Grant Agreement number: FP7-613551

Project acronym: LEGATO

Project title: LEGumes for the Agriculture of TOMorrow

Funding Scheme: Collaborative Project

Date of latest version of Annex I against which the assessment will be made: 05 January 2015

Periodic report: 1st X 2nd □ 3rd □ 4th □

Period covered: from 1st January 2014 to 30th June 2015

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¹ Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.
² The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.
Declaration by the scientific representative of the project coordinator

I, as scientific representative of the coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached periodic report represents an accurate description of the work carried out in this project for this reporting period;

- The project (tick as appropriate)³:
  - X has fully achieved its objectives and technical goals for the period;
  - □ has achieved most of its objectives and technical goals for the period with relatively minor deviations.
  - O has failed to achieve critical objectives and/or is not at all on schedule.

- The public website, if applicable
  - X is up to date
  - □ is not up to date

- To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 3.4) and if applicable with the certificate on financial statement.

- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 3.2.3 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of scientific representative of the Coordinator: Dr Richard Thompson

Date: 30/08/2015

For most of the projects, the signature of this declaration could be done directly via the IT reporting tool through an adapted IT mechanism.

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³ If either of these boxes below is ticked, the report should reflect these and any remedial actions taken.
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1. Publishable summary

1.1 Summary description of project context and objectives
The LEGATO project has been conceived to promote the culture of grain legumes in Europe by identifying priority issues currently limiting grain legume cultivation and devising solutions in term of novel varietal development, culture practices, and food uses. LEGATO will develop tools and resources to enable state of the art breeding methodology and to exploit fully the breadth of genetic resources available. The project will focus on a small number of key characters not previously explored in depth and complementary to other ongoing European and national projects. These topics covered include disease and pest resistance, where in addition to marker development for major fungal and viral pathogens, a focus on emerging insect pests is planned. The impact of end-of-season drought and heat stress on the rhizobial symbiosis, and its consequences for plant performance, will be studied. Two characters that can influence grain legume yield, autofertility and number of flowering nodes, will be investigated. The potential for improving legume nutritional and organoleptic quality by identification of desirable traits and innovative selection methods will be investigated. LEGATO will conceive sustainable legume-based cropping systems adapted to different pedoclimatic zones, respecting local constraints. The project has been constructed around the participation of commercial partners including SMEs in the areas of marker development, plant breeding, and legume food processing, who will benefit from the advances made in these areas in LEGATO. Promising legume varieties (Pea, faba bean, grass pea, white lupin and lentil) and cropping systems will be tested at a series of pan-European sites to favour the widest possible take-up in agriculture, and the partners potentially concerned will participate in a stakeholder forum convened regularly during the project.

1.2 Description of the work performed since the beginning of the project and the main results achieved so far
In WP1 “Genetics and new breeding tools and material”, the main efforts are in trait mapping and marker generation. LEGATO has focused on two traits that may improve yield, the more flowering node (MFN) gene(s) and loci conferring autofertility in Faba bean. 20 germplasm accessions that contrast for this trait have been examined, and allelic variants in a candidate gene identified. Crossing work for introgressing the trait into elite pea varieties is progressing. Faba bean is characterized by intermediate levels of cross-fertilization, resulting in heterogeneity. Complete self-fertility (aimed to breed pure lines), or obligate cross pollination (aimed to fully exploit heterosis), would allow for innovative breeding strategies. To fine map genomic regions associated with autofertility, experimental work has started to screen the autofertility potential of lines of a Recombinant Inbred Line (RIL) population (from a Vicia faba x V. paucijuga cross).

Studies in WP1 are designed to generate markers for Marker-Assisted Selection (MAS), and also to assess the value of MAS in a reference situation. To assess Genetic gain from marker-assisted selection of pea for drought tolerance a genomic selection model for higher grain yield under severe drought stress is under development for a reference population field-phenotyped in the ArimNet project REFORMA. Molecular data have been obtained on this population by a Genotyping-By-Sequencing (GBS) procedure optimized for pea. Progress was also made on breeding for reduced anti-nutritional in grain legumes by creating populations in which the responsible genes can be mapped, for Oxalylaminopropionic acid (ODAP) content of grass pea, and vicine-convicine content in faba bean [Figure 1]. These studies will also yield markers for MAS in WP6. Pea genome sequencing, once completed, will offer unique opportunities for pea improvement. In order to benefit from the incipient availability of the sequence, however, high-density Single Nucleotide Polymorphisms (SNPs) data is needed. To support, and exploit the pea genome project, a large RIL population (760 lines) from a cross between contrasting genotypes has been developed and is being genotyped by GBS to produce a high-density map. For the parents, we have generated ~130M reads in 2x126bp format, and library preparation on the mapping population is in progress. Furthermore, to access useful genes from pea species closely related to cultivated pea, two backcross populations that introgress P. fulvum or P. elatius into P. sativum, are under development [Figure 2]. A subset of 96 lines has been preliminarily genotyped by 40 gene-based markers by the partner AMBIS Ltd, and phenotyped for several traits (displaying wide morphophysiological variation).

Finally, WP1 also aims to widen our knowledge of white lupin genetic structure and contribute to marker development. Material from two germplasm sets (genetic resources and RIL population) has been multiplied, and DNA for GBS extracted. Phenotyping data were obtained on the RIL population and pre-exist for the global landrace collection.
In WP2, “Biotic stress resistance” is being approached in the project by screening germplasm collections of pea, faba bean and grass pea under field conditions to identify sources of resistance to weevils (By the partners Agritec, INRA and CSIC), aphids (By the partners Agritec, CSIC and IFAPA), to major fungal diseases and parasitic weeds (By the partners IFAPA and CSIC) and to viruses (By the partner UPOL). Several promising sources of resistance have been identified [Figure 3]. Root exudate may participate in the control of soil-borne pathogens and be an important pre-penetration resistance mechanism. Thus we developed a method to collect root exudates and to test them on the soil-borne pathogen Fusarium oxysporum. Screening of a pea collection with this method identified several pea accessions with root exudates that inhibited F. oxysporum germination. Culture filtrates of Didymella pinodes, Botrytis fabae and Ascochyta lathyr i isolated from diseased leaves of the relevant crop have been extracted and purified to obtain the main phytotoxin (necrotrophic effector), named pinolidoxin, regiolone and cytochalasin B, respectively. These phytotoxins were provided for the planned sensitivity assays on pea and faba bean. Several further bioactive compounds were extracted and sent to the partner CSIC to assay their insecticidal activity against weevils and their antifeedant activity against aphids.

Inoculated plant material has been generated (By the partner CSIC) to be used in the forthcoming period by the partner GXP in the transcriptomic study (using Massive Analysis of cDNA Ends (MACE)) to identify candidate genes controlling resistance in pea and for SNP genotyping, by the partner CSIC for proteomics and by the partner UNINA for metabolomics studies for marker identification.

WP3 “Optimizing plant adaptation to abiotic stress” is dedicated to the identification of plant ideotypes which perform well under drought stress, and in particular, continue to fix nitrogen efficiently. During the reporting period, a series of the required methods was developed. These now permit high-throughput fluxomics studies in the soil-plant-atmosphere continuum, Magnetic Resonance Imaging (MRI, see Figure 4) and Positron Emission Tomography (PET) imaging of roots and seeds within pods, among other plant organs, and split-root analyses to characterize the compensatory responses to localized drought. Rhizotrons adapted to high throughput, and the phenotyping cabins, were subjected to a series of steps of validation. A comparison of the field performance of different legume crops in terms of nitrogen household (fixation/assimilation) was also carried out using 15N labelling. Five genotypes contrasted for their root system architecture and for their differential response to water stress were chosen from the MediLeg project and their response to water stress was studied in the high-throughput phenotyping platform during spring 2015. Sample analyses are in progress in order to decipher which ecophysiological traits promote better acclimatization and resilience after water stress. In a complementary approach, Quantitative Trait Locus (QTL) analysis of a pea RIL population subjected to drought stress has pinpointed ten loci accounting for up to 57% of the response seen. In studies on pea genes of potential adaptive value to drought stress, recent results suggest a role for the vacuolar sulfate transporter SULTR 4.1, which is being further investigated. A second gene studied via mutant analysis is a putative pea orthologue of the Deeper ROoting (DRO) gene which confers drought tolerance in rice.

WP4 “Defining traits adapted to consumers’ expectation” is engaged in an assessment of a series of quality characteristics for grain legumes that can be used to define consumer preferences. A representative collection of 400 grain legume accessions, (plus 100 harvested from a network of field trials of promising varieties), was gathered and multiplied for comprehensive chemical-physical, organoleptic and rheological/end-user analysis [Figure 5]. Initial pilot studies on legume flour-reinforced breads have enabled the development of a protocol for the sensorial analysis to be coupled with an experimental market to assess consumer preferences at the hedonic and willingness-to-pay (WTP) levels.

Increasing grain legume cultivation offers the potential to reduce the environmental impact of agriculture. To what extent this can be achieved depends on development, optimization and application of legume-based cropping systems, the theme of WP5 “Grain legume cropping system management”. The multi-criteria sustainability assessment model (MASC) tool has been used to conceive innovative cropping systems in France, Sweden and Spain [Figure 6]. The next step will be to initiate workshops with stakeholders to discuss the assessment and determine the weights for the different components of the sustainability assessment in the specific region. Cropping systems with grain legume variety mixtures, intercropping of grain legumes and cereals and grain legume species have been used to determine the effect of diversification on biotic stresses from diseases, pests and weeds [Figure 7]. Due to variable disease pressure, results are limited at this stage, but in one study aphid infestation declined faster in intercropped pea than in sole crop pea [Figure 8]. To evaluate the need for inoculation with rhizobial strains, which may be advantageous in soils at the limits of current cultivated areas, rhizobial community composition/diversity, size and N-fixation efficiency is being
The project will generate breeding and management tools to reinforce the cultivation of legumes in European agriculture. It is focusing on the main bottlenecks for grain legume cultivation, which are low and variable yields, mainly due to certain abiotic and biotic stress factors. By smart breeding based on NGS sequence data for pea, and the introduction of previously unexploited germplasm, the development of new varieties that are more competitive will be accelerated. In addition, the benefits of legume cultivation will be more fully exploited by re-designing cropping systems to have legumes as the central element, to benefit from the ecological services they provide. The cropping systems are expected to have diverse end-users: initially bio/organic farms, zones of limited area of production (short circuits), and ecologically sensitive zones such as water catchment areas, but with the expected evolution of agricultural policy rendering them more economically competitive, to see more general application.

LEGATO will help to increase the availability of legumes in Europe and diversify protein supply for food and feed purposes. By evaluating the factors affecting acceptability of legume varieties for human consumption, the project will help to diversify local protein supply and promote domestic legume use. Work on legume-based cropping systems and novel disease resistance loci in will secure a position for legumes as a protein source on the feed market. Collectively, these actions will contribute to reduce the geopolitically sensitive deficit in European plant protein production.

The project results will help to take advantage of the positive effects of legumes on the environment. The “ecological services” which legumes provide include atmospheric nitrogen fixation. LEGATO investigates and optimizes the legume-Rhizobium symbiosis, including the option of inoculation that is responsible for nitrogen fixation in different soil-climate zones in Europe. The results will lead to a reduction in synthetic N-fertilizer use, and in the incumbent energy costs. By carrying out a Europe-wide network of trials of major grain legume crops, the most promising ideotypes adapted to various soil-climatic zones, will be identified and with the involvement of local panels, be incorporated into recommended cropping regimes.

In order to translate LEGATO research and innovation into market applications, the project is driven by nine SMEs. GXP is at the forefront of novel genome analysis techniques and applies its expertise to an ambitious
analysis of gene expression in a population derived from a wild pea relative. AMBIS Ltd carries out marker-assisted selection for various traits and directly supports commercial plant breeding. Two associated SMEs, Decolonne and Patrimvs produce and evaluate legume-enriched bakery products. The outcome of their projects should pave the way for using grain legume flour as a protein source in the bakery industry. The other SME partners, Agrovegetal, Agritec, SZG, PGRO-RL and NPZ are involved with breeding and will benefit directly from the availability of information on markers for key traits and the agronomic data produced by variety trials across Europe as single stands, in rotations and intercropping.

1.4 Project logo

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

1.6 Relevant contact details
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1.7 Illustrations
Figure 1. (T12.3, Identification of markers for anti-nutritional compounds). Diagram of the saturation of the vicine-convicine (v-c) region in faba bean. Previous maps (Satovic et al. 2013) show that v-c is between Medtr2g019260 and Medtr2g008050 in the extreme of the faba bean long chromosome I. The target region, spanning 6 Mb, is being saturated with gene markers from the syntenic Medicago truncatula (Mt) chromosome II. Fourteen Mt candidate genes are being assayed for the presence of the SNPs (Single Nucleotide Polymorphisms) and further genotyped in the whole F2 population. Allelic variations of gene Medtr2g008220 cosegregating with the v-c content (ranging from 0.04 to 0.5), are presented for several individuals. Mapping of additional target genes in this area will provide closest flanking markers to identify the responsible v-c gene. (IFAPA, Cordoba).
Figure 2. (T1.3.1, Construction and characterization of pea introgression lines). Schematic representation of low density genotyping of selected subset of pea introgression lines (P. sativum cv. Terno x P. fulvum WL2140) analyzed by CSSL Finder software. (Univ. Olomouc, Czech Republic).

Figure 3. (T2.1.1 and T2.1.2, Field phenotyping of resistance to pests and diseases). Symptoms of main pea diseases or pests on susceptible (left) and resistant (right) pea accessions: (A) broomrape; (B) ascochyta blight; (C) powdery mildew; (D) rust; (E) aphid (F) weevil. (CSIC Cordoba).
Figure 4. (T.3.1.1, Symbiotic traits optimizing pea and faba bean performance under drought). Root system of pea and common bean monitored with MRI. a) root system of pea 19 days after sowing and b) 36 d. after sowing. Peas were grown in 56mm x 200 mm containers. Voxel size 0.5 x 0.5 x 1 mm. Common bean plant c) 32 d. after sowing with apparent nodules, voxel size 0.39 x 0.39 x 1 mm grown in a container 90 x 250mm, d) section of the plant shown in c) with voxel size 0.23 x 0.23 x 0.46 mm, substrate a 1:2 mix of agricultural soil and sand (Forschungszentrum Jülich GMBH).

Figure 5. (T.4.1.4, Studies on grain legume nutritional quality: carbohydrate metabolism variants). Identification of novel variants in pea germplasm. Seeds of the four lines shown in the main photograph are wrinkled-seeded. The upper two lines are r and rb mutants. While both JI 2110 and JI 1417 have simple starch grains like JI 399, they lack a mutation at the rb locus. JI 2110 does not complement any of the five known mutations in the starch biosynthetic pathway leading to a wrinkled-seeded phenotype. Compound and simple starch grain morphology is shown in the small images (John Innes Centre, Norwich).
Figure 6. (T5.1.2, Cropping system design involving grain legumes) Objectives and key actions described during the first MASC on-site workshop (INRA Paris-Grignon).

Figure 7. (T5.2.1, Intercrops and varietal mixtures with pea, faba bean, lupin and grass pea). Overview of experimental plots with different pea and faba bean varieties, evaluated as sole crops, variety mixtures and intercrops with cereals (purple plants in the background: lacy Phacelia grown on field margins around the experiment), (SLU Alnarp).
Figure 8. (T5.3.1, Effect of cropping system on pests and pathogens). Changes in pea aphid numbers on field pea tops in the course of the assessment period (28.5. - 24.6.14; AGRITEC 2014 - complete version of the trial = 10 treatments). Treatments compared on the graph: P100 = field peas, standard amount of seeds; P+W = field peas + spring wheat; P+B = field peas + spring barley; P+F = field peas + faba bean; P50 = field peas, half amount of seeds; P+FP = field peas + fodder peas; P+O = field peas + oat; P+W+FP = field peas + spring wheat + fodder peas; P+B+FP = field peas + spring barley + fodder peas; P+O+FP = field peas + oat + fodder peas. (Agritec, Czech Republic).

Figure 9. (T6.1, Evaluation of innovative genotypes and new breeding tools). Demonstration of LEGATO trials with pea varieties at Agritec, Sumperk, Czech Republic, Jana Poslusna speaking. (Agritec, Czech Republic).