and quality, N uptake during periods critical for leaching and no negative effects on subsequent crops.

- **Session 3, plenary: Beneficial legume-microbe interactions**

Two partners of the LEGATO project were speakers in this session.

Virginie Bourion (INRA, France) reported on partner choice in a core collection of pea inoculated by a mix of five *Rhizobium leguminosarum* ssp. *viciae* strains (Rlv). The crop's ability to fix atmospheric N<sub>2</sub>

as a main nitrogen resource in plant nodules is a major component of its attractiveness. However, the symbiosis may not be optimal because natural Rlv populations are quantitatively and qualitatively heterogeneous, with strains varying in competitiveness and efficiency of nitrogen fixation. The variability of pea-Rlv partner choice was investigated within a collection of 104 pea accessions co-inoculated with a mix of five diverse Rlv strains. Analyses of the genetic structure of the pea collection - genotyped using the GenoPea 13K SNP Array - uncovered different genetic groups representative of the pea geographic origin or history of selection. Proportions of nodules formed with each strain were estimated in each pea accession. Differences in the Rlv choice were observed between the different pea genetic groups, revealing changes in partner choice during domestication and breeding selection. Additional experiments performed on a subset of pea accessions showed that in most cases competitiveness for nodulation of a given pea-Rlv symbiotic association could not be related to its nitrogen fixation efficiency. Further studies involving larger pea panel and mix of Rlv strains together with a higher density SNP genotyping will be carried out to identify specific loci underlying the partner choice trait.

The presentation of Marc Lepetit (INRA, France) was entitled "Improving adaptation of legume-rhizobium symbiosis to the soil environment". A major hallmark of legume crops is adaptation to mineral N limitation by developing root symbiotic nodules with N<sub>2</sub>-fixing rhizobia. Their use may reduce nitrogen fertilizer inputs. However nodules are highly sensitive to soil constraints. Soils do not necessarily shelter symbiotic partners for optimal N<sub>2</sub> fixation. Local water deficit and other stresses suppress rapidly the activity of nodules directly exposed to them in soils. Consequently yields of symbiotic legumes are frequently highly fluctuating. There is a need for breeding strategies. A first strategy is to select rhizobium/legume associations that display the most efficient N<sub>2</sub> fixation and allow success for rhizobium inoculation at sowing. Bacteria forming nodules are not necessarily efficient and may display different levels of competitiveness for nodulation when they are in mixture, as is generally the rule in soils. The second strategy is to identify genetic determinants that allow plants to compensate a partial suppression of their symbiotic activity due to a localized soil constraint (notably drought). Several systemic mechanisms adjusting symbiotic capacity to the plant's N demand are involved. Compensatory responses may vary according to the symbiotic partner. Recent progress toward these two objectives using *Medicago truncatula*/Sinorhizobium as model system and pea/Rhizobium leguminosarum as agronomical target will be presented. This work has been supported by Peamust (ANR) and Legato (EU FP7 KBBE) grants.

- **Session 7, plenary: Legumes in food and feed and other alternative uses**

M.R Bronze (LEGATO partner) gave the first talk on: "The hidden phenolic content of faba beans". Faba bean (*Vicia Faba*, L.) is rich in carbohydrates, proteins, fat and have significant amounts of phytochemicals, such as (poly)phenols, known for their health benefits, namely in cardiovascular diseases, type II diabetes and cancer. Faba beans are particularly rich in flavonoids, as (+)-catechin, (-)-catechin and (-)- epigallocatechin, and hydroxycinnamic acids, such as ferulic and pcoumaric acids. Phenolic content of faba beans is usually based on the free phenolic fraction. However, in legumes some phenolics are not directly extractable, as they are part of cell walls, considered as dietary fibres, due to the rich content in polysaccharides. As conjugated and bound phenolic compounds may be bioavailable after ingestion of faba beans in humans, in order to study the soluble-conjugated and the bound phenolic compound fractions, a 50% ethanolic extract was prepared from a faba bean genotype. The extract and the residue were submitted to acidic and alkaline hydrolyses and the total phenolic content of the (1) soluble-free, (2) soluble-conjugated and (3) insoluble fractions were determined by Folin-Ciocalteu method. Characterization of the main phenolic compounds was performed by LC-MS. The antioxidant activity of these fractions was also determined by ORAC. Results are discussed in terms of bioaccessibility and health benefits of faba bean consumption.

C. Domoney (John Innes Centre), a further LEGATO speaker in this session, presented genetic diversity in pea and its impact on strategies for seed quality improvement. Considerable genetic variation exists but remains under-utilised in breeding programmes. The fundamental research made on seed quality in pea identifies allelic variation in genes that control some economically important traits, and is providing tools and resources, which may be exploited ultimately within breeding programs to improve end uses. Natural germplasm and induced mutant populations of pea are equally valuable resources for isolating variants of genes involved in determining seed quality. High-throughput screening methods were deployed to identify induced mutations and natural variation in

genes that control seed compositional and visual traits. Although reduced seed trypsin-chymotrypsin inhibitor mutants were recovered among induced mutant populations, a high-throughput germplasm screen identified an extremely rare null mutant in a *Pisum elatius* accession. Combining this variant with null seed lectin and pea albumin 2 mutants provides opportunities for considerable gain in nutritional quality in pea seeds. Visual traits can influence economic value for food crops. Loss of color from seeds may be reduced by disrupting the regulation of the chlorophyll degradation pathway, while avoiding perturbations in chlorophyll turnover, which can diminish yield.

A poster made by L. Narits (Estonian Crop Research Institute) presents results from LEGATO project. The poster reported on identification and testing of novel legume breeding lines of major grain legumes cultivated in Europe possessing valuable characters such as disease and pest resistance and quality for human consumption. Twelve field pea varieties and seven breeding lines from six European countries were tested in field trials in Estonia. Evaluation of morphological and economical characters, resistance for biotic and abiotic stresses and quality was carried out. According to the results, two breeding lines are comparable to better varieties holding the potential for the future. Work with lines is continuing.

Another poster presented LEGATO project results: D. Živanov and collaborators worked on intercropping legumes with legumes and its effects on yield components. The experimental trial was established in the experimental field of the IFVCNS, using completely randomized block design with 3 replications. The plot size was 5m<sup>2</sup> and the seeding ratios was 50:50%. Pea +faba bean, pea + fenugreek and grass pea + white lupin were mixed together, and mechanically sown in rows at the same depth. A total of ten plants randomly chosen per plot in 3 replications were estimated for the yield components (the number of pods, number of seeds and seed weight per pod). The average number of pods (7.6/8.2), number of seeds (26.9/30.1) and seed weight (6g/7.2g) per pea plant in a mixture with faba bean and fenugreek was significantly higher than in sole crop, where the average number of pods was 5.2, number of seeds 19.2, and seed weight 4.7g. However, in the same mixture the average number of pods (12.3/13.8) and seeds (29.7/57.7) per faba bean and fenugreek plants were significantly higher than in intercrop. Moreover, the fenugreek had the significantly higher seed weight (0.9g) in mixtures than in a sole crop. The sole crops of grass pea and white lupin had significantly higher numbers of pods (18.5/9.2), seeds (44.1/29) and seed weight (7.25/13.2) than in mixture.

In the LEGATO project, S. Katanski and collaborators worked on intercropping field pea with perennial legumes for forage production. One of the most important problems in establishing perennial legumes are weeds in the early growth stages of the legume. Field pea is a short term, annual crop and can reduce weed invasion, and also it can provide increased forage yield in the first cut in the first year. The goal of this study was to examine the potential of intercropping field pea with perennial legumes for forage production. The trial with 3 replications was carried out at IFVCNS Novi Sad at Rimski an\_evi in 2015. Alfalfa and red clover were the undersown crops, and field pea was the companion crop. Control variants were pure stand of alfalfa and red clover and their mixture with oat. At the harvestable stage of pea, forage yield (t ha<sup>-1</sup>) and weed proportion (%) were monitored. On average, intercropping with oat produced the highest green forage yield (22.6 t ha<sup>-1</sup> alfalfa and 18.9 t ha<sup>-1</sup> red clover). Forage yield was higher when perennial legumes were sown with pea than grown as the pure crops (10.0 t ha<sup>-1</sup> vs. 6.4 t ha<sup>-1</sup>, 10.8 t ha<sup>-1</sup> vs. 8.7 t ha<sup>-1</sup>). The lowest weed proportion has been achieved in sowing with oat (0.4 % alfalfa and 0.3 % red clover), while pure crops had the highest weed percentage (13 % and 46 %, respectively).

- **Session 8, plenary: Frontiers in legume genetics and genomics**

This session was introduced by the key lecture of Judith Burstin (INRA, France): Towards the genome sequence of pea: a tribute to Mendel, who reviewed the various stages reached in the field of the genetics since Mendel until today and the contributions of the genomics. LEGATO is supporting informatics analysis of the pea genome sequence in J. Burstin's group.

P. Annicchiarico (CREA, Lodi) showed the interest of genotyping-by-sequencing and its exploitation in forage and grain legume breeding. Genotyping-by-sequencing (GBS), compared with SNP array platforms, may drastically reduce genotyping costs, and is applicable also to relatively small experiments. However, it may require optimization for specific crops, to maximize the number of usable markers. Exploiting GBS-generated markers may require optimization too (e.g. to cope with

missing data). This study aimed (i) to compare the value of different GBS protocols for three legume crops (alfalfa, pea, white lupin) that contrast for genome size, ploidy and breeding system; (ii) to show various successful applications of GBS data on legume species and some of their challenges. Preliminary work on alfalfa highlighted the greater interest of ApeKI over PstI:MspI DNA digestion. We compared all different combinations of KAPA or NEB Taq polymerase with primer extensions that are progressively more selective on restriction sites, identifying GBS protocols of general value across the three legume species. Genomic selection accuracy was assessed for predicting forage yield-based alfalfa breeding value and pea grain yield in different growing environments, considering different missing value imputation strategies and statistical models (Ridge regression BLUP, SVR, Bayesian methods). The usefulness of GBS data to assess alfalfa cultivar distinctness was evaluated, which offers advantages over phenotypic trait-based distinctness. GBS also proved useful for exploring the genetic structure of white lupin landrace germplasm.

In a poster, C. Ferrandiz (CSIC-UPV) and collaborators worked on mutations in the MFN genes which lead to increased production of pods and seeds in pea. The production of pods and seeds depends on the number of flowering nodes formed by the plant. Two genes have been identified, named MORE FLOWERING NODES (MFN), encoding homologous transcription factors that control the period of time in which the primary inflorescence apex is active and, therefore, the number of flowering nodes. Homozygous plants with null mutations in MFNa or MFNb, grown under optimal greenhouse conditions, produce up to twice the number of pods and seeds as the wild-type line, without affecting flowering time. To understand whether the MFN genes can be used to increase the production of seeds in pea, the phenotype of the mfn mutants was analyzed in detail. In addition, to analyze their performance in commercial varieties, the mfn mutant alleles were introgressed into several pea elite cultivars. Finally, new mfn alleles were searched for, potentially more advantageous, in collections of wild pea accessions and cultivars to establish their possible correlation with the number of flowering nodes. MFN genes offer potential for increasing yield in grain legumes.

- **Session 10, plenary: Frontiers in legume genetics and genomics**

An oral presentation was made by M.-C. Vaz Patto. Today's consumers are increasingly concerned with food quality and sustainability. Grain legumes could play an important role in answering these concerns. Grass pea (*Lathyrus sativus*) is a promising source of calories for drought-prone areas while common bean (*Phaseolus vulgaris*) is the most important edible legume worldwide. Yet the nonalignment of breeding objectives and end-users preferences is hampering their use in Europe. Breeding for improved end-user's quality is a complex task due to trait interaction. Particular metabolites act as anti-nutrients as well as health-promoting agents, influencing both taste and consumers' acceptability. Under the scope of LEGATO, BEGEQA and QUALATY projects, the genetic basis of several of these dual action bioactive compounds (as phenolic compounds, phytic acid or -ODAP) was studied as a primary step for devising innovative quality breeding approaches for common bean and grass pea. To reach these goals, 100 grass pea and 150 common bean accessions were repeatedly field trialed. Seed metabolite contents were analyzed using different analytical approaches and DNA screened using NGS-based SNP genotyping. A genome-wide association study, relating contents of bioactive compounds with genomic information, is ongoing using mixed linear models to account for population structure and family relatedness. Functional markers will be identified conferring consumer-driven quality traits, to redirect breeding towards more attractive varieties.

An oral presentation by A.-M. Torres (IFAPA, Córdoba, Spain) focused on advances in identifying candidate genes controlling low vicine-convicine levels in faba bean (*Vicia faba* L.). Vicine and convicine (v-c), limit the use of faba bean as food and feed. A single gene *vc-*, responsible for a 10-20 fold reduction in v-c, 10 cM apart from the white hilum has been described (Duc et al. 1989; 2004). Khazaei et al. (2015), reported a QTL for v-c content on chromosome I (Chr I) and although several closer markers were identified (Gutierrez et al. 2006) no candidate genes are so far available. The identification of suitable candidate genes is limited by the lack of knowledge of the pathway of v-c biosynthesis and the large faba bean genome size (~13 Gbp). In an attempt to determine which enzymes or transcriptional regulators could be encoded by the *vc-* gene, a combination of genetic linkage and comparative genomic approaches is being applied. To facilitate high-throughput genome profiling DarTSeq (Kilian et al. 2012) was applied to a RIL population from the cross Vf6 (high v-c) x *vc-* (low v-c) generating more than 10.000 markers. For assignment of the linkage groups to specific chromosomes, 58 EST anchor markers from the reference consensus map (Satovic et al. 2013) were assayed and 14 of them could be mapped. On the other hand, 37 SNPs, from the KASPar assay



platform (Semagn et al. 2014) belonging to Chr I, were genotyped and 9 of them, were polymorphic. The moderate conservation of the faba bean Chr 1 with the Medicago Chr 2 localized the target region between Medtr2g005900 and Medtr2g026550. For fine mapping the v-c position, primers for 61 new candidates were designed using both the Medicago (29) and the faba bean (32) transcriptome sequences (Ocaña et al. 2015) and 12 genes could be mapped. The forthcoming linkage analysis may provide potential candidate genes for the target trait.

A third oral presentation was made by P. Smykal on the use of wild pea *Pisum fulvum* and *Pisum elatius* chromosome segment substitution lines in cultivated *P. sativum* genetic background. Plant evolution under domestication has altered numerous traits, introducing domestication bottleneck resulting in high degree of relatedness, leading to narrower genetic base of cultivated germplasm, prone to pests and diseases. The study of genetic diversity showed that although wide diversity is captured among cultivated pea, wild material provides yet broader diversity (Smýkal et al. 2013, 2015). The chromosome segment substitution lines (CSSL) containing genomic segments of wild pea (*Pisum fulvum* WL2140 or *Pisum elatius* L100) in the cultivated pea (*P. sativum* subsp. *sativum* cv. Terno or cv. Cameor) genetic background were developed. These lines have been molecularly analyzed using microsatellite and gene-specific markers at 2 to 82 cM spacing at early generations (BC2-3F2-4). There were 5 to 14 segments per line, with mean of 9.6. Higher density genotyping of 50 selected BC3F6 *P. fulvum*/Terno CSSL lines using pea 13.2k Pea SNP (Tayeh et al. 2015) and further 100 lines using DARTseq approach is in progress. Establishment of such a permanent introgression library will allow phenotypic characterization of an unlimited number of target traits, which, coupled with higher density markers, will provide means for QTL and gene identification and subsequent incorporation in desired genotypes. Field testing of agronomical performance of 50 lines of *P. fulvum*/Terno CSSL lines is under way.

- **Session 10, plenary: Frontiers in plant and crop physiology**

R. Metzner (IBG, Germany) presented his work on in vivo monitoring of the development of legume root system, nodule and pod development. Quantitative non-invasive measurement of structural and functional development of plant organs allows for deep phenotyping and dynamic investigation of plant performance under stress. While this is straightforward for leaves or stems, other plant parts, such as seeds enclosed in pods or roots and nodules hidden in soil, are more difficult to investigate. Their development, however, is critical for yield and performance under stress and direct observations in conjunction with genetic and metabolomics approaches may therefore hint on the underlying mechanisms. A set of three non-invasive techniques for studying such developmental processes was presented. 1) Low-field nuclear magnetic resonance relaxometry with portable devices (pNMR) is used to monitor dry matter and water content in pods over periods of several weeks. 2) Magnetic Resonance Imaging (MRI) is taken to study the structural development of roots and nodules in soil filled pots over several weeks. 3) Positron Emission Tomography (PET) with the short-lived radiotracer <sup>11</sup>C is used to analyze the partitioning of photoassimilates and its dynamics among roots and nodules. The application of all three techniques to pea and bean plants grown in soil was illustrated, and their potential for evaluating the effects of genotype or rhizobial strain on plant performance under stress and on biological nitrogen fixation was discussed.

- **Session 12, parallel: Integrated pest and disease management**

A poster was presented by A. Cimmino (Univ Napoli, Italy) on necrotrophic effectors produced by fungal pathogens of legume crops. Necrotrophic fungi, including essentially *Ascochyta* and *Botrytis* species, are among the main biotic constraints in legume crops. Both fungal genera are well known phytotoxin producers. These toxins, belonging to different classes of natural compounds, are frequently involved in the development of disease symptoms. The chemical and biological characterization of the necrotrophic effectors produced by *Didymella pinodes* and *Botrytis fabae* pathogens of pea and faba bean respectively, was presented. Furthermore, studies aimed at identifying novel resistance sources in the respective hosts by sensitivity assays were discussed.

Another poster was presented by A. Evidente (Univ Napoli, Italy) on biocontrol of emerging insect pests of legume crops. Pea weevils (*Bruchus pisorum*) and pea aphids (*Acyrtosiphon pisum*) are insect pests of great economic importance for agriculture and represent a major cause of damage and loss in legume crops. *Bruchus pisorum* is a specialist, feeding and developing almost exclusively on pea. *Acyrtosiphon pisum* feeds on several species of legumes worldwide and ranks among the most

serious aphid pests in agriculture. Recently, germplasm collections of pea have been screened under field conditions to identify sources of resistance to weevils and aphids in pea. The identification of compounds with phagodeterrent activity, which are able to interfere with aphid-host plant selection and host acceptance, is currently becoming of great interest for the design of innovative biotechnical strategies in control of phytophagous insects. Results obtained on the identification of metabolites responsible for antixenosis and/or antibiosis in pea resistant accessions to weevils and aphids were reported. The effects of natural metabolites on these pests was also discussed.

M. Seidenglanz (AGRITEC, Czech Republic) and collaborators and colleagues (CSIC, Spain) presented a poster on the effect of intercropping field pea with spring cereals on temporal changes in pea aphid (*Acyrtosiphon pisum* Harris, 1776) abundances and distribution in crops. In the course of 2013 - 2015 abundances of pea aphid (*A. pisum*) and their natural enemies found on field pea plants, grown either as a monoculture or intercropped with spring cereals and some other legumes, were compared. Two types of trials were used: small plot trials and large plot trials. Pea aphid colonies in field peas intercropped with cereals developed differently to those in pea monocrops. However, the small plot trials results differed from those obtained in large plot trials. In the small plot trials on intercropped plots pea aphid numbers were higher during the first part of crop colonization, but the decline in aphid populations also happened earlier. In the large plot trials pea aphid abundances were significantly lower in field pea – barley intercrops compared to monocrops during the whole duration of crop infestation. Pea aphid colonies encountered syrphid larval attacks more frequently in intercrops than in monocrops.

- **Session 13, plenary: Frontiers in legume breeding**

In this session a poster was presented by G.B. Kiss (AMBIS Ltd, Hungary) and colleagues on generation of linked markers for low vicine-convicine (lvc) and zero tannin (zt-1) traits and their use in Marker Assisted Selection (MAS). Polymorphic PCR based genetic markers tightly linked to low vicine convicine content (lcv) and zero tannin (zt-1) traits were developed for several faba bean (*Vicia faba*) parents. These markers (VfVc12, VfVc13, VfZt12) were used to genotype individuals in several breeding populations to help marker assisted selection for these traits.

- **Session 14, plenary: Frontiers in legume agronomy**

Eric Justes (INRA, France) gave the key lecture of this session on the effects of grain legume insertion and cereal-grain legume intercrops in low input cropping systems in Southern France. The results show that intercropping with pea and faba bean increased durum wheat grain yield by 8 % compared to sunflower and diminished N fertilizer by 45 kg N.ha<sup>-1</sup>. There was no effect of soybean.

E. Pelzer (INRA, France) made an oral presentation on design and assessment of legume-based cropping systems with stakeholders in Europe. Legume-based cropping systems have numerous environmental benefits, but stakeholders are often not aware of all of them. Moreover, there is a large range of available species that can be grown with various practices in the fields (sole or cover crop, intercrop, etc.). Their aim was thus to design legume-based locally adapted cropping systems, in three European regions, respecting the diversity of legumes species and practices. After describing the most frequent cropping systems in each region, local improvement targets were defined. Then, during a design workshop gathering scientists and a few advisors and farmers, knowledge on legume crops was shared, and then innovative cropping systems were designed. With the aim of assessing them with the multi-criteria tool @Masc (tool accounting for user's preferences on sustainability), stakeholders in each country (29) were surveyed to gather their preferences on ranking of performance criteria (weight sets) and to collect their opinions on the feasibility of designed cropping systems. These weight sets were synthesized into 4 MASC trees and each innovative cropping system was assessed according to those trees. In the end, legume-based cropping systems were identified for each region, which were assessed for sustainability by the 4 stakeholder trees. The study further revealed lack of knowledge on legume crops and their effects on the following crops that should be completed for promoting legumes in Europe.

G. Carlsson (SLU, Sweden) reported on the participatory development of grain legume-cereal intercrops for enhanced productivity and reduced weed abundance in organic crop production. Grain legumes such as beans, lentils, lupins and peas are highly appreciated for their symbiotic nitrogen fixation and for providing high-quality food and feed, but perceptions of low or variable yields may limit

farmers' interest in growing grain legumes. In addition, despite documented benefits of crop diversification in terms of yield stability and resource use efficiency, European agriculture is still mainly based on sole crops. Results were presented from an ongoing project together with organic farmers in southern Sweden, designed to evaluate the empirical generalizability of production benefits in intercropping systems. The work started by agreeing on objectives concerning evenness in crop maturity, low weed abundance and high crop quality, that need to be fulfilled in order that the participating farmers grow more intercrops. Grain legume-cereal combinations that appeared promising according to farmers' experience and scientific results were then identified, and their performance tested in on-farm trials and research-station field experiments. The results obtained so far show clear reductions in weed abundance with increasing proportion of the cereal component in lentil-oat and lupin-wheat intercrops, and higher total yields in intercrops than in sole crops. The results and experience from the project were discussed in the context of promoting the implementation of intercropping and enhancing the sustainability of Swedish organic grain legume production.

- **Session 18, parallel: Resistance to biotic and abiotic stresses**

D. Rubiales (CSIC, Spain) presented the use of wild relatives in pea breeding for disease resistance. Pea (*Pisum sativum* ssp. *sativum*) is a cool season legume crop produced worldwide, mainly in temperate regions whose seeds can be used to feed livestock or for human food. Pea yield can be constrained by a number of pests and diseases to which there is insufficient genetic resistance available. Wild relatives, including related subspecies of *P. sativum*, or even the most distant *P. fulvum* offer a reservoir of sources of resistance that can be exploited in pea breeding. At CSIC Córdoba large germplasm collections of *Pisum* were screened looking for resistance to ascochyta blight, powdery mildew, rust, broomrape, fusarium wilt, aphid and weevil. This resulted in the identification of valuable sources of resistance that have been introduced in breeding programs. As an example, a new gene (Er3) for powdery mildew resistance was identified in *P. fulvum* and introduced in pea, with a resistant cultivar ('Eritreo') already protected in the European catalogue. Similarly, the first two broomrape resistant cultivars are now protected, and a number of breeding lines are being developed. Parallel to this applied approach, some of the identified resistances have been characterized at the histochemical, genetic and proteomic levels.

C. Polanco (Univ Leon, Spain) presented a poster on the characterization of Mildew resistance locus (Mlo) genes in wild species of lentil. Powdery mildew is a widespread disease that can cause significant harvest losses in crop plants in temperate climates. Loss-of-function alleles of specific MLO genes confer broad-spectrum powdery mildew resistance in monocots (barley) and dicots (*Arabidopsis thaliana*, tomato, and pea). SNARE genes are required for mlo-specified resistance. MLO genes are present as small families in plants and 14 MLO gene family members were identified in the lentil genome (*Lens culinaris culinaris* cv. CDC-Redberry) by sequence similarity-based homolog searches using the available sequences in pea and soybean. The phylogenetic analysis revealed that three genes (LcMLO\_01, LcMLO\_02, LcMLO\_10) were homologous to MLO genes associated with powdery mildew resistance in other species. A set of primers was designed in order to amplify the complete LcMLO\_01 gene sequence from interesting lentil accessions and wild relative species. SNARE gene candidates in lentil and the sequences of LcMLO\_01 in *L. c. orientalis*, *L. odemensis*, *L. tomentosus*, *L. nigricans*, and *L. lamotei* were also presented. Most of the nucleotide differences were detected in the 14 intronic regions but amino acid changes were also identified. The deduced protein sequences showed the presence of characteristic and conserved transmembrane motifs and a conserved calmodulin-binding domain. A phylogenetic tree was constructed using the corresponding nucleotide sequences of this gene including cultivated lentil lines.

The poster presented by N.F. Almeida (Univ Lisboa, Portugal) with collaborators and colleagues (Camara Municipal de Alvaizere and Simoes & Ramos, Portugal and CSIC, Spain) was dedicated on the pursuit for resistance sources to biotic stresses in *Lathyrus sativus*. Grass pea (*Lathyrus sativus*) is a feed, food and fodder crop, with great adaptability to inauspicious environments, high protein content and resistance to serious diseases. It is one of the most promising sources of calories and protein for the drought-prone and marginal areas of Asia and Africa, being an alternative for marginal soils and for cropping systems diversification in Europe. As part of the European LEGATO and the Portuguese QUALATY projects, a search for resistance sources to weevils and fungal diseases was undertaken on a collection of hundred grass pea genotypes from diverse origins, including Europe, Africa and Asia. Along with the phenotypic observations (resistance level) on field and controlled

conditions, DArT-seq and E-SSRs were used to access the genetic diversity within and among grass pea accessions and relate it to resistance level. The acquired data will reveal valuable diverse sources of resistance for the development of modern grass pea cultivars, improving the attractiveness of this underused but promising crop.

M. Prudent and collaborators (INRA, France) presented a poster on the effect of soil microbial community diversity on resilience of pea plants after drought. It was hypothesized that the stability (resistance and resilience) of pea production under drought might be enhanced with increasing the diversity of soil microbial communities. An experiment was conducted under greenhouse conditions where pea plants were grown during three months under three levels of soil microbial community diversity, obtained by a dilution approach. For half of them, a water stress was applied during two weeks at vegetative stage followed by a rewatering until the end of the growth cycle, while the other plants were well watered throughout the experiment. Traits measured reflected plant development and growth, plant resource acquisition and allocation among compartments and seed production (i) after drought to evaluate the resistance of the plant, and (ii) at physiological maturity to evaluate the resilience of the plant. The results showed that plant biomass depletion due to drought did not vary with the level of microbial diversity. Adaptation of the root system and plant transpiration was however dependent on the level of microbial diversity: under low diversity level, root traits and stomatal conductance were less affected by drought than under high diversity level. After rewatering, plants in interaction with a higher diversity level had a higher biomass, indicating a better resilience. Seed production was also enhanced by the higher microbial diversity level.

A poster on a transcriptomic approach to identifying candidate genes for drought tolerance during the reproductive phase in pea was proposed by V. Vernoud (INRA, France) and collaborators and colleagues from Univ Paris-Saclay, France. Drought is a major environmental factor limiting the productivity of crop plants. In pea drought stress occurring during the reproductive phase can greatly affect seed yield and quality. The response of pea plants (var. Caméor) subjected to water stress during the seed filling period, a phase when massive remobilization from the vegetative organs occurs to sustain seed high-nitrogen demand was investigated. Pea plants were subjected to drought stress at the beginning of the seed filling period of the first two nodes for 8 days. Total and one-seed biomass decreased by 35% and 20% respectively by this limited water stress. Nitrogen allocation to the different plant compartments was also affected, with an increased N allocation to the leaves from the vegetative nodes and a decrease to the root and seed compartments. Transcriptomic changes in water-stressed leaves from the vegetative nodes were analysed by hybridization of a 40k pea microarray: 178 genes were at least two fold up-regulated in water-stressed samples compared to well-watered samples, whereas 55 genes were downregulated. Among the most strongly up-regulated genes were those encoding a glutamine amidotransferase, a sucrose transporter from the SWEET family, a phosphoenolpyruvate carboxykinase and a carotenoid cleavage dioxygenase involved in strigolactone biosynthesis.

P. Winter (GenXPro, Germany) and colleagues from CSIC (Spain) reported the use of MACE technology to identify positional and expressional candidate genes for resistance to *Didymella pinodes* in pea. MACE (Massive Analysis of cDNA Ends) allows ultra-deep transcription profiling without PCR introduced bias by combining second generation sequencing and the TrueQuant technology. In MACE each cDNA molecule is represented by one sequence (the tag) of 94 bp, originating from a region around 100–500 bp from the 3' (poly-A) end of the transcript. High-throughput sequencing of tags provides numerical gene expression values and allows the identification of SNPs and INDELS from the 3'ends of the genes. In the frame of "LEGATO" project, MACE is being used to identify positional and expressional candidate genes for resistance to *Didymella pinodes* in pea. MACE has been used to perform a detailed transcriptomic study to identify genes differentially expressed in a resistant reaction (accession P665) compared to a susceptible one (cv. Messire). The resulting sequences will be used to identify SNPs between P665 and Messire for these differentially expressed genes. These SNPs will be further genotyped in the RIL population P665 x Messire in a high throughput way by sequencing MACE libraries from 94 RIL families. This will allow the mapping of these differentially expressed genes in the P665 x Messire genetic map and the study of their possible colocalization with the QTLs associated with resistance to *D. pinodes* that have been already identified in this population.

#### **4. Main conclusions and eventual actions to be taken**

The 2ILS Conference was a unique occasion to publicize the first results from the LEGATO project at an international level. The next step now is to analyse all the results obtained in the LEGATO project and to transfer them to the various stakeholder during the final meeting (Joint LEGATO-EUROLEGUME International conference) in 27 and 28 September 2017 in Serbia.

**Annex 1: Third Stakeholder meeting program (2ILS Conference Program)**

October 12<sup>th</sup> 2016

08:00 Registration;

09:00-09:30 Welcome addresses

**09:30-10:30 Session 1, plenary: Legumes value chain: market requirements and economic impact.** Chaired by Eduardo Rosa (UTAD, Portugal) and Adrian Charton (FERA, UK)

09:30-10:00 Key lecture 1: Hakan Bahceci (Int. Pulse Trade and Industries Confederation)

10:30-11:00 Coffee break

**11:00-12:00 Session 2, plenary: Legumes and environment.** Chaired by Richard Thompson (INRA, France) and Diego Rubiales (CSIC, Spain)

11:00-11:30 Key lecture 1: Marie-Hélène Jeuffroy (INRA, France)

11:30-12:00 Highlighted presentation

12:00-13:00 Poster viewing

13:00-14:30 Lunch

14:30–16:00 Parallel sessions:

**Session 3, parallel: Mechanisms of beneficial legume-microbe interactions.** Chaired by Douglas Cook (UCDavis, USA) and Florian Frugier (CNRS, France)

14:30-15:00 Key lecture: Jens Stougaard (Aarhus University, DK)

**Session 4, parallel: Genetic resources.** Chaired by Hari Upadhyaya (ICRISAT) and Margarita Vishnyakova (VIR, Russia)

14:30-15:00 Key lecture: Jeff Doyle (Cornell Univ., USA)

16:00-16:30 Coffee break;

16:30-17:30 Parallel sessions:

**Session 5, parallel: Legumes value chain: market requirements and economic impact (cont.).** Chaired by Fred Muel (Terres Inovia, France) and Aden Aw Hasan (ICARDA)

**Session 6, parallel: Root diseases.**

17:30-18:30 Poster session 1

Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters on the sessions of the day

20:45 Third International Legume Football Cup

October 13<sup>th</sup> 2016

**8:30-10:00 Session 7, plenary: Legumes in food and feed and other alternative uses.** Chaired by M.C. Vaz Patto (ITQB, Portugal)

08:30-09:00 Key lecture: Frédéric Marsolais (Agr & Agri Food Canada)

10:00-10:30 Coffee break;

**10:30-12:00 Session 8, plenary: Frontiers in legume genetics and genomics.** Chaired by Charles Brummer (Univ California, USA) and Marta Santalla (CSIC, Spain)

12:00-13:00 Poster session 2  
Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters from the sessions of the day

13:00-14:30 Lunch

14:30-16:00 Parallel sessions:

**Session 9 parallel: Legumes in food and feed and other alternative uses (cont.).** Chaired by Ruta Galoburda (Latvia) and Tom Warkentin (Univ. Saskatchewan, Canada)

**Session 10 parallel: Frontiers in legume genetics and genomics (cont.).** Chaired by Bernadette Julier (INRA, France) and Kevin McPhee (North Dakota State Univ. USA)

16:00-16:30 Coffee break;

16:30-18:00 Parallel sessions

**Session 11, parallel: Frontiers in plant and crop physiology.** Chaired by Christophe Salon (INRA, France) and Luis Aguirrezábal (INTA, Argentina)

16:30-17:00 Key lecture: Phil Mullineaux (Univ. Essex, UK)

**Session 12 parallel: Integrated pest and disease management.** Chaired by J. Davidson (Australia) and Weidong Chen (USDA-ARS, USA)

16:30-17:00 Key lecture: Seid Kemal (ICARDA)

17:45-19:00 ILS General Assembly

20:45 Third International Legume Football Cup

October 14<sup>th</sup> 2016

**8:30-10:00 Session 13 plenary: Frontiers in legume breeding.** Chaired by Wolfgang Link (Georg-August-University, Germany) and Gerard Duc (INRA, France)

08:30-09:00 Key lecture: Scott Jackson (Center for Applied Genetic Technologies, USA)

10:00-10:30 Coffee break;

**10:30-12:00 Session 14, plenary: Frontiers in legume agronomy.** Chaired by Eric Jensen (SLU, Sweden) and Susana Araujo (ITQB, Portugal)

10:30-11:00 Key lecture: Kadambot Siddique (University of Western Australia)

12:00-13:00 Poster session 3

Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters from the sessions of the day

13:00-14:30 Lunch

14:30 – 16:00 Parallel sessions:

**Session 15, parallel: Frontiers in legume breeding (cont.).** Chaired by Pooran Gaur (ICRISAT) and Paolo Annicchiarico (CREA, Italy)

**Session 16 parallel: Advances in legume agronomy (cont.).** Chaired by Fred Stoddard (Univ. Helsinki, Finland) and Claudio Porceddu (CNR, Italy)

16:00-16:30 Coffee break;

**Session 6, parallel: Legumes and environment (cont.).** Chaired by Christine Watson (SRUC, UK)

**Session 18 parallel: Resistance to biotic and abiotic stresses.** Chaired by Weidon Chen (USDA) and Lournet Gentzbittel (CNRS, France)

18:00-19:00 Concluding session

Posters and oral presentations awards

ILS Honorary member's awards

20:00 Farewell Dinner

October 15<sup>th</sup> 2016 (private sessions)



**Annex 4: Picture of the participants at Troia, Portugal**

