Executive Summary:
In the past 50 years global N-fertilizer use increased by 800% (Foley et al, Nature 478, 337), increasing energy use and causing environmental pollution. N-fixing Grain legumes (GL) can reduce N-fertilizer use, while providing a healthy local protein source. LEGATO was conceived to promote GL cultivation in Europe, where they are currently minor crops, by a wide spectrum of activities. A major effort in LEGATO was devoted to developing and identifying molecular markers to support breeding. Two developmental traits related to yield, flowering node number (mfn), and autofertility, have been worked on intensively.
Markers were identified for use in integrating these traits in breeding lines, and for mfn, indications of yield increases were obtained. Certain GL contain antinutritional compounds that may be problematic for human consumption. In LEGATO, markers tightly linked to the faba bean Vc- (vicine and convicine) locus were identified, and the first known markers for ODAP content in grass pea were obtained. Furthermore, several high-density SNP Marker sets have been produced from crosses between wild and cultivated pea lines, and a HD marker collection for the white lupin has been built (Ksiazkiewicz et al, Sci. Rep. 7, 15335), both of which will transform breeding activities in these two crops.

GL are subject to a range of pests, which contribute to low and variable yields, and damaged, unsaleable seeds. Insect pests including bruchid and sitona weevils, and aphids, are a growing problem as insecticide use is increasingly restricted. Aphids also transmit viruses, a further disease source. To overcome this, for bruchid beetles and aphids, novel sources of resistance have been identified in germplasm collections, and markers developed that are suitable for following their introduction into cultivated varieties. Germplasm collections have also been screened to identify sources of resistance to major fungal diseases (Ascochyta, mildew, rust, fusarium wilt, chocolate spot). For Ascochyta, resistance is presently multilocus and quantitative. To refine resistance markers, 1. Expressional markers have been identified, and 2. SNPs in genes within the known QTL intervals have been demonstrated. For Fusarium, progress has been made in identifying pea defense mechanisms that may unveil gene candidates for resistance.

Terminal drought is one of the principal stresses of GL cultivation, and both genetic and ecophysiological approaches were directed at this. Three complementary strategies were used in pea for selecting resistant lines. One involved genomic selection with GBS-generated SNP markers on 311 RILs subject to terminal drought, which generated a predictive model for favorable loci. A second exploited GWAS to develop a MAS criterion for selection for intrinsic drought tolerance, while a parallel targeted strategy identified gene candidates for a role in the drought response. The effect of drought on nitrogen fixation is critical to GL use in durable cropping systems. The capacity of legumes to cope with water shortage and fix N depends on the establishment of an adequate root system with sufficient nodules. It was shown that limiting mineral N penalizes legume root development and N-fixing performance later on, but that the response varies with different legume crops. The possible benefit of rhizobial inoculation was investigated for pea and faba bean at several sites including those where legumes had not been previously grown for many years. Innovative MRI and PET imaging tools were developed for studying non-invasively the water and metabolite dynamics of the root (including individual nodules) and aerial compartments. The interaction between drought and high temperature stresses has been studied in pea, biomarkers for each stress identified, and evidence for different strategies of response depending on the genotype, adaptation and resilience, has been obtained.

In order to increase GL consumption, genetic variability for nutritional, organoleptic and technological traits was evaluated in more than 500 accessions assembled from LEGATO germplasm banks. Classical physico-chemical assays were done in parallel with three different NIR-based methods. The results provide hitherto unavailable detail about the variety of compositional profiles available. They will also allow NIRS to be used for routine quality evaluation during selection in place of laborious assays. To investigate consumer acceptance of GL-supplemented foods, GL flour-fortified bread was prepared and tested for consumer acceptability on tester panels (sensorial and willingness-to-pay). Diverse legume-based cropping systems (CS) were investigated for the ecological services they could
provide. This included use of a design method, MASC®, to devise CS adapted to a given location, testing weed control using variety mixtures in pea, and showing reduction in pathogen incidence (faba bean rust and pea powdery mildew) over pure stands, by using varietal mixtures and interspecies intercropping. A pan-European trials network was set up. Two years of trials results of leading varieties of GL were obtained and made available, including a G x E analysis revealing the limiting parameters for a given location. Finally, Marker-Assisted-Selection was tested for several traits at four commercial breeding sites.

Project Context and Objectives:
The LEGATO project was conceived to address some of the main obstacles to increasing grain legume (GL) cultivation by tackling the relevant research. It was divided into five research workpackages, WP1 to 5, one stakeholder interface WP, WP6, and outreach (WP7) and Management (WP8) WP. GL cultivation is in part limited by the lack of robust high-yielding varieties. In order to support breeding and improve the choice of varieties available, several approaches were taken in WP1 of LEGATO. One focused on two traits that limit yield, pod number in pea and autofertility in faba bean. For pod number, the approach taken was to investigate the potential of the more flowering node (mfn) loci, which in Arabidopsis increase the number of flowering nodes and thence pods per plant. Trials in the greenhouse and in the field have confirmed the positive effect of the pea orthologues of mfn1 and mfn2 on pea yield. As allelic variation (or the variation for mfn1 and mfn2 genes) was not found in currently used pea germplasm, this represents a novel source of variation for breeding.

To enlarge the area sown, it may be necessary to cultivate GL in climatic zones where pollinating insects are limited or absent. Therefore, studies of autofertility were undertaken in faba bean, which requires bee pollination and is characterized by intermediate levels of cross-fertilization. Genes that control variation in autofertility have been investigated. The knowledge obtained should allow the marker-assisted selection (MAS) either of pure autofertile lines or alternatively, of synthetic varieties that maximize heterosis via obligatory cross-pollination.

Selection for reduced anti-nutritional content is a priority in several GL, including faba bean and grass pea. Marker tools have been generated to assist the selection for low content of vicine and convicine in faba bean (Vc locus), and low content of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in grass pea (Lathyrus sativus). In both cases, lines with reduced levels of these anti-nutritionals exist, but introgression of the genes responsible has been limited by the absence of tightly linked markers. In LEGATO, QTLs for Vc were greatly refined, leading to the identification of candidate genes, and in grass pea, the first QTLs for ODAP content were mapped.

Despite its considerable importance, genetic variation in pea drought tolerance, identified through genome- or marker-enabled approaches, has hardly been investigated. In WP1, two complementary approaches for this trait were pursued. A search for allelic variation in candidate genes whose expression alters during drought treatment yielded several responsive genes. Among these, evidence was obtained for a role for strigolactone synthesis in pea’s response to drought. The second approach taken involved genomic selection for pea tolerance to severe terminal drought using material identified in a previous, Era-Net project, REFORMA. While the former approach focused on the effect of a few major genes contributing to tolerance, the latter took into account both major and minor genes in a statistical model for marker-based selection. Finally, in WP1, high-density marker collections and genetic maps were produced both in pea and in white lupin. These provide key tools for more effectively exploiting germplasm, and pursuing MAS and genomic selection in these crops.

WP2 has trawled the rich genetic resources of pea and faba bean, and their close relatives, in the quest
for new sources of resistance to major pests and pathogens. Insect pests such as the weevils Sitona lineatus and Bruchus rufimanus for faba bean, and B. pisorum for pea, and the aphids Aphis fabae for faba bean and Acrithosiphon pism for pea and grasspea are major factors in legume cultivation. Chemical treatments, are environmentally hazardous and inefficient, while biological control and other alternative management practices give today unreliable results. Unfortunately, little resistance is available among current cultivars. Germplasm collections of pea, faba bean and grasspea were screened under field conditions and the mechanisms of resistance studied in the most promising accessions with a view to their integration in breeding schemes.

Likewise, legumes are susceptible to a number of diseases for which little genetic resistance is present in cultivated varieties. Germplasm collections, particularly of Pisum spp., but also of faba bean and grass pea, have been thoroughly screened for resistance to fungal diseases (ascochyta blight, powdery mildew, rust, fusarium wilt, chocolate spot), viruses and parasitic weeds (broomrape). As a result, valuable sources of resistance to the various diseases have been identified, that are now being introduced in crop breeding programs, and data on the loci concerned made available for translational application in other legume species.

WP3 was focused on understanding the determinants of performance in terms of nitrogen fixation and yield, of GL subjected to abiotic stresses, in particular drought stress. Symbiotic fixation of legumes is modulated by soil nitrate availability and frequently limited by their high sensitivity to water stress. Nodule production imposes further carbon costs, which further limit root growth, and consequently, water and nutrient uptake. By studying N2 fixation and root architecture development of pea, faba bean and lupin, under varying N-regimes, species-specific root characteristics were uncovered. Abiotic stresses such as drought or heat stress are known to penalize biological nitrogen fixation and seed filling. We have developed non-invasive imaging methods (MRI and PET) to study metabolite and water dynamics in structure and function in nodules and pods of pea during plant development, allowing one to follow the physiological responses to these applied stresses. These methods provide a new perspective for knowledge-based breeding. Legume plants encounter variable stresses, including heat and drought, which most of the time are both occurring simultaneously and sequentially. These constraints are likely to occur more often due to climate change. The physiological mechanisms and the associated molecular basis of drought acclimation and resilience were investigated. This involved assessing gene expression in roots and nodules subjected to single or multiple water deprivation events. Plants’ morphological responses were characterized after three weeks treatment period, and differing strategies of responses established for different legume species. 400 genes, which were differentially expressed in response to drought, are being studied in more detail to assess their utility as biomarkers for drought responses and/or tolerance to drought events.

In order to increase consumption of GL in Europe, consumer preferences in terms of taste have to be comprehensively evaluated and matched to the corresponding compositional profile of a suitable cultivar. Furthermore, the technological properties of the flour for transformation in food products have to be assessed. In WP4 of LEGATO, more than 500 accessions of the most important European culinary grain legumes were assembled from germplasm banks and breeders working collections and subjected to a comprehensive chemical-physical, organoleptic and rheological/end-user analysis, as sources of high nutritional value (such as protein content and quality), adequate health-beneficial component contents, interesting organoleptic properties (such as aroma volatiles and sugar contents), and adequate processing-related traits, for breeding programs and genetic studies on quality. A detailed sensorial
analysis of legume fortified cereal breads (wheat and maize) was performed in two different European countries (France and Portugal).

The feasibility of cost efficient spectroscopic screening was investigated by comparing FT-IR, NIRS and NIR imaging measurements with the classical quantifications determined above. Thanks to the appropriate calibrations, it was shown that for several components, rapid NIR spectroscopy or imaging analysis could replace the classical methods for seed compositional profiling in legume breeding programs.

The food industry seeks to introduce new ingredients for nutritional and health-promoting purposes or with interesting functional properties, for instance rich in protein or fibre, and antioxidants. These growing trends offer an opportunity to identify novel food uses for whole grain legumes as well as to develop new ingredients and products derived from them. The comprehensive dataset obtained should facilitate identification of suitable germplasm for a given application. Acceptability being a further vital criterion, we have determined consumers' willingness to pay for foods fortified with grain legume ingredients using legume flour-fortified bread as an example, based on tests by sensory panels and an experimental auction.

In order to benefit from the many environmental benefits of GL crops, including positive effects on accompanying or following crops, new cropping systems involving GL were designed and evaluated in WP5. Using the MASC® tool, durable crop rotations, adapted to local pedoclimatic conditions and preferences, were designed with a panel of local stakeholders, for three sites, in Sweden, France and southern Spain.

Crop diversification in space and time is key to enhancing ecosystem services and reducing dependency on inputs. We showed a mixture of pea (Pisum sativum L.) varieties with two types of leaf morphology increased yield and competitiveness against weeds over single variety stands. Field experiments involved four sites over three years: Serbia, Sweden, and France (close to Paris, and Toulouse).

Biotic stresses are a major challenge for the incorporation of legume crops in agro-ecosystems. We tested the effect of crop diversification in faba bean and pea crops on two major diseases, rust and powdery mildew, respectively. Both intercropping the legume with barley, or using a varietal mixture of the legume, brought about a significant reduction in disease incidence for both rust in Faba bean and powdery mildew in Pea.

Although inoculation of pea and faba bean with Rhizobium leguminosarum symbiovar viciae (Rlv) is not required in most European soils, it may be necessary on fringe/new cultivation areas. The strain composition and N-fixing performance of local rhizobial strains was determined for five test sites. Then, the requirement for inoculation was tested at those sites, using strains of known efficiency, and compared to non-inoculated samples. No clear requirement for inoculation on yield was shown although on one site hosts for Rhizobium had not been sown for >20y. This indicates that a requirement for inoculation is unlikely to be an obstacle to extending the area sown to GL.

In WP6, dedicated to interactions with stakeholders, a range of activities was pursued. A pan-European GL trials network was run by 15 breeders from Estonia and the UK in the North, through to Greece, Spain and Portugal in the South, covering 3 climatic zones, and testing 81 varieties of six GL species. The objective was to allow an informed decision as to which species and varieties to grow at each site in future in place of imported soya (Description of trials network at http://intranet.iamz.ciheam.org/forms/Legato/WP6/files/Field_Trial_Protokol_5.1.2016.pdf).
The feasibility of integrating marker-assisted-selection in commercial breeding programmes was investigated by developing, testing, and successfully applying molecular markers for six traits (vicine/convicine content, Fusarium, powdery mildew, PSbMV and PEMV resistances), in breeding programmes, implicating four breeders.

WP6 also organized four stakeholder meetings during the project, at partners’ sites, with different objectives. The meetings programmes were constructed with numerous external speakers. The first meeting dealt with the planning of the trials network, whilst the last meeting examined the results of the trials and their exploitation. Other meetings, held in Sweden and Spain, diagnosed the limits to GL production, and offered solutions in terms of cultivation practices and development of food and feed uses.

The Final International Conference (Abstracts at: [http://www.legato-fp7.eu/FinalConference/pdfs/Book_of_abstracts_LEGATO_EUROLEGUME.pdf.](http://www.legato-fp7.eu/FinalConference/pdfs/Book_of_abstracts_LEGATO_EUROLEGUME.pdf)) held jointly with the sister project FP7 Eurolegume, also provided a forum for exchange with plant breeders and food processing representatives, as well as with advisory bodies, including two round-table sessions that gave stakeholders the opportunity to flag future directions.

Outreach activities in LEGATO, including media interactions, were attributed to WP7. As part of this task, a database of relevant institutions/organisation/individuals, used to communicate all LEGATO events, was set up and regularly updated. The project website [http://www.legato-fp7.eu](http://www.legato-fp7.eu) was set up and maintained. The website hosts all publicly available project documents, including reports, and deliverables, newsletters, and result sets such as the field trials data. It will continue to be maintained for five years after project end. An annual Newsletter was prepared and distributed electronically to the mailing list, and as hard copies during meetings. WP7 organized the Advanced Training course on “Advances in breeding and agronomy for improving sustainability and quality of grain legume crops”, 35h of courses with 24 participants. The participants mainly came from pan-Mediterranean countries where legume cultivation plays an important role, and the expertise regrouped in LEGATO could be usefully transmitted. The Final International Conference (132 registered participants from 45 different countries), was organized together with the sister project FP7 Eurolegume. 40 oral presentations and 15 flash presentations were delivered, that will be the basis of a future issue of the stakeholder journal “Legume Perspectives”. In addition to production of a brochure and poster describing the contours of the project and intermediate results, a brochure “best practice guidelines for sustainable legume production and use” has been prepared to communicate on the principal outcomes of the project for stakeholders.

The combined efforts of 29 partners have enabled a much broader treatment of the subject than could be achieved by a single partner or member state, and permitted transfer of expertise and access to resources otherwise geographically restricted. The process of conversion of European agriculture to sustainable/durable/agroecological systems is underway but will require accompaniment by subsequent projects such as those supported by H2020. LEGATO has close links with the most relevant H2020 projects to ensure that the project results are exploited as fully as possible in future. As LEGATO consisted largely of pre-competitive research and development, the number of examples of successful applications is necessarily limited, but in many cases the impact will become clearer in coming years (see also impact section).

Project Results:
Key result no 1: Genes regulating the plant reproduction in pea and faba bean
Workpackage: WP1

Research aims and background
Earlier work identified in a pea (Pisum sativum) mutant population the MORE FLOWERING NODES (MFN) genes mfn1 and mfn2, which increased sharply the number of fertile nodes and increased the seed production in the single and the double mutant. Project work by CSIC aimed to identify new allelic variation (or the variation for mfn1 and mfn2 genes) in different pea germplasm.

Faba bean (Vicia faba) features intermediate levels of cross-fertilization that complicate breeding by preventing both the selection of cross-pollinating varieties capable of thorough heterosis exploitation (because of the partial self-pollination) and the selection of fully self-fertile pure lines. However, variation occurs in the level of cross-fertilization. Work by IFAPA aimed to identify genes that control this variation and to increase the opportunities for marker-assisted selection (MAS).

Results and applications
The work on pea implied the molecular characterization of 28 wild accessions and 10 modern pea cultivars that maximize the phenotypic variation in number of flowering nodes. New polymorphisms that lead to amino acid changes in the encoded proteins were found for mfn1 and mfn2 genes in both wild and modern pea, but these alleles showed no correlation with the number of flowering nodes. Hence, the effect of these alleles was insufficient to modify the function of the relevant proteins and to cause a trait change comparable with that of the original mfn1 and mfn2 alleles.

The work on faba bean exploited earlier phenotyping data and new data for a recombinant inbred line (RIL) population, focusing on six traits that dissected the transformation of flowers into pods and of ovules into seeds in the absence of cross-pollination. Fine mapping analyses revealed two quantitative trait loci (QTLs) for the number of flowers per node in chromosome I and a few QTLs for the transformation of ovules into seeds that collocated in chromosome VI, 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 in chromosome IV. Self-sterile material exhibited production of largely abnormal pollen and narrower style-ovary angle than self-fertile one.

Significance and benefits
The work on pea indicated that mfn1 and mfn2 mutants are the main genes at hand for improving the node fertility, and found no evidence for their occurrence in modern varieties or wild material. These mutants have been introgressed into four recent cultivars via repeated backcrosses. The work on faba bean has opened the way to the identification of self-fertility genes by future candidate-gene approaches that will exploit the generated information on stable, fine-mapped QTLs, as well as allowing for a deeper understanding of physiological mechanisms contributing to self-fertility.

References
Results are given in the Deliverable 1.4. A few articles for international journals will be produced.

Key result no 2: Marker-assisted selection for reduced anti-nutritional content in faba bean and grass pea Workpackage: WP1

Research aims and background
This research aimed to generate marker tools to select for low content of vicine and convicine in faba bean
and low content of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in grass pea (Lathyrus sativus). Work on faba bean was performed by IFAPA and INRA, while AMBIS designed and applied MAS tools to a few breeding programs. ITQB was responsible for grass pea work. Vicine and convicine (v-c) have anti-nutritional effects in monogastric animals and potential toxicity in humans. There is a single gene (vc-) responsible for a 10-20 fold reduction, but its identification by a candidate-gene approach is complicated by lack of knowledge on the v-c synthetic pathway, the large faba bean genome and the lack of a reference genome. ODAP induces the neuron degeneration syndrome known as lathyrism in humans and animals that eat its grains frequently. Plant material with lower ODAP content was identified and there is evidence for ODAP as a quantitative trait, but information is lacking on genes and/or linked markers for MAS approaches.

Results and applications
The work on faba bean implied the characterization for v-c content and the genetic analysis of the F2 progeny of a bi-parental cross between contrasting genotypes. Synteny between M. truncatula chromosome 2 and faba bean chromosome 1 helped restrict the genomic region hosting the vc- gene. Fine mapping based on a genetic map of over 4000 KASPar and DarTSeq markers identified two markers closely linked to the gene, which could explain about 86 % of the trait variation and showed 100 % efficiency in selecting for the low v-c allele. Candidate genes within the genomic interval of these markers are being defined for future gene expression studies. AMBIS designed primers linked to low v-c and applied them to breeding material of two partner institutions.

Work on grass pea included the phenotyping for β-ODAP content in two sites and the molecular characterization by about 3500 polymorphic DArTSeq markers of 115 accessions representative of the worldwide species distribution. Marker-trait analysis revealed six markers with statistically significant linkage to the trait that could be useful for MAS. A RIL population was developed from a cross between genotypes with contrasting trait levels, and its F5 generation is undergoing field evaluation with the aim of detecting QTLs for β-ODAP content within a future genetic linkage map.

Significance and benefits
The faba bean work achieved the objective of producing a reliable molecular marker tool to select for low vicine-convicine content, and set the basis for future candidate-gene research with high chances to identify and sequence the vc- gene (and, generate, thereby, the most reliable marker-based tool). The dense genetic map that was produced can be useful for future genetic studies. The grass pea work identified the first molecular markers that assist the selection for low β-ODAP. The definition of these markers from a broad genetic base should ensure their wide applicability, while awaiting for the development of gene-based markers (or other markers closer to the gene).

References
Results are given in the Deliverable 1.4 and will be reported in two international journal articles.

Key result no 3: Genome-based approaches to select pea for drought tolerance
Workpackage: WP1

Research aims and background
Drought is expected to increase in Europe and the Mediterranean basin. Despite its considerable
importance, pea drought tolerance by genome- or MAS approaches has hardly been investigated. Work by INRA implied the search for allelic variation in candidate genes, whereas work by CREA, implied the validation of a genomic selection procedure for tolerance to severe terminal drought that was developed within the Era-Net project REFORMA. The former work can be useful to exploit the effect of a few major genes with high positive effect on the tolerance trait. The latter, which capitalizes on both major and minor genes by estimating their effect (as accounted for by linked markers) in a statistical model, is very promising for complex traits (such as drought tolerance) but its application has been hindered until now by its need for large marker numbers.

Results and applications
Project work focused on two candidate genes selected from earlier experiments and information on Medicago and Arabidopsis orthologs., which encoded (1) a glutamine amidotransferase, and (2) a carotenoid cleavage dioxygenase named RMS1 involved in strigolactone biosynthesis. Loss-of-function mutant alleles were identified within a pea TILLING (Targeting Induced Local Lesions in Genomes) population. Plants with the RMS1 mutant gene proved more susceptible to drought in two experiments, suggesting that strigolactone biosynthesis could play a key regulatory role in the plant’s response to drought. Work in progress is assessing the importance of the other gene, and defining a regulatory network connecting sulfur nutrition and drought to locate candidate genes.

A genomic selection model with high predictive ability was defined using thousands of Genotyping-by-Sequencing (GBS)-generated SNP markers and phenotyping data under managed severe terminal drought in Lodi and rain-fed field conditions in Morocco of 315 RILs belonging to three populations. Also, a MAS criterion for intrinsic drought tolerance (i.e. tolerance not depending on early flowering) based on SNP variation at six independent loci was defined by a genome-wide association study (GWAS). Genomic selection and MAS approaches, assessed on the basis of yield responses in managed-stress experiments of different sets of independent lines, were able to produce remarkable genetic progress. Genomic selection proved at least as efficient as phenotypic selection in terms of yield gain per selection cycle.

Significance and benefits
The candidate-gene research work provided evidence for the important role of strigolactone biosynthesis for pea drought tolerance, although the relevant allelic variation available in modern pea germplasm requires exploration before finalizing the definition of MAS tools. Work in progress may produce useful MAS tools for other key traits associated with drought tolerance. For genomic selection, displaying yield gain per selection cycle similar to phenotypic selection is very promising, considering that it is at least 4-fold less expensive per line and implies at least 2-fold shorter selection cycle than phenotypic selection. However, it may require specific models for other populations. The MAS tool is of special interest to select for autumn-sown, cold-prone environments, where selection for early flowering may produce cold-susceptible material.

References
Results are given in the Deliverables 1.3 and 1.5. They have been reported in two scientific articles listed below and a few additional ones planned for international refereed journals.


Annicchiarico et al. 2017. Genotyping-by-sequencing and its exploitation for forage and cool-season grain

Key result no 4: High-density marker collections of pea and white lupin, and trait-marker studies in lupin
Workpackage: WP1

Research aims and background
The work on pea aimed to create new germplasm with extensive molecular characterization, for use as a genomic resource in future studies. It included: (1) a collection of Chromosome Segment Substitution lines (CSSL) derived from crosses of cultivated pea with two wild species (P. fulvum and P. elatius), developed by UPOL and characterized with GenXPro; (2) a large RIL population originated by contrasting parents developed by Aberystwyth Univ., whose high-density sequence-based map can be integrated with other maps and support INRA’s genome sequencing effort.

Work on white lupin (Lupinus albus) performed by CREA and IPG Poznan aimed at: (1) producing a dense genetic map and exploit it to develop MAS procedures for a few key agronomic traits; (2) characterizing with high marker numbers a comprehensive landrace world collection, and assessing the species diversity and genetic structure. Genetic structure information can be important to optimize the exploitation of global lupin genetic resources by breeding programs.

Results and applications
Some 145 CSSL were selected by introgressing P. fulvum into cultivated pea via three backcross generations. Genotyping by DARTseq markers produced about 49000 SNP and 56000 PAV markers, of which 1880 could be placed into linkage groups using synteny with Medicago. Fifty additional lines were genotyped by a 13k pea Illumina assay. A subset of 50 lines was phenotyped for morphologic, agronomic and grain quality traits. A transcriptomic study of seed coat was performed using MACE technology.

Introgression of P. elatius into cultivated pea produced over 100 CSSL that await molecular characterization. A pea RIL population of 760 lines was developed, which, after partly unsuccessful GBS genotyping, is genotyped by a 90k Affymetrix Axiom array.

White lupin RIL data produced a dense consensus linkage map with about 3600 GBS-generated SNPs and 465 markers from previous maps. Mapping of sequence-based markers onto the newly-sequenced narrow leafed lupin (NLL, Lupinus angustifolius) genome revealed matching syntenic blocks for 79 % of the white lupin genome. Linkage analyses identified four QTLs for response to vernalization, two for anthracnose resistance, and one for alkaloid content, which differed from mapped NLL loci. Landrace diversity analysis based on 6200 GBS-generated SNPs of over 300 genotypes belonging to 86 landraces from 8 historical cropping regions detected fairly large variation between regions, landraces within region, and genotypes within landraces (the latter being unexpected for a predominantly self-pollinated crop).

Landrace diversity reflected a longitudinal gradient, and was outstanding in the Near East (where the crop may have originated). Variety diversity represented a fairly limited and separate portion of the overall crop diversity. Multi-environment phenotyping data were used to verify the predictive ability of genomic selection, which proved high for grain yield and several other traits.

Significance and benefits
The two sets of pea CSSL, of which one thoroughly genotyped, can be precious genomic and/or genetic resources for future studies of traits to which wild pea may provide useful variability (e.g. tolerance to biotic and abiotic stresses), or for domestication traits that need be selected for when crossing modern and wild...
pea (whose efficient MAS selection would be particularly attractive. The large pea RIL population, once genotyped, will also be an important genomic resource.

The lupin genetic map can be useful for future studies, whereas markers linked to three key traits can be exploited for MAS. The different control of key traits in white lupin and NLL suggests a limit for our ability to exploit information from the close, sequenced species NLL, reinforcing the need for white lupin genome sequencing. The genetic structure of white lupin landraces can drive their exploitation, which is encouraged by the modest portion of genetic variation exploited by modern varieties. The high predictive ability of genomic selection for yield and other traits is promising.

References

Results are given in the Deliverables 1.1 1.2 and 1.6. Some results have been reported in the publication below, while at least three future articles for international journals have been planned.


Key result no 5: New sources of resistance to insect pests identified and characterized
Workpackage: WP2

Research aims and background

Insect pests such as aphids and weevils are becoming increasingly important in legume crops. The most damaging ones are the weevils Sitona lineatus and Bruchus rufimanus for faba bean, and B. pisorum for pea, and the aphids Aphis fabae for faba bean and Acrystosiphon pisum for pea and grasspea. Only a measure of control can be achieved via repeated chemical treatments, which is uneconomical and environmentally hazardous. Biological control and other alternative management practices give today unreliable results.

This encourages an investment in the development of resistant cultivars. Unfortunately, little resistance is available among the cultivars presently available to farmers, necessitating searches in germplasm collections. With this aim, germplasm collections of pea, faba bean and grasspea were screened under field conditions and the most promising accessions were further studied under controlled conditions in dual-choice and no-choice tests to discern levels of antixenosis and/or antibiosis.

Results and applications

A Pisum spp. germplasm collection was screened for resistance to B. pisorum in multiple years and locations, allowing the identification of accessions with reduced seed infestation and/or larval development. As the study pointed to a possible major role of no-preference, a second study discerned further possible genotypic effects on insect fecundity and oviposition describing unattractive or repellent genotypes for oviposition. These accessions have already been introduced in a crossing program to exploit their resistance in cultivar breeding.

Unfortunately, no resistance to S. lineatus or to B. rufimanus was detected in the V. faba collections studied. However, some genetic variability for tolerance to S. lineatus was identified, suggesting escape mechanisms. A study of the F2 progeny of the cross 2080 (the most tolerant) x 1913 (the most sensitive) suggested quantitative inheritance.

Resistance to Acrystosiphum pisum and to Aphis fabae was identified in pea and faba bean, respectively.
The most resistant accessions were further studied under semi-controlled conditions assessing aphid preference and plant damage. Two of the resistant pea accessions were further characterized in a no choice and dual choice experiments showing that resistance of one accession is based on antibiosis by diminishing aphid proliferation whereas on another one is based on antixenosis by deterring aphid feeding.

Significance and benefits
Resistance to Bruchus pisorum and to Acyrthosiphum pisum is now available in pea germplasm and readily accessible for pea breeding programs.

Successful applications
Resistant accessions identified are already introduced in crossing programs outside LEGATO. QTLs are already being identified (in prep.) which will facilitate MAS programs

References
Aznar-Fernández T & Rubiales D, .... Influence of flower and pod genotype on pea weevil (Bruchus pisorum) oviposition capacity and preference on host and non-host species. Annals of Applied Biology, submitted.

Key result no 6: New sources of resistance to diseases
Workpackage: WP2

Research aims and background
Legumes can be affected by a number of diseases for some of which little genetic resistance is available among presently cultivated varieties. Resistance may be available in landraces and in wild relatives that can be exploited in crop breeding.

Results and applications
Germplasm collections, particularly of Pisum spp., but also of faba bean and grass pea, have been thoroughly screened for resistance to fungal diseases (ascochyta blight, powdery mildew, rust, fusarium wilt, chocolate spot), viruses and parasitic weeds (broomrape) paying special attention to the search for
novel mechanisms of resistance acting at early stages of infection. As a result, valuable sources of resistance to the various diseases have been identified, that are now being introduced in crop breeding programs.

Significance and benefits
The novel sources of resistance identified will serve as donors of resistance in legume breeding programmes.

Successful applications
The resistant accessions have already been introduced in crossing programs outside LEGATO, and identification of QTLs and markers is already completed or in progress in some instances.

References
Bani M, Pérez-De-Luque A, Rubiales D & Rispail N, .... Physical and chemical barriers in root tissues lead to quantitative resistance to 4 Fusarium oxysporum f. sp. pisi race 2 in pea. Submitted to Frontiers in Plant Sciences
Rubiales D, 2018. Can we breed for durable resistance to broomrape?. Phytopatologia Mediterranea, submitted

Key result no 7: Markers for Ascochyta blight resistance
Workpackage: WP2

Research aims and background
One of the major fungal disease constraints of most cool season legumes are ascochyta blights. Genetic
variation for resistance has been reported in most legume crops and is being used by breeders. However, available resistances are quantitative and controlled by multiple genes, hampering breeding for resistance to this disease using traditional crossing schemes. Marker assisted selection (MAS) is a promising approach for accelerating the development of varieties with increased resistance to this disease. Genetic studies have identified a number of genomic regions (QTLs) associated with ascochyta blight resistance in most crops, but progress in delivery of markers usable for tracking these QTLs in MAS has been slow. The aim of this study was the identification of genes involved in resistance to ascochyta blight in pea and faba bean and the development of different kinds of markers that could be used to select resistant plants in legume breeding programs.

Results and applications
Using different high-throughput techniques, markers useful for selecting plants resistant to ascochyta blight have been identified. These included 1) expressional markers: genes whose level of expression can be used to distinguish resistant from susceptible individuals; 2) positional markers: SNPs in genes located within the QTLs associated with resistance that can be used to track the alleles promoting resistance in breeding programs; and 3) Peptide markers: peptides whose constitutive quantity could be used as biomarkers to identify the resistant individuals.

Significance and benefits
The level of expression of some selected genes, and/ or the constitutive level of some target peptides have been explored to assist breeding for resistance to ascochyta blight. These approaches, together with the more usual approach of identifying SNPs located in QTLs associated with the trait have resulted in a set of markers expected to speed up resistance breeding programs. The usefulness of these markers for identifying resistant individuals in segregating populations has been already checked in the framework of LEGATO project using a RIL (Recombinant Inbred Line) population segregating for resistance to ascochyta blight

References
Key result no 8: Identification of mlo homologous genes
Workpackage: WP2

Research aims and background
Powdery mildew is one of the most widespread and damaging fungal pathogens in many crops including legumes. A knock-out mutation in one member of the MLO family has led to durable and broad spectrum resistance against this disease in several crops including barley, tomato and pea. Lack-of-function of this MLO has been associated with higher efficiency of papilla formation, impeding cell penetration by a wide range of pathogenic powdery mildew isolates. Although mlo-based resistance is exploited in pea, this resistance mechanism has not yet been exploited in other legumes. Our aim was the identification and characterisation of candidate mlo homologues in legume crops as a first step to exploiting mlo-based resistance.

Results and applications
By mining their genome databases, 13 to 15 mlo genes were identified in temperate legume crops including barrel medic, chickpea, lupin and lentils while tropical legume crop such as common bean, pigeon pea or cowpea possessed 18 to 20 mlo members. Through phylogenetic analysis, three candidate MLOs with potential function in powdery mildew resistance were identified in chickpea, barrel medic, lentil and lupin, while six were identified in common bean and pigeon pea. Comparison of these genes allowed the identification of several clade-specific motifs hallmarking each MLO clade. Based on these results, candidates for conferring mlo-based resistance to powdery mildew were also identified in faba bean, and chickling pea, with the genomic sequence amplification of the whole gene for chickling pea and lentil. The identified candidate mlo in these legume crops can be used as a biomarker to breed for powdery mildew resistance. Targeted mutation or editing of these genes may also contribute to increase the level of resistance in these species. They could also serve to isolate novel mlo homologues in other legume crops.

Significance and benefits
Identification of novel candidate mlo genes in legumes is crucial for future legume breeding for powdery mildew resistance. They will be the base for the development of gene-based molecular markers for MAS, and for the creation of new cultivars with higher levels of resistance through the identification of natural variants in these genes, or the modification of their expression by genome editing approaches.

References
Polanco C, Sáenz de Miera LE, Bett K & Pérez de la Vega M. A genome-wide identification and comparative analysis of the lentil MLO genes. Submitted to PLoS ONE
Rispail N, Almeida N, Gutierrez N, Torres A, Vaz Patto C, Polanco C, Saenz de Miera L, Pérez de la Vega

Key result no 9: Root and symbiotic traits of pea, fababean and lupin
Workpackage: WP3

Research aims and background
Legumes play a major role in the evolution of agriculture towards sustainable management of resources. Nevertheless, in European cropping systems, the agronomic services linked to N fluxes provided by legumes are poorly taken into account in crop rotations, we lack references for reintroducing them. Although there are many legume species, their specific biological properties linked to N fluxes have been mainly studied on pea, the main grain legume crop studied and cultivated in recent decades in Europe. However, lupin and faba bean can be adapted to specific climatic conditions and markets in Europe. Symbiotic nitrogen fixation (SNF) of legumes is modulated by soil nitrate availability and frequently limited by its high sensitivity to abiotic stresses, amplified in the context of current climate change. Among soil stresses, SNF is particularly sensitive to water stress. Furthermore, the nodule development induces additional carbon (C) expenditure, which presumably limits the growth of their roots and therefore their ability to retrieve water and nutrients from soil. This limits in fine legume yields. In this context, our objectives were to characterize and compare the SNF abilities and root architecture characteristics of pea, fababean and lupin, considering their sensitivity to soil N. For this, we conducted complementary field and greenhouse experiments, using both phenotyping and modeling tools to identify species-specific root characteristics explaining differences among species.

Results and applications
In field experiments total amount of N accumulated by pea, fababean and lupin was similar despite different seed yield and N concentration among species. For all species but varying among them, the percentage and quantity of N derived from SNF decreased with the level of N supply. The level of soil mineral N supply in the field had no impact on pea, fababean and lupin growth and N uptake. This indicates that these three legume species compensated low levels of N availability by the onset of SNF. Still, the increased C cost induced by SNF could occur at the expense of root growth. Hence, greenhouse experiments showed significant and consistent effects of nitrate supply on root and nodule traits. As such, for pea and fababean, nitrate supply resulted in an increase in tap root length, number and length of longest first order lateral root and length of longest second order lateral root. For lupin, nitrate supply increased the number of first and second order lateral roots, and the length of longest first order lateral roots only. As such, for lupin, the impact of nitrate mainly occurred through an increase of the number of roots. The increase in the length and number of roots with nitrate supply is consistent with previous observations showing an increase of root growth in presence of high levels of soil nitrate for many species. It is also consistent with observations on legume plants showing decreased root growth when nodulation occurs, due to the high C cost induced by nodule production.

Significance and benefits
The field and greenhouse characterization of various legume species allowed identifying species-specific symbiotic and belowground traits and their response to soil mineral N which could be related to differential
ability to retrieve soil N and be consistent with varying level of soil nitrate remanig after harvest. This will help in the choice of best legume species adapted to a given soil nitrate availability and associated risk of nitrate leaching.

In addition, our greenhouse experiments using different experimental set ups will help defining the best experimental design for measuring the most robust and accessible root traits. Indeed, their measurement can be tedious and time consuming. As an example, the effects of nitrate on root traits could be observed either using the whole detailed information from root profiles in rhizotrons, or using simplified measurements on the two main roots only in hydroponic culture.

Successful applications
Calculated root parameters reflect the potential root architecture. Combined with root biomass as an input of carbohydrates, they together determine the actual root architecture. This data set will be of high relevance to fully simulate root profiles, whilst breeding for improved root phenotypes.

References
A publication is in preparation and will be submitted in February 2018.

Key result no 10: Development of non-invasive methods to study the dynamics in structure and function in nodules and pods of pea
Workpackage: WP3

Research aims and background
In many important production sites, drought or heat stress have a detrimental influence on SNF, their mechanistic effects on resource allocation to nodules and roots and seed filling in pods. In the case of nodulated roots, it is both the structure and the function, in terms of SNF, that is of interest for investigating the effects of stress. It is not only interesting what the size or volume of the pod is but also what the dynamics of the contents, such as water and protein or carbohydrates, are, and how they develop and respond to stress.

Methods for investigating the developmental and functional dynamics of such structures would be very useful in investigating development with and without stress of all legume crops, which would allow a new perspective for knowledge-based breeding approaches and optimisation of rhizobial strains and inoculation protocols. Also development of the yield-relevant organs of other crops, such as wheat ears, are an important field of study for academia and breeders alike.

Results and applications
We focussed on developing two different methods:
1) For the development of the root-nodule system and the function of the nodules as a site of SNF, we applied a combination of magnetic resonance imaging (MRI) and positron emission tomography (PET). MRI can image roots and nodules non-invasively in 3D and was used to monitor the development of the roots and nodules of individual plants. PET is a 3D non-invasive technique as well, but requires the application of a radiotracer. We here used 11C, administered as 11CO2 to the leaves. The tracer was fixed and transported as photoassimilate towards sink regions such as roots and nodules. The spatial resolution for the root system was sufficient to identify the individual nodules (Deliverable 3.1). The allocation of C tracer towards nodules and roots was monitored over four days and stable under control.
conditions. To simulate stress, we applied nitrate in an amount sufficient to strongly affect SNF. This resulted in a significant drop of tracer allocation towards the nodules and an increase in allocation towards the lower roots.

2) seed filling in pods with a mobile nuclear magnetic resonance (NMR) sensor that we developed. This sensor allows for monitoring the water- and solid matter content in individual pods in a fully automated manner over extended periods of time. The method was successfully tested in the greenhouse with different plants of various genotypes, and multiplexed to allow up to six plants to be measured in parallel. In this manner control and stress treatments or different genotypes could be investigated simultaneously.

Significance and benefits

Nodule function is usually evaluated after harvest, at a time scale of days to weeks, by measuring growth and estimating colour. Carbon allocation could only be measured after harvest as well, using stable isotope tracers or long-lived radiotracers. Such destructive methods are not suited to study dynamic processes and require large numbers of plants. The application of 11C tracers and PET allows for the first time to study the dynamics of C allocation in response to abiotic stresses in 3D, non-invasively and at a timescale of hours to days, both in roots and nodules. Due to the short half-life of the tracer, multiple experiments can be done on the same plant. In between PET measurements other methods can be applied as well. After final harvest the plant remains available for complementary analysis, such as gene expression and enzyme activity essays.

Seed filling and development in pods has so far been studied destructively and gravimetrically. The NMR sensor makes it possible now for the first time to monitor the dynamics of seed filling in terms of water- and dry matter content in the living pods. It thus makes it possible to directly test for the resilience of seed filling against abiotic stresses.

References

D3.1 D3.3 and Milestone M17

Key result no 11: mfn mutations potential in relation to improvement of agronomic traits
Workpackage: WP3

Research aims and background

Previously to LEGATO, we had identified mutant alleles of the pea MORE FLOWERING NODES (MFN) genes, which encode two homologous transcription factors. In a preliminary study, carried out under our standard (optimal) greenhouse growing conditions, we have observed that loss-of-function alleles of these genes, mfn1 and mfn2, increased the number of flowering nodes in pea, without apparently affecting the number of vegetative nodes, and led to an increased production of pods and seeds in the single and the double mutants. Therefore, MFN might constitute a promising genetic resource to increase seed yield in pea that deserves to be further studied. The question addressed here was whether the mfn1 and mfn2 alleles do have the potential to increase seed yield in pea under different growing conditions, including field trials, and whether the mfn mutations affected seed quality.

Results and applications

The two mfn mutants originally available were obtained from a screening of a pea EMS mutagenized population. For that reason, before carrying out a detailed phenotypic characterization, the mfn mutations
were "cleaned" through backcrosses (BC) with their parental wild-type genotype (Cameor cv). BC5 seeds were obtained but the phenotypic characterization has been carried out with BC2 plants. The increase in seed yield observed, in the preliminary studies, as result of mfn mutations was confirmed in the detailed characterization with the "clean" mutant alleles. This has been clearly confirmed under standard greenhouse growing conditions. The mfn mutations did not significantly affect flowering time. Also, studies under greenhouse conditions suggested that the plants carrying mfn mutations were more productive than the wild-type parental under conditions of moderate heat stress (23.5 °C max tº). The increase of seed yield observed under greenhouse conditions ranged from ~100% for mfn2, 85% mfn1 mfn2 and 15% for mfn1.

Importantly, the concentration of basic components of seed composition (ash, fat, fibre and protein) was the unaffected in the mfn mutants. Possibly, the most encouraging result of our study is the analysis of the mfn mutants in a field trial (carried out in collaboration with the group of Dr Tom Warkentin (University of Saskatchewan, Canada). It demonstrated that the three mutants (mfn1, mfn2, and the mfn1 mfn2 double mutant) clearly displayed a higher production of pods in the main stem and in the rest of the plant’s branches, resulting in a seed yield notably higher in all the mutants, the double mutant being the most productive genotype (where the yield was double that in the wild-type parental, Cameor cv.), followed by mfn2 and by mfn1.

Significance and benefits
The results obtained strongly suggest that the MFN genes could represent a novel genetic resource with a strong potential for improving seed yield in pea (and other legumes), that could benefit from an increased production of flowering nodes/pods under certain growing environments.

Successful applications
The mfn alleles have been introgressed in pea elite varieties that should be soon ready to be evaluated in field trials. The potential area of this research is the increase of yield in pea and, quite possibly, also in other grain legumes.

References
References to the LEGATO related publication(s) and LEGATO deliverable(s):
Planned publications: one publication on the MFN genes describing their identification, molecular characterization, mutant phenotype, and possible impact on yield (possibly, including field traits performed by Tom Warkentin's group, U Saskatchewan at Saskatoon) for 2018. LEGATO deliverables: D1.4 and D3.3

Key result no 12: Classifying key molecular and germplasm targets towards better adaptation of legume plants abiotic stresses
Workpackage: WP3

Research aims and background
Legume plants encounter variable stress, including heat and drought, most of the time occurring simultaneously and sequentially. Research herein conducted aimed at identifying:
A) for pea, the physiological mechanisms and the associated molecular basis related to drought acclimation and resilience. Identifying key targets for plant improvement under multiple and successive
drought events involves assessing gene expression in roots and nodules (i.e. tissues which are the closest to environmental condition changes, morphological or functional response being the key targets to plant improvement) of plants subjected to i) single drought/rewatering environmental conditions then ii) more realistically occurring situation in the field where multiple episodes of drought are followed by subsequent increased water availability;

B) for a variety of legume plants, their responses to various environmental conditions through contrasted treatments comprising either a water stress, a high temperature stress or a combined high temperature x water stress at various periods of their phenology. To that purpose, a range of legume species and genotypes was chosen: i) faba bean, lentil, pea and lupin most cultivated variety in France and ii) pea mutants for transcription factors (MFN, more flowering nodes) which increase pod and seed number per plant and screened for their potential increased yield and its stability according to the environmental treatment. Plants morphological responses were characterized after three weeks treatment period.

Results and applications
The series of experiments on pea (genotype Cameor) where drought period(s) were followed by subsequent rewatering period(s) provided i) an exhaustive overview of the expressed genes at the beginning and/or the end of each period and the identification of the most differentially expressed ones ii) highlights of their expression during fine kinetics studies where analysis were temporarily more frequenced. Most of the 400 genes which were significantly differentially regulated in roots and nodules as a result of drought, were still differentially expressed after a subsequent rewatering period, but in an opposite way. The most highly deregulated genes were subsequently analysed for their expression patterns in response to successive drought/ recovery periods. Although among biomarkers found for acclimation and resilience to drought, none were common in both roots and nodules, a biomarker was common in roots and nodules for acclimation to drought. All the highly deregulated biomarkers in roots were common to acclimation and resilience conditions, while in nodules, only 3 biomarkers of the 7 for acclimation could be used as biomarkers for resilience.

Greenhouse trials allowed classifying responses of a range of legumes to factorial combination of drought and high temperatures. This study revealed the differential behavior of roots in response to temperature or water stress, depending on the four legume species, thus underlying the need to focus on how the root system architecture is regulated or on the different physiological processes behind the variety of root functions. Legume species presented a variety of shoot or root related response, reacted differentially to stresses in the above ground and the underground parts of the plant. Interestingly, lentils increased their nodule number and nodule biomass under high temperatures while the others species maintained (in pea) or decreased their N2-fixation structures (in faba bean and lupin).

Significance and benefits
Such studies allows identifying common and specific metabolic regulatory processes involved in pea drought tolerance and recovery after water stress. Methods which were used in the molecular studies conducted in these experiments can be readily transferred to either other species or stress. The putative function of these biomarkers, already involved in a large range of abiotic as well as biotic stresses, has to be further studied to i) assess their specificity towards drought stress, ii) understand the associated physiological mechanism. Biomarkers screening studies performed in more realistic conditions such as in the field will ultimately contribute to identify candidates genes for enhancing legume tolerance to drought. The differential response to temperature or water stress of roots from the various legume species
highlights the need to focus on the regulation of their root system architecture. This can be pursued using root image analysis and will lead to identify putative morphological root traits (number of roots, root length,...) which could promote a better tolerance to abiotic stresses.

In conclusion, both studies enriched our knowledge concerning both structural and functional response of a variety of legume plants subjected to various environmental stresses, occurring either alone or combined in a sequential and/or repetitive manner.

References
References to the LEGATO related publication(s) and LEGATO deliverable(s): D3.2 and D3.6.

Key result no 13: Grain legumes nutritional, organoleptic and processing potential quality breeding disclosure
Workpackage: WP4

Research aims and background
Grain legumes are important sources of proteins that have recently gained attention as contributing to disease prevention. Additionally, the potential of grain legumes for incorporation in food products depends on their technical ability for processing and these food products acceptability will depend on their taste, also influenced by different legume components. The European grain legume germplasm has great potential for nutritional and organoleptic quality improvement. However, the lack of a comprehensive characterization of the existing genetic variability for the nutritional, organoleptic and processing quality has hampered their exploitation in breeding. A collection of more than 500 accessions of the most important European culinary grain legumes was assembled from LEGATO germplasm banks and breeders working collections and subjected to a comprehensive chemical-physical, organoleptic and rheological/end-user analysis. The main objectives of the work were to quantify the existing variability and identify outstanding grain legume accessions, as sources of high nutritional value (such as protein content and quality), adequate health-beneficial component contents, interesting organoleptic properties (such as aroma volatiles and sugar contents), and adequate processing-related traits, for breeding programs and genetic studies on quality.

Results and applications
For the basic nutritional and health-beneficial related traits analysed in the entire collection of grain legume species, significant variability was found among the five species (pea, faba bean, chickpea, lentils and grass pea), but also within species. This was the case not only of the major constituent-related traits such as protein content and quality (accessed by individual amino acid contents), fat and fibre contents, but also of the minor component-related traits such as carotenoids and tocopherols, phenolic components and the associated antioxidant capacity, or the resistant starch content. In addition, significant variation was detected for the traits measured just on some of the grain legume species such as the different cell wall polysaccharides contents (neutral and acid sugar contents) in peas and faba beans, and the sucrose and sucrose-derived metabolites content on faba bean. Significant variability was also detected among species for whole flour aroma volatiles. However, the capacity to differentiate between accessions within each legume species studied depended on the grain legume. In relation to whole flour dough viscosity profiles, some faba bean and pea accessions displayed peak viscosities similar to those of cereal flours, and particular grain legumes species, such pea, showed higher diversity of viscosity parameters.
Chickpea accessions displayed the higher water absorption capacity. Cooking time was also very variable between accessions. All this variability should provide good opportunities for trait improvement through conventional legume breeding. Additionally when this compositional/processing variability was analysed taking into consideration seed morphological traits with influence on market class definition, it provided important guidelines for quality improvement in these grain legume breeding programs.

Significance and benefits
With this comprehensive characterization of quality-related traits of this five grain legume species collection we gained a deeper understanding of the trait variation contributing to nutritional, organoleptic and processing quality, and we can now judge if the existing genetic variability allows their improvement through conventional plant breeding, and also highlight the most interesting sources of these traits to be included in future quality breeding programs, as well as to identify the most contrasted accessions for future genetic studies.

References
LEGATO associated Deliverables: 4.2 and 4.3. Two published articles, six articles for internationally refereed journals being prepared.

Key result no 14: An array of legume fortified cereal breads: Alternative and well accepted convenient ways to increase legume consumption
Workpackage: WP4

Research aims and background
One main determinant of food choice is taste. In order to identify the most important quality traits for legume consumers, chemical analysis must be correlated with sensory evaluation, and prior to this, legumes should be processed. Due to the emergence of new food habits and increased consumer awareness of the nutritional merits of legume components, sensorial analyses of innovative food formulations involving legumes, were considered in LEGATO. As case studies, maize and wheat traditional breads were fortified with different grain legume flours as a convenient way of increasing legume consumption. The main objectives were the development of innovative food formulations incorporating grain legumes (legume fortified maize and wheat breads) and their optimization taking into account the most important quality traits for consumers through a sensorial analysis in two different European countries (France and Portugal).

Results and applications
In what concerns the wheat bread, several dough mixes, with different proportions of wheat/pea flours were developed in order to make a legume-fortified (French) baguette bread. When compared to the standard wheat bread, major impacts were detected on the rheological properties and on the coloration of the bread. Nevertheless sensory analyses were very promising, with no significant differences in perception between the different proportions of pea flour incorporation. Once optimized, this procedure
should permit the development of a high protein content-claimed bread with good acceptance by the baguette consumers. Concerning the maize bread, several composite maize “broa” breads were produced with four different grain legume (faba bean, pea, lentils and chickpea) incorporation on four different maize flours (hybrid and traditional varieties). “Broa” is one of the Portuguese ethnic breads made with more than 50% of maize mixed with wheat or rye flours. The optimization of the maize bread formulation by consumer liking and descriptive profiling provided a sensory map of the samples. Major changes in “broa” sensory profile appeared related to apparent humidity and bread crumb cohesiveness. Significant differences were found between the different maize flours, but no significant effect was found for the legume flour incorporated, creating an array of alternative fortified formulations for the development of nutritiously enriched “broa”, qualifying for a high-protein-content-claim and being well accepted by consumers.

Significance and benefits
Overall, this approach conducted at industrial scale with consumers engagement proved that the incorporation of legume flours into different cereal breads appears to be an acceptable strategy for diet diversification, since an array of different formulations for high-protein-claimed cereal breads, nutritiously enriched and well accepted by consumers, was developed, as well as the identification of the most important components affecting consumer choices. This will allow future fine-tuning of legume breeding objectives to match consumer preferences.

References
LEGATO associated Deliverable: 4.3. One article for internationally refereed journal being prepared.

Key result no 15: High-throughput spectroscopic tools for routine implementation of quality objectives in grain legume breeding
Workpackage: WP4

Research aims and background
Breeding for improved grain legume quality is a complex task due to trait interaction. Particular metabolites act as antinutrients as well as health-promoting agents, influencing both taste and consumers' acceptability. The European grain legume germplasm has great potential for quality improvement. However, in addition to the lack of a comprehensive characterization of the existing genetic variability for quality related traits, also the lack of fast screening tools for these traits hampers their exploitation in breeding. Spectroscopy techniques offer a reliable cost-effective alternative approach for rapid and easy analysis of bio-molecules, with little need for sample preparation, being therefore an appealing technology for breeders. The LEGATO project had the objective to develop fast, reliable and cost efficient spectroscopic screening tools to routinely implement selection for quality in legume breeding programs.

Results and applications
Attenuated Total Reflectance Fourier Transform infrared, ATR/FT-IR (ITQB, Portugal), Near-infrared Hyperspectral imaging, NIR-HSI (INRA, France) and Near-infrared spectroscopy, NIR (INIAV, Portugal) spectra from a collection of about 500 different accessions from five grain legume species (chickpea, faba bean, grass pea, lentil and pea) were collected and combined with the physical-chemical quality related traits data measured through standard methods on the same accessions. Multivariate analysis of this combined data set allowed the identification of spectrally outstanding species and accessions and the
development of species-specific predictive models for key quality parameters in grain legumes. High diversity was detected among accessions ATR/FT-IR spectra, being grass pea and pea the most differentiated grain legumes. Through NIR spectra, outstanding accessions were identified for all the species. The multivariate analysis of NIR-HIS spectra allowed the clear separation between species and also the identification of outstanding accessions in all the species. Some of these accessions were simultaneously identified by different IR approaches and could be related to a specific biochemical composition. NIR prediction equations were optimized for moisture, fat, protein and fibre content, and new NIR-HIS prediction equations were developed for proteins, fat, total phenolic content and antioxidant activity, applicable to all the grain legumes under study, and for arabinose content in faba bean or pea.

Significance and benefits
In conclusion, the work developed validated the use of spectroscopic predictive models as high throughput tools for key quality traits routine evaluation in grain legumes. The developed predictive models may be used onwards by the project partners as quick and simple quality screening methods. This will allow future fine-tuning of legume breeding objectives to match consumer preferences.

References
LEGATO associated Deliverable: 4.4. Two future articles for international refereed journals are planned.

Key result no 16: Analysis of consumers’ willingness to pay for novel grain legume food uses
Workpackage: WP4

Research aims and background
Current European consumers choices highlight the search for nutritional and health benefits in food without neglecting the sensory and convenience aspects of food. These demanding choices are a challenge for the current food industry, which seeks to introduce new ingredients for nutritional purposes and also health promoters with functional properties in their products. These growing trends offer an opportunity to the grain legumes industry in the development of convenient alternative food and industrial products. Indeed, consumers believe that grain legumes’ health and environmental attributes are important, but are they willing to pay more for the benefits they provide? Do consumers accept the fortification of traditional food with grain legume ingredients? Is signalling the benefits of grain legumes perceived and valued by consumers? Are consumers willing to trade-off taste for health benefits? The main objective of work developed under LEGATO was to measure consumers’ willingness to pay for foods fortified with grain legumes.

Results and applications
Consumers’ behaviour was analysed through experimental markets (auctions), using LEGATO developed innovative fortified maize bread formulations, with a partial replacement of cereal by different grain legume flours. A theoretical research, empirical work and experimental markets study was developed in the field of experimental economics to reveal the values of consumers’ willingness to pay for legume fortified bread and their interpretation. In the theoretical stages the principal concepts and incentive mechanisms of experimental economics were identified as well as the main publications on the study of consumers’ preferences for fortified breads. The combination of sensory techniques and experimental economics mechanisms was highlighted and reinforced the interest in the joint use of these methods. The developed
experimental markets contributed to the identification of consumer preferences for breads fortified with different grain legume flours. Knowledge was obtained on consumers’ acceptance (also based on the economic evaluation of the products) of the incorporation of grain legumes ingredients into a traditional food product and on the impact of information (sensorial, composition and nutritional) on willingness to pay for these types of breads.

Significance and benefits
The experimental market results revealed that sensorial attributes were essential in the confirmation of consumers’ monetary valuations; but the non-sensorial information, was important in strengthening the position of each fortified bread in the hierarchy of consumer preferences. This approach contributed to closing the knowledge gap between the recognition of the nutritional and health benefits of grain legume food products and the most important components determining consumer choices.

References
LEGATO associated Deliverable: 4.5. One article for an international refereed journal is being finalized.

Key result no 17: Design and Assessment of legume-based cropping systems
Workpackage: WP5

Research aims and background
Grain legumes included in cropping systems result in many agronomical and environmental benefits such as reduction of greenhouse gas emissions and fossil energy consumption, due to the reduction of N fertilization. Legumes also have positive effects on the following crops: N fertilization may be decreased, and the yield of the following crop is higher compared with other preceding crops. Current European agriculture is now dominated by simplified cropping systems, i.e. short crop sequences and high use of chemical inputs. Our aim was to design locally adapted innovative cropping systems including grain legumes (several species and types of cultivation) for three European local pedo-climatic contexts (from north to south: Scania in Sweden, Paris Basin in France, and Andalusia in Spain). We used the prototyping method, and discussed the feasibility of designs with a large panel of stakeholders in each local context, based on their description and assessment with the MASC tool, accounting for stakeholders’ point of view on sustainability. Our hypothesis is that even if legumes are not grown in large areas in Europe, legume-based cropping systems could be implemented and would contribute to increased sustainability compared with the current cropping systems if the conditions are met. The methodology for the design and assessment of locally adapted cropping systems with legumes is described and could be applied to other contexts.

Results and applications
Four to five cropping systems were designed in each local context, with crop sequences longer than references. They included at least two grain legumes (pea, faba bean, chickpea, lentil or lupine), as sole crops or intercropped with cereals. Overall sustainability was similar or improved in 71% of the legume-based cropping systems compared with their corresponding references. Stakeholders from different countries, with different activities and different priorities proposed four weight sets corresponding to four different points of view on sustainability. However, it was possible to find cropping systems including grain legumes that could be implemented in each region as they were considered feasible by stakeholders.
Significance and benefits
Despite the differences between the three local contexts in terms of pedo-climatic conditions, socio-economical context, local improvement target and crop species, the same methodology was successfully applied, and led to diverse and interesting innovative cropping systems, with improved sustainability assessment results, generating fruitful discussions during the design and stakeholders workshops. The cropping systems are area-specific even if the methodology for design, assessment and discussion on feasibility of cropping systems could be applied in any other areas of the world.

Successful applications
The principles of MASC analysis of cropping systems involving stakeholders is used in national research projects.

References

D5.1. Modified MASC model adapted to the diversity of legume crops and crop management plans in Europe.
D5.2. Novel prototypes of cropping systems with GL and ranking of new cropping systems that appear acceptable and efficient for both stakeholders, farmers and collectors on a multi-criteria point of view.

Key result no 18: Pea variety mixtures for enhanced yield stability and reduced weed infestation
Workpackage: WP5

Research aims and background
European conventional agriculture is often based on simplified systems and a large input of agrochemicals. Crop diversification in space and time is a key component in the design of resource-efficient cropping systems with enhanced use of ecosystem services and reduced dependency on inputs of synthetic agrochemicals. Diversification of cropping systems offers opportunities to the improved use plant growth resources, reduced pest, weed and disease problems, and enhanced resilience to stress and yield stability. Within-species diversification by variety mixtures may increase yield stability, has been studied in cereals and is now used in practical agriculture. Enhancing the yield stability of European grain
Results and applications
The results showed that a variety mixture of pea can combine the beneficial traits of the individual varieties with maintained total pea grain yield relative to the highest-yielding variety. However, in some experiments, it was observed that the land equivalent ratio (LER) was greater than 1.1 indicating more than 10% advantage from the variety mixture. Results from the four sites indicated the variety mixtures' yields were more stable than those of sole crops, but the data require further analysis. Analysis of the competitive interactions between the two cultivars showed that the normal-leafed cultivar was more competitive than the semi-leafless cultivar at maturity. However, the benefits in terms of weed reduction and standing ability were not as strong in variety mixtures as was observed in pea-wheat intercrops included in the same experiments. Lodging resistance of the variety mixtures was observed to be intermediate between the individual varieties. At one site pea variety mixtures have proved effective in reducing the incidence of pea powdery mildew. In another site growing an early variety at the edges of a pea field resulted in the damaging Sitona weevil being concentrated on the early-flowering variety. This points to a possible use of the early type of varieties as trap crops. These results must be confirmed in further experimentation.

Significance and benefits
Pea variety mixtures may enhance grain yield stability as a result of compensating effects of the cultivars, which have differential responses to abiotic and biotic stresses. Variety mixtures of GL may be more applicable than species mixtures with GL in systems with herbicides, and may potentially reduce the requirements for herbicides in conventional agriculture, but further experimentation and analysis are required to determine these potential benefits from grain legume variety mixtures.

Successful applications
Results will be utilized within the H2020 ReMIX project.

References
D5.3 Crop diversification for enhanced yield, yield stabilization and management of biotic stresses of GL based crops

Key result no 19: Disease management in grain legumes by crop diversification
Workpackage: WP5
Research aims and background
Biotic stresses are a major challenge for the incorporation of legume crops in agro-ecosystems. Diseases may significantly reduce yields, adding uncertainty to the return of the investment that farmers make. Chemical control is usually too costly both in economic and environmental terms. Crop diversification may be a more efficient alternative for reducing the incidence of diseases in legume crops. It has previously proved useful in other pathosystems, so we decided to test it in two important legume crops, faba bean and pea, focusing on two of their major diseases, rust and powdery mildew, respectively. A satisfactory control of both diseases would be an important step towards the implementation of crop diversification in legume crops.

Results and applications
Two different strategies for introducing diversification in agricultural systems were used: intercropping, by mixing different crops in the same field, and variety mixtures, i.e. growing together different varieties of the same crop. In the case of intercropping, the main crop (faba bean or pea) was mixed with either one of two cereals (wheat and barley) or another legume (pea or faba bean). As for varietal mixtures, the experimental design consisted of mixing different proportions of a resistant monogenic variety with a susceptible one. Alternate-row mixing was the system employed in both cases. Results point to a significant reduction of the incidence of both diseases in the intercropped treatments compared to that of the monocrop. Both in the cases of rust in faba bean and powdery mildew in pea the best results are obtained when the legume is combined with barley. Varietal mixtures also bring about a significant reduction in the levels of both diseases in the susceptible variety. A proportion of 50/50 (resistant/susceptible) appears to be the most efficient combination, balancing adequately disease suppression and increase in genetic variability. This increase in genetic variability also contributes towards the durability of the genetic resistance.

Significance and benefits
This is the first time to our knowledge that the effects of crop diversification have been tested in these pathosystems. The positive results open the door to further testing with other diseases, aiming to offer to the farmer a comprehensive system of disease control by crop diversification. The employment of two different strategies (intercropping and variety mixtures) also increases the opportunities for implementation of diversification. In particular, the employment of variety mixtures needs the conjunction of another relevant strategy in sustainable agriculture, which is the use of resistant varieties. This synergy of different strategies paves the way for a more environmental-friendly control of diseases in legumes.

Successful applications
Results will be utilized in the H2020 ReMIX project.

References
References to the LEGATO related publication(s) and LEGATO deliverable(s):
D5.3 Crop diversification for enhanced yield, yield stabilization and management of biotic stresses of GL based crops.
Key result no 20: Assessment of the requirement for inoculation of pea and faba bean
Workpackage: WP5

Research aims and background
It is well-established that inoculation of pea and faba bean with Rhizobium leguminosarum symbiovar viciae (Rlv) is not required for obtaining symbiotic capacities in most European soils, except on very acid soils. This is because large populations of Rlv strains are normally present. However, recent studies from other parts of the world have shown that inoculation of pea with efficient Rlv may be required at low population densities or when indigenous strains are poorly effective. The requirement for inoculation was tested in five European pedo-climatic conditions. Rlv was collected at sowing from the five sites and was characterised by developing a generally-applicable method for reproducible extraction of nodD DNA from soil and estimating the rhizobial abundance and diversity. We also determined the efficiency of indigenous Rlv, and the genetic composition of indigenous nodulating populations from the five soils by PCR on molecular markers. Efficiency and competiveness of predominant genotypes was evaluated by nodulation assays in controlled conditions. Finally, the inoculation strategy was evaluated on field experiments with pea and faba bean crops, with and without inoculation, on the five sites.

Results and applications
Estimates of nodD sequence abundance in the soil samples were approximately ten times higher than most probable numbers of nodule-forming units estimated from plant test. The abundance of nodD varied from $5.8 \times 10^4$ to $1.7 \times 10^8$ copies per gram of soil, indicating that all the soils had presumably enough rhizobia for effective nodulation of the crops. The Rlv populations from the five European sites were characterized after trapping on pea and faba bean. A total of 214 Rlv isolates (131 pea/83 faba bean) have been collected and nodD sequenced. They belong to the five geographic origins: 59 from France (39/20 pea/faba bean), 70 from Sweden (50/20), 54 from Czech Republic (31/23), 24 from Serbia (11/13), 7 from Spain (0/7). Representative Rlv isolates were used in inoculation experiments under controlled conditions to estimate efficiency to fix nitrogen. A large variation was found within locations according to the host plant, confirming that symbiotic potentials of the soils strongly vary and are not equivalent with pea or faba bean. Variations were found between isolates at the same location and on the same host plant, indicating that isolates of one geographic origin and belonging to closely related soils may display contrasted capacities to promote efficient symbiotic activity. Selected Rlv strains efficient for pea or faba bean from each of the five European sites were used as inoculum in comparison with a reference strain inoculum, and no inoculation, in field experiments at the five sites in 2017. Unfortunately, only two of the five experiments were successful. In these experiments, the inoculation with local efficient strains or the reference strain did not significantly affect pea or faba bean yields, even though on one of the sites hosts have not been grown for 20-30 years.

Significance and benefits
This work has isolated the largest collection of Rlv strains available so far. The collection provide a valuable basis for investigating the effect of variability of Rlv partner on pea and faba bean symbiotic traits in the future. Further experimentation is required to test whether an inoculation strategy consisting of determining the Rlv population size, diversity, efficiency and selecting efficient local strains for inoculation
may prove valuable for enhancing or stabilizing yields in pea and faba bean.

References
References to the LEGATO related publication(s) and LEGATO deliverable(s):
Deliverable 5.4 Assessment of the requirement for inoculation of pea and faba bean in different agro-pedo-climatic conditions in Europe

Key result no 21: Genotypes for European climatic zones
Workpackage: WP6

Research aims and background
Due to trade deals made in the 1980’s, the EU has started to import cheap US-grown soya as a source of protein for livestock. Recent policy changes have favoured its replacement by home-grown pulses (for protein). Along with this, the EU has funded grain legume research projects and LEGATO is one of these. The aim of these projects has been to increase collaboration between European grain legume researchers and to disseminate findings to farmers and consumers (food and feed) throughout Europe. LEGATO in part aimed to increase the awareness of the suitability of home-grown grain legumes within 3 climatic zones across the EU, Continental, Maritime and Mediterranean. A trials network of plant breeders across Europe ranging from Estonia and the UK in the North, through to Greece, Spain and Portugal in the South, was established. Eleven countries were involved. The network also identified the priorities for trait selection. Species were defined (mainly by breeders), for specific climatic zones within Europe. The results of the trial series are available to growers to allow an informed decision as to which species and varieties to grow in the future, and also to encourage users to move away from imported soya, in favour of sustainable home grown grain legumes.

Results and applications
Spring peas were well adapted to all climatic zones, but winter peas were less so in the Continental zone due to the more extreme winters. The same reason prevented winter beans from being grown in the Continental zone. Winter beans can be successfully grown the Maritime and Mediterranean zones. Spring Beans are well adapted for the Continental and Maritime zones, but not in the Mediterranean zone where they are sensitive to the lack of water and high temperatures in the spring and parasitic plants such as Orobanche. Other more niche grain legumes are less widely distributed and less adapted to most zones. There are species of grain legumes adapted to every climatic zone within Europe, with spring peas being the most widely adapted.

Suitability of species within climatic zones based upon Legato trial series 2015-2016
The table attached (Figure 51 in Deliverable D6.3) gives an indication of the adaptability of species/types to the three climatic zones. Adaptability is based on the ratings given below.

Successful applications
Data has the potential to be further interrogated by future projects, and H2020 projects such as LegValue and TRUE have shown interest. Raw trials data is publically available on the LEGATO website.
Statistical analysis of pea data by the DIAGVAR method has the potential to be adapted to faba beans.

References
Deliverable 6.1. Trials and analysis of Genotype x Environment interaction
Deliverable 6.3 Definition of adapted ideotypes (genotypes x cropping systems) for different European regions, with seed quality fitting users requirements: document of synthesis

Key result no 22: Marker-assisted selection
Workpackage: WP6

Research aims and background
Faba bean seeds contain high levels of vicine and convicine which are considered as having anti-nutritional effects on human and some domesticated animals. The consumption of faba bean varieties with high vicine/convicine (Hvc) content is limited in these groups. Faba bean lines which have low vicine/convicine contents have been identified. Breeding (e.g. introgression) for low levels of vicine/convicine is hampered by the lack of suitable molecular DNA markers. Using markers it is possible to predict vicine/convicine content at a very early stage of plant development if cotyledons are genotyped by gene-specific or flanking genetic markers. The MAS approach could simplify the determination of vicine/convicine content and is becoming widely used to help breeders to speed up and rationalise plant selection. Trait-specific DNA-based flanking genetic markers were developed for low vicine-convicine content in WP1. Screening work and subsequent crosses were carried out by NPZ and SZG using the available markers. Also tested were four major traits relating to plant protection in peas. These markers were adapted to detect resistance or sensitivity to four types of economically damaging viral and fungal pathogens, pea seed-borne mosaic virus P1 (PSbMV), pea enation mosaic virus (PEMV), Fusarium oxysporum race 1 and powdery mildew (Erysiphe pisi f.sp. pisi). Initial screening work was conducted by Agrovegetal and Agritec, but all work was later transferred to Agritec.

Results and applications
Within the markers used for Hvc, clear results were obtained in the PCR assay, but there was not a complete correlation with the reference (photometric) analysis. This may be due to the genetic structure of the material. Work is continuing to fine-tune the marker or marker combinations for an optimal correlation between genotypic and phenotypic data. Four pea parental lines and 43 crosses were tested for the 4 fungal/viral pathogens. Lines of interest will be continued within the Agritec breeding programmes. The crossing material derived within the project will now be used for the validation of the developed markers.

Significance and benefits
Traditional plant breeding can take many years to integrate a desired trait into a breeding programme but MAS provides a useful tool for plant breeders looking to speed up the selection process. Firstly, this will benefit breeding companies by allowing them to produce better varieties with specific traits more quickly, also the grower, by providing new varieties with improved agronomic performance and finally the consumer (food or feed) by improving nutritional benefits.
Flanking as well as gene-specific DNA-based genetic markers were used for genotyping the breeding material. Based on the genotypes of the individuals of the segregating populations, potential elite single
plants were identified and will be used further in the appropriate breeding programs of NPZ, SZG and Agritech. This could ultimately improve the uptake of grain legume production within Europe.

Successful applications
MAS provides a useful tool for plant breeders looking to integrate new traits into their breeding programmes more quickly and to produce better varieties. Ultimately this will benefit the grower and consumer (food and feed) by providing new varieties with improved agronomic performance and nutritional quality benefits.

References
Deliverable 1.7 A public document on what has been achieved in the WP
Deliverable 6.2 Marker-assisted selection results.

Key result no 23: Impact of Stakeholder meetings
Workpackage: WP6

Research aims and background
The content of the stakeholder meetings was defined in the DOW, but flexibility in the topics allowed for local bias and current topics, particularly in terms of policy changes that occurred during the period of the project.

The first meeting in Alnarp, Sweden, on the 26 February 2015 was jointly organized with a local group, LegSA and topics were aligned accordingly. It was dedicated to the analysis of the brakes and levers in grain legume production. Invited speakers and LEGATO partners presented examples of innovation in marketing grain legumes for human consumption, which could provide important niche markets for relaunching GL production. The second meeting in Cordoba, Spain on the 1 December 2015, focused on the use of legumes as pasture and forage in the Mediterranean region, as well as animal feeding in different European countries (Spain, France and UK) from an economic point of view, and underlining techniques which can improve the economic value of legume seeds. Priorities expressed by the Andalusian stakeholders were addressed. Meeting 3 took place in Troia, Portugal on the 12 to 14 October 2016 along with the 2nd International Legume Society (2ILS) Conference. It covered themes from within LEGATO and was a platform for presenting the first results. Several LEGATO partners were selected for oral and poster presentations. Meeting 4 was organized on the 17 May 2017, in Czech Republic, and hosted by Agritec. This event was in part dedicated to breeding. Finally, although not declared as a stakeholder meeting, the International Conference held jointly with Eurolegume in September 2017 (Abstracts at: http://www.legato-fp7.eu/FinalConference/pdfs/Book_of_abstracts_LEGATOEUROLEGUME.pdf) also provided a forum for exchange with plant breeders and food processing representatives, as well as with advisory bodies such as Demonet (Demonstrationsnetzwerk Erbse/Bohne).

Results and applications
The EU has the ability to produce a significant proportion of protein from pulses, however, this depends upon many factors; cheap soybean imports, more profitable crops, policy and disease and abiotic stress. Research projects such as LEGATO can help by providing innovative genetic material better adapted for
food and feed, and low-input agriculture. The use of legumes as pasture and forage in the Mediterranean was discussed in meeting 2. Their use in animal feed may be increased by regular supply of quality products (Eg dehulling faba bean). Faba bean may reduce the use of soya in animal diets in UK. End user engagement in projects was crucial. At ILS2 first results from LEGATO were presented and communicated to an international audience. The final meeting was attended by EU-based pulse breeders and advisory bodies. Three Czech breeders presented breeding strategies and the registration of varieties was explained. The prospects for increased EU production were discussed as a general topic.

Significance and benefits
The Stakeholder meetings provided a unique opportunity to provide interaction between LEGATO Stakeholders and other industry-related professionals. A breakdown of participants from academia, industry, Inter-professional bodies, students, farmer/advisor, policy, SME and other is given in the deliverable reports. One message from breeders during the stakeholder meetings was that, whatever the economic context, significantly improved resistance to major pathogens and pests will be necessary to convince farmers to invest in GL cultivation, in view of the significant risk of variable yields with current varieties. This underlined the importance of research in these areas, as 1. Resistances to several important pathogens and insect pests are scarce in currently used material, and 2. Farmers will be less and less able to rely on plant protection for disease and pest control, due to severe reductions in permitted products.

More generally, the four meetings gave a high profile platform to communicate messages within LEGATO. More widely, messages about production, use, policy and support were discussed in a broad EU context.

References
Deliverable 6.4 Stakeholder topic meeting report n°1
Deliverable 6.5 Stakeholder topic meeting report n°2
Deliverable 6.6 Stakeholder topic meeting report n°3
Deliverable 6.7 Stakeholder topic meeting report n°4

Potential Impact:
LEGATO was conceived to facilitate uptake of project results by commercial stakeholders, in particular, breeders. Thus several breeding companies were part of the consortium, and others were associated in the field trials network. Other stakeholder groups were either project partners in the food transformation area (Decollogne, Patrimvs), or in the molecular biology service area (GenXPro, AMBIS), or were invited to the annual stakeholder meetings. The field trials network included additional breeders and locations besides those of project partners, thus widening the communication of project.

Dissemination activities

Stakeholder meetings:
Stakeholder meetings were organized to: provide an interface between project partners and external stakeholders, with a programme focussed on a topic of interest to both groups, and also reflecting local priorities. They were organized, with one exception, synchronously with the annual project meetings to ensure widest possible interaction.

The first stakeholder meeting (reported in D6.4 http://legato-fp7.eu/deliverables_and_publications.html website link) was held on the campus of the Swedish Agricultural University at Alnarp, jointly with the
Scandinavian network LegSA (Legumes for Sustainable Agriculture). The main topic of the meeting was EU grain legume consumption. The speakers were asked: What potential do European-produced legumes for food have to supply our protein needs? And how can their consumption be promoted? There were two sessions: - Session 1 “Levers for boosting EU Grain Legume (GL) production and quality”, with two conferences on “Improving key quality traits in GL” and “Brakes and levers on GL production for food and feed” and Session 2 “Optimizing GL for human consumption” which dealt with “Defining traits adapted to consumers’ expectations”, and “Which traits for human consumption? – Consumer perception”, followed by presentations on “SME experience and feedback”.

In Session 1, it was pointed out that food legumes have a higher market value than those for feed, but the quality standards are higher. Specifically, for faba bean exported to Egypt, only beans free of bruchid beetle damage are acceptable. As bruchid infestation is a major problem, and pesticide control problematic (A UK report assessed that 40 active substances are deemed likely to be lost or restricted in their use), the motivation to find genetic sources of resistance is high. A further major focus is on elimination of antinutritionals, vicine/convicine and tannin. In both cases suitable loci are available and are being introduced into current cultivars, but better molecular markers are needed to accelerate varietal selection. Among the brakes on GL production is declining yields, partly due to soil-persisting diseases such as Aphanomyces. The presentation showed with these examples the current potential for genetic improvement of seed quality once high-resolution markers become available, and exploitation of seedbank diversity. Among the levers, hope for the future appears in the CAP 2014-2020, which offers some opportunities for protein crops (minimum 3% of ecological interest area, crop rotation encouragement to increase diversification (environmentally friendly), and favorable national policies in certain member states (e.g. Plan Protéines Végétales pour la France 2014-2020).

Research projects such as LEGATO can help to fuel this positive impetus by providing innovative genetic material better adapted for food and feed, and for low-input agriculture, as well as locally-adapted cropping systems built around grain legumes that optimize their ecological services.

The meeting participants provided several innovative examples of how grain legume consumption can be promoted for human consumption through both the development of novel products and marketing strategies, focusing on the merits of local sourcing and the use of traditional varieties and/or recipes. This theme was continued with further examples in the LegSA meeting, which took place the same afternoon.

The second stakeholder meeting took place in the morning of 1st December 2015 at the University of Cordoba in Spain, and was synchronized with the LEGATO second annual meeting. The stakeholder meeting topic was: “Legume cultivation: economic models, agronomic techniques, and promotional measures” In this context, after presentations of levers for increasing the proportion of legumes in agrosystems and on their interest as pasture and forage crops in the Mediterranean Region, the use of legumes in animal feeding in Spain, France and UK was analyzed from an economic point of view. To conclude, the evolution of EC support for protein crops and its potential impact on EU protein crop areas was reviewed. The meeting began with a Diagnostic –why grain legume cultivation has declined over the past 20 years. Several issues were raised:
1. Increase of short rotations
2. Lower productivity of GL compared to main arable crops
3. Economic return on following crops is rarely considered,
4. A lack of advice on legumes provided to farmers
5. Genetic progress is lower and less rapid than with the main arable crops (less investment),
6. Strategies of collecting firms and processing industry reinforce the dominant crops

A presentation from a Portuguese forage crop breeder reminded of the importance of legumes in forage cropping systems in arid Mediterranean regions. The necessary plant adaptation / resiliency requirements can be met by using Sown Biodiverse Legume Rich Permanent Pasture and Forage Crops (SBLRSPP&FC), which will improve animal production and recover degraded lands in a sustainable way. A second session focussed on the value of faba bean in replacing soybean in feed constitution: Whatever the price context, dehulling can improve the economic value of faba bean. A UK project, OPTIBEAN was described. Its objective was to replace imported Soya with home grown protein and to optimize faba bean breeding, production and use. The results showed that incorporation of faba bean in feed of salmon, poultry, swine, and ducks allowed at least partial replacement of soybeans, with greatly reduced carbon costs. The meeting was completed by a presentation from DG Agriculture and Rural Development on the support measures given to GL which allow member states some flexibility to increase support for GL if wished.

The third stakeholder meeting was held concurrently with the 2nd ILS conference, in Troia, Portugal (Abstract book at: http://www.itqb.unl.pt/meetings-and-courses/legumes-for-a-sustainable-world/abstracts#content). 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. This meeting was the largest international event on LEGATO topics held during the contract. It attracted mainly research but also commercial participants and provided a shop-window for the first project results, LEGATO partners featuring prominently in the programme. As a result of this conference, an issue of the stakeholder journal Legume Perspectives was produced which summarizes the presentations (available at: http://ils.nsseme.com/#about see also 1.7).

The fourth stakeholder meeting was held in the Czech Republic, at Velke Losiny, hosted by a LEGATO partner, Agritec. The programme of the meeting was focused on breeding grain legumes and registration of legume varieties with particular attention to the Czech Republic. It was shown how the use of biotechnology and Marker-Assisted selection could help pea and faba bean plant breeding programs. It was also highlighted that specific cropping systems, such as intercropping of legumes with cereals, may limit insect pests. A report on the two-year pan-European trials network managed in LEGATO was presented, with a debate on the methods of analysis of the results. Furthermore, the event allowed a good interaction with breeders from central Europe, and those involved in the procedure of varietal selection and registration in the Czech Republic.

International conference:
The final conference was held in Novi Sad, Serbia, from 27-28. September 2017, jointly with EUROLEGUME. The conference was divided into 6 thematic sessions: 1. Genetics and breeding, 2. Biotic stress resistances, 3. Enhancing legume quality - novel food & feed, 4. Defining ideotypes root phenotyping and microbial interaction, 5. Agronomy, 6. Climate changes, stress adaptation & mitigation measures. Five invited plenary lecturers, chosen for their complementary expertise relative to the two projects’, gave presentations on subjects ranging from genomics to LCA and consumer preferences: Professor Timothy Close (UC Riverside, USA), Professor Moira Dean (QU Belfast, UK), Stephanie Mittermaier (Fraunhofer IVV, München, D), Professor Mark Peoples (CSIRO, Canberra, AU), Dr. Thomas Nemecek (Agroscope, Zürich, CH).
At the conference, there were 132 registered participants from 45 different countries. In total, 40 oral presentations were held and 15 flash presentations. The book of abstracts contains 130 abstracts written by 369 authors. Each participant received a copy of the Book of Abstracts, which is available at: http://www.legato-fp7.eu/FinalConference/pdfs/Book_of_abstracts_LEGATO_EUROLEGUME.pdf. During the conference, two round tables were held, dedicated to the cultivation and use of legumes. The discussions were based on interventions by stakeholders representing different interest groups (varietal development, composition and processing, inoculation, cold climates cultivation, outreach). A press conference was organized at which regional and national media were represented. From press clipping data, it is estimated that information from the conference was displayed in around 500,000 households (including primary and secondary sources).

Advanced training course:
The 5 days international advanced course “Advances in breeding and agronomy for improving sustainability and quality of grain legume crops” was jointly organized by LEGATO and EUROLEGUME FP7 projects, and hosted at the CIHEAM Institute IAMZ in Zaragoza. A total number of 24 participants attended the full course, coming from 11 countries, mainly bordering the Mediterranean. Participants were graduates with previous experience in basic or applied research, working in universities, research centres or private companies. The 36 hours programme dealt with a wide spectrum of scientific topics reflecting the multidisciplinary research of the two projects:
- Quality of grain legumes and technological innovation in legume products and byproducts
- Advances in plant-microbial communities’ interactions
- Advances in agronomy
- Advances in breeding and genomics
- New phenotyping methodologies, with emphasis on roots
Deliverable 7.7 contains a detailed report of the advanced course.

Newsletter:
An annual newsletter was produced to report on the main project-related events and to highlight recent results arising. The electronic newsletter was circulated via a mailing list of more than 400 contacts, and issues are downloadable from the project website (http://legato-fp7.eu/newsletters.html). Copies were also distributed during the annual Project meetings and the International conference.

Website:
The project web site was set up in the first two months of the project and is the main vehicle for providing information to the general public. As such, it hosts a project overview, the partners in the consortium, and project structure, as well as all public documents (Deliverables and Publications, copies of the newsletter and Guidelines for legume use), field trials database generated during the project, events schedule and links of general interest about legumes.

Guidelines:
One of the project deliverables is a Guidelines brochure on sustainable legume cultivation and use (http://legato-fp7.eu/index.html). The objective has been to indicate where innovations from LEGATO research may contribute to promoting legume production and consumption, and not to duplicate the excellent guidelines for growing legumes that already exist. The brochure also lists links to resources
created during the project and links to other useful sites.

Issue of legume perspectives magazine (based on Troia meeting):
The proceedings of the International Legume Society meeting in Troia, Portugal (ILS2) were published in the form of an issue of the stakeholder journal LEGUME Perspectives (volume 14). This is available for downloading as a PDF on the ILS website (http://ils.nsseme.com/#about)

Impact and Exploitation of results

Development of new markers for rapid breeding:
WP1 included several studies aimed at identifying marker for traits of agronomic interest. The new markers developed during the project are available on the legato Website (http://legato-fp7.eu/index.html). Among these markers are those very close to the Vc locus for vicine/convicine in faba bean, that will facilitate selection of this important trait, markers for the rms1 locus in pea, which plays a role in response to drought, mfn1 and mfn2, that control the number of flowering nodes in pea, and have a positive effect on yield in the initial yield trials performed ; and identification of molecular markers linked to the anti-nutritional compound β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in grasspea (Lathyrus sativus).

Integration of marker-assisted selection in commercial breeding programmes:
Markers, in part created within WP1, were used for marker-assisted selection in commercial breeding programmes. They included markers for low vicine-convicine content. Screening work and subsequent crosses were carried out by breeding companies NPZ and SZG, with technical support from AMBIS. Also tested were four major traits relating to plant protection in peas, adapted to detect resistance or sensitivity to four types of economically damaging viral and fungal pathogens, pea seed-borne mosaic virus P1 (PSbMV), pea enation mosaic virus (PEMV), Fusarium oxysporum race 1 and powdery mildew (Erysiphe pisi f.sp. pisi). The screening and selections were conducted by breeding companies Agrovegetal and Agritec, with technical support from AMBIS. Thus the feasibility of integrating MAS in the varietal selection was established, and connections made with AMBIS who could provide support in future breeding schemes.

Establishment of lupinus albus genetic map, and exploitation of genetic diversity:
Work on white lupin (Lupinus albus), performed in LEGATO WP1, has (1) produced a dense genetic map, and exploited it to develop marker-assisted-selection (MAS) procedures for a few key agronomic traits, and (2) characterized with high marker numbers a comprehensive landrace world collection, and assessed the species diversity and genetic structure. Genetic structure information at the global diversity level is thus available for the exploitation of white lupin genetic resources by breeding programs, notably for important agronomic traits such as vernalization requirement, alkaloid content and anthracnose resistance, for which QTL data were obtained.

Testing and validation of mfn as a gene promoting yield:
mfn (more flowering nodes) was a mutant phenotype studied in WP3 of LEGATO for its potential effect on yield. After confirming a positive effect on yield per plant in the greenhouse, in collaboration with Dr. T. Warkentin (Univ. Saskatchewan at Saskatoon), the mutants were grown in the field. As a result of the
higher production of pods in the main stem and in the rest of the branches of the plant, the yield (weight of seeds per plant) was notably higher in all the mfn mutants, the double mutant being the most productive genotype (where the yield was double that in the wild-type parental, Cameor cv.), followed by mfn2 and by mfn1. The mfn material is now being introgressed into current performing cultivars to exploit its potential for increasing yield.

Disease/Pest resistant lines for pre-breeding:
Bean and grasspea sources of resistance to fungal diseases (ascochyta blight, powdery mildew, rust, fusarium wilt, chocolate spot), viruses, parasitic weeds (broomrape) and insect pests (aphid and weevil) obtained are being introduced in crop breeding programs.
Ascochyta blight resistance sources are multilocus. Progress from MACE sequencing has yielded SNP markers usable for following QTL in breeding programs.
New sources of resistance have been identified to Broomrape (Orobanche crenata) in Faba bean and pea, and introduced in crossing programs (doi.org/10.1007/s13593 resulting in the recent release of the first resistant cultivars.
Resistance to Bruchus pisorum (https://doi.org/10.1007/s10340-017-0925-1) and to Acyrthosiphum pisum (DOI: 10.1111/aab.12417) is now available in pea germplasm which is readily accessible for pea breeding programs. QTLs have been identified (in prep.) which will facilitate MAS programs.

Spectroscopic methods for rapid seed content profiling:
In LEGATO WP4, classical methods of determination of seed constituents were compared to FT-IR spectroscopy, NIR spectroscopy and NIR imaging measurements. A good correlation was found for NIR values for several components compared with the classically determined values (Table 1 in attachment). NIR spectroscopy and NIR imaging were shown to be useful to predict some biochemical compounds with a good degree of accuracy, whereas FT-IR spectroscopy does not seem to be able to predict the constituents studied. NIR technology (spectroscopy or imaging) can be integrated in a future screening tool in order to quantify the following parameters: moisture, fiber, proteins, fat, antioxidant, total phenolic content and arabinose, substituting for the classical methods, and saving much time and material investment.

Experience of bread fortification with legume flours:
Two SMEs in LEGATO have developed and tested legume-flour fortified breads; Patrimus (Broa-type Portuguese maize bread) and Decollogne (french-style wheat baguette). The bread loaves were subjected to sensorial testing by naïve tasters (Deliverable 4.4). In both cases, formulations were identified that were well-accepted by the tasting panels. The perspectives for marketing products based on these prototypes will now depend on the economic viability of the corresponding product lines.

Willingness to pay (WTP) and sensorial evaluations by consumer panels:
In parallel studies in LEGATO, the willingness to pay (WTP) for a legume flour-fortified bread loaf was tested on tasting panels, and found to be satisfactory (Deliverable 4.5). Furthermore, labelling of the loaves to highlight the environmental merits of legumes in agriculture was rated as an asset by the panel. In order to use such labelling, a regulatory framework would have to be approved.

MASC protocol and examples:
MASC is a method for designing and evaluating cropping systems for their sustainability. The MASC protocol allows one to design cropping systems adapted to local conditions, and on interaction with local stakeholders. MASC was shown to be suitable for designing cropping systems that incorporate legumes. The experience obtained within LEGATO showed that at three sites (Scania, Paris basin, Andalucia), cropping systems could be conceived that met the criteria of sustainability, integrated local crop needs, and were favorably rated by local actors. MASC seems therefore to be a good basis for introducing legume-based cropping systems, and as such could be exploited widely by stakeholders in the area of agricultural counsel.

Diagnostics and Recommendations for rhizobial inoculation:
A generally applicable method was developed for reproducible extraction of nodD DNA from soil and estimating the rhizobial abundance and diversity. The molecular QQAD technique (ms in prep.) makes it possible to determine whether an effective soil population exists, and to estimate its genetic diversity. LEGATO studies showed that European agricultural soils generally have substantial established populations of rhizobia that are able to nodulate pea and faba bean effectively. These populations are genetically diverse, and strains differ in the level of benefit they provide to the host plants. Furthermore, their relative performances on pea and on faba are not necessarily the same. Inoculation with an elite strain should, in principle, be beneficial, although it will not always lead to a significant yield increase compared to nodulation by the established soil population. Selection of efficient bacteria, requires specific selection processes based on efficiency and competitiveness for nodulation of the associations. These traits have been measured for rhizobial isolates obtained from agricultural soil samples collected at several European sites. Thus the information is available to prepare inocula that will guarantee the contribution of the Rhizobial partner under suitable conditions of cultivation.

Databases: molecular markers developed during the project, and seed composition and physicochemical properties of grain legume genetic resources:
Molecular markers for individual characters and collections of markers generated during the project have been summarized in a table available on the LEGATO website (http://legato-fp7.eu/index.html). A database containing the composition and physicochemical properties of Grain legume accessions analysed in WP4 is in preparation and will be accessible on the LEGATO website (http://legato-fp7.eu/index.html) once an associated publication has been accepted.

List of Websites:
Project public website
The website address is the following: http://www.legato-fp7.eu

Project logo (in attachment)

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