Guidelines on sustainable legume cultivation and use

LEGATO

LEGumes for the
Agriculture of TOmorrow

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LEGumes for the Agriculture of TOmorrow

Why legumes?

Grain legume cultivation in Europe has declined over the past twenty years to reach levels where they represent minor crops. This is largely due to competition as a source of protein feed for livestock from cheap soybean imports, which, despite available subsidies, often render grain legume cultivation unprofitable. The cultivation that remains is mainly providing niche markets (organic, "locally produced" labels, special local varieties related to traditional dishes..), or in attempts to diversify crops produced in sustainable farming systems. Grain legumes are rarely economically competitive in high-input cropping systems. Their merits as fixers of Nitrogen depend on being cultivated on nitrogen-poor soils, where they may be useful, or in a rotation either before or after a nitrogen-demanding crop such as maize or oilseed rape. They are usually rain-fed and usually do not require irrigation, but when under moisture stress they will respond to irrigation during pod filling. By judicious choice of legume species and varieties (see later), a suitable crop can be found for all European climatic zones.

Building a value-chain with legumes

In order to increase profitability, the grain legume producer has to conceive a new value-chain that will give added value to the crop produced. To do this, the ecological services provided by legumes in agroecosystems need to be considered and exploited as far as possible. These can include: provision of fixed nitrogen, weed suppression, reduction in pest and pathogen transmission, provision of a local protein source, reduction in water consumption/pollution, increasing biodiversity, for example by being a food source for bees. For all of these reasons, they will have a key role in the conversion of farming systems to cope with climate change, an increasingly urgent need.

By judicious exploitation of naturally occurring sources of resistance, and adapting cultivation methods, use of plant protection products can be reduced or eliminated in some cases. This approach is particularly promising for resistance to bruchid weevils, where resistant lines have been identified in faba bean, also for non-host response to broomrape in pea, for resistance to certain viruses (pea), and for mlo-type resistance to mildew in pea. By stacking partial resistances to Aphanomyces, an effective management of this serious pathogen may be achievable. For other pathogens, the mechanism of infection is now better understood (Ascochyta blight, Fusarium), but effective sources of genetic resistance are not currently available. Even when a good source of resistance is available, it will generally be in an exotic genetic background and require several generations of backcrossing to introgress it into a new cultivar. In this context, loss-of-function sources of resistance (for example those afforded by mlo orthologues) are particularly interesting as they may be induced directly in cultivars by mutagenesis.

In addition to the benefits afforded by legume cultivation, the merits of legumes in the diet need to be more widely appreciated. This involves on the one hand, varietal selection to minimize anti-nutritional factors and facilitate processing, and on the other hand, the development of novel food products that fully exploit grain legumes as protein sources and thus render them more attractive for consumers.

The merits of legumes in agriculture are probably most easily exploited in organic or other low-input farming systems. Legumes as a major source of proteins are important in vegetarian/vegan nutrition. Once well-established in these 'niche' markets, a basis for expansion may be promotion of the health benefits of legumes in the human diet, for which in the long term recognition by labelling may be necessary. Although not part of the activities mandated by the LEGATO project, it is certainly an area requiring increased support in future. Within LEGATO, a study of experimental markets showed that consumers value this type of labelling for legume-fortified breads, and that it increases their willingness to pay a premium.

Research perspectives

➢ *Intercropping*

Intercropping grain legumes and cereals has demonstrated multiple agronomic and environmental benefits, in previous research as well as in LEGATO experiments (in WP5). Notably, intercropping reduces weed abundance compared to grain legume sole crops, leads to higher and/or more stable combined grain yields, and can reduce the severity of pest or disease problems in both the legume and cereal components. While yield increases in intercropping were not consistent across all sites in the LEGATO experiments, the associated increases in yield stability observed at several sites can be of major importance – especially in low-input cropping systems. Practical questions around establishment, management (e.g. weed and pest control) and harvest can be solved by taking both farmers' and scientists knowledge into account. On the other hand, logistics for sorting the harvested mixed grains need to be solved by promoting collaboration between the concerned actors, notably farmers and facilities for drying, sorting and storing grains. The practical implementation of intercropping thus requires continued and enhanced multi-actor collaborations and innovative logistics solutions.

➢ *New sources of resistance*

Legumes can be affected by a number of pest and diseases for some of which there is little genetic resistance available. This however may exist in landraces and in wild relatives that can be exploited in crop breeding. In WP2 of LEGATO, germplasm collections, particularly of Pisum spp., but also of faba bean and grasspea have been thoroughly screened for resistance to fungal diseases (ascochyta blight, powdery mildew, rust, fusarium wilt, chocolate spot), viruses, parasitic weeds (broomrape) and insect pests (aphid and weevil), yielding the identification of valuable sources of resistance that are being introduced in crop breeding programs.

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 incomplete and of complex inheritance, controlled quantitatively by multiple genes. Genetic studies have resulted in the identification of a number of QTLs in most crops, but progress in delivery of markers usable in MAS has been slow. This, together with the insufficient understanding of the variability of the pathogen populations has complicated resistance breeding (http://ils.nsseme.com/assets/LegumPerspect8.pdf). Recent knowledge generated in this project by MACE sequencing has allowed the identification of SNPs of differentially expressed transcripts that can be used as positional markers to identify the individuals carrying these resistance QTLs in breeding programs.

The major constraint for legume cultivation in Mediterranean areas is broomrape (Orobanche crenata). Faba bean cultivars with various levels of resistance have been released in various countries, the resistance always being derived from ICARDA materials that were mainly based on Giza402 line. Relying on a single source of resistance is risky and broadening the genetic basic of resistances deployed is a major need in order to increase durability of resistance. During the project, additional sources of resistance have been identified and characterized, and introduced in crossing programs (doi.org/10.3389/fpls.2016.01747). Some levels of resistance have been identified in Pisum spp. and successfully transferred to cultivated pea (doi.org/10.1007/s13593) by crossing and selection, resulting in the recent release of the first resistant cultivars.

Resistance to insect pests such as aphids and weevils is increasingly important. 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.

Quality and breeding for specific nutritional ends

Grain legumes are highly nutritious. Nevertheless, in order to better promote the production and consumption of grain legumes, we must first know the variability existing in the nutritional make-up of these crops and make their nutritional benefits accessible to consumers and other end-users through breeding of new varieties of higher quality. The possibility of a particular legume component acting as an anti-nutrient, as well as a health-promoting agent, sometimes influencing processing and eventually taste and consumer food acceptance, adds extra layers of complexity in devising strategies for quality improvement in food legumes.

In the WP4 of the LEGATO project, a multidisciplinary approach was applied, integrating a comprehensive characterization of different quality traits of food legumes (nutritional, health beneficial, organoleptic and processing related), in representative collections of the different culinary species (peas, chickpeas, faba beans, lentils and grass peas) in use by the European grain legume breeding community. This approach allowed the identification of outstanding germplasm with interesting nutritional and associated sensorial and processing quality traits to be included in breeding programs focusing on quality.

The same collections were subjected to high throughput spectroscopic analysis such as NIR (Near Infra-Red), NIR-his (Near Infra-Red Hyperspectral Imaging), or FT-IR (Fourier Transform Infra-Red), being some of them also screened with molecular markers (GBS based SNP screening). This allowed the development of breeding/selection tools (such as spectroscopic methods or associated molecular markers) for quality characteristics, facilitating the routinely implementation of quality objectives in legume breeding programs. Examples are the identification of ODAP content-associated SNP markers in grass pea, or the calibration of NIR, NIR-HIS and FTIR-predictive models for protein, fat or total phenolic content selection in the five grain legume species studied (peas, chickpeas, faba beans, lentils and grass peas).

Simultaneously, due to the emergence of new food habits and increased consumer awareness of the nutritional merits of legume components, sensorial and consumer behavior analysis were considered as well in LEGATO WP4. As a case study, maize breads fortified with grain legume flours were used on sensorial and experimental market analysis. This approach contributed to close the knowledge gap between the recognition of the nutritional and health benefits of grain legume food products and the most important components affecting consumer choices. This will allow in future fine-tuning of legume breeding objectives to match consumer preferences.

➢ *Adaptation of legume crops to drought and high temperature stresses*

Grain legume cultivation in Europe is mainly rainfed and often subject to drought and high temperature stresses. Root development is key in enabling the plant to respond to these stresses and exhibit a degree of tolerance. In LEGATO WP3, a range of studies were carried out on responses to drought/high temperature of different legume crops, using the high-throughput phenotyping facility and in particular, the rhizotubes which allow a non-destructive examination of root morphology in real-time. Advanced metabolite imaging techniques were also employed to follow metabolite flux in growing plants. These approaches revealed some general principles of the responses to the applied stresses:

- The plant's responses to drought and temperature are different
- Roots and nodules respond differently to these stresses
- Nitrogen supply during root/nodule development is critical
- Different legume crops react differently in their efficiencies of recovery of low soil [N], reflecting differences in root architecture

Among the perspectives arising out of these results:

- in the long-term, use of models of root and nodule development to predict root profiles and performances of given genotypes
- dynamic studies of metabolic responses to water/temperature stress of a given plant genotype using MRI and PET imaging

These approaches will provide valuable new tools for varietal selection.

Inoculation with rhizobia

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. The traits have been measured for rhizobial isolates obtained from agricultural soil samples collected at several European sites (in WP5 of LEGATO). The molecular Quantitative Qualitative Amplicon Diversity (QQAD) technique they have developed (P. Young, Univ. York, ms in prep.) makes it possible to determine whether an effective soil population exists, and to estimate its genetic diversity. If uncertainty about the need for inoculation remains, it will generally be more cost effective just to inoculate, which may have substantial yield benefit and seldom has any negative effect.

Resources

Markers developed and /or characterized in LEGATO project

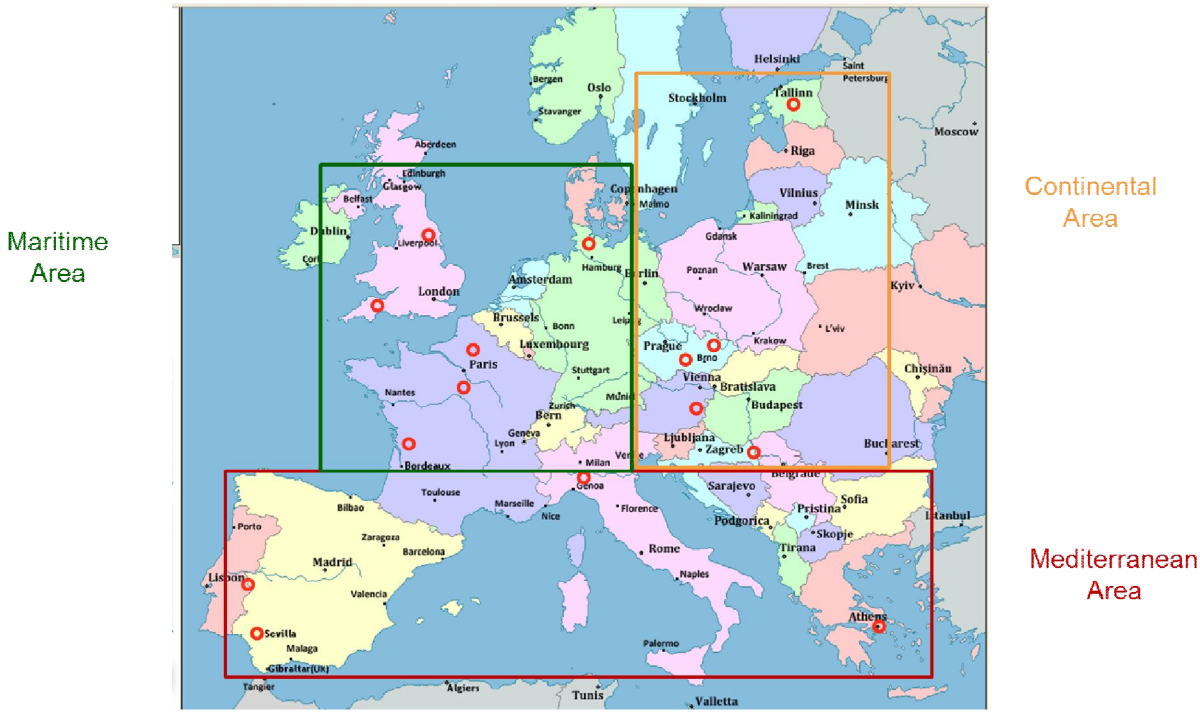
Species	Genotype	Trait	Marker designation	Marker type	Source to be contacted for availability	Remarks (eg., used for MAS)
Pisum sativum	Caméo	Drought tolerance candidate gene	RMS1	SNP	INRA (V. Vernoud)	
Pisum sativum	Attika	Drought tolerance	TP78343, TP13485, TP6268, TP68525	SNP	CREA (P Annicchiarico)	MAS
Pisum sativum	Isard	Drought tolerance	TP94476	SNP	CREA (P Annicchiarico)	MAS
Pisum sativum	Kaspa	Drought tolerance	TP51372, TP63677, TP6885	SNP	CREA (P Annicchiarico)	MAS
Pisum	*Pisum fulvum* x *sativum* cv. Terno BC3F4		49,085 markers	SNP	UPOL (P. Smykal)	1880 mapped on pea genome w r t Medicago synteny
Pisum	*Pisum fulvum* x *sativum* cv. Terno BC3F4		56,323 markers	PAV	UPOL (P. Smykal)	1880 mapped on pea genome w r t Medicago synteny
Pisum sativum		PsMBV-resistant lines			UPOL (P. Smykal)	
Pisum sativum	various	More flowering nodes	mfn1	SNP	CSIC (F. Madueño & C. Ferrándiz)	
Pisum sativum	various	More flowering nodes	mfn2	SNP	CSIC (F. Madueño & C. Ferrándiz)	
Vicia faba		Vc- (low vicine/convicine)		Kaspar SNP silico dARts	IFAPA (A. Torres)	
Lathyrus sativus	various	Reduced ODAP content	6 new markers	DArTseq-based SNP	ITQB (C. Vaz Patto)	
Lathyrus cicera	various	Rust resistance	12 new markers	DArTseq-based SNP	ITQB (C. Vaz Patto)	
Lupinus albus	various	New *L. albus* genetic map	3600 new (+465 old)	SNP	IPG-PAS, (M. Książkiewicz) CRA	
Lupinus albus	P27174	alkaloid content	TP16854	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	alkaloid content	TP447859	SNP	IPG-PAS, CREA	

Species	Genotype	Trait	Marker designation	Marker type	Source to be contacted for availability	Remarks (eg., used for MAS)
Lupinus albus	P27174	alkaloid content	TP22150	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	alkaloid content	TP70046	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	alkaloid content	TP309728	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	anthracnose resistance antr04_1, antr05_1	TP23903	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	anthracnose resistance antr04_1, antr05_1	TP229924	SNP	IPG-PAS, CREA	
Lupinus albus	P27174	anthracnose resistance antr04_1, antr05_1	TP47110	SNP	IPG-PAS, CREA	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Contig17163	SNP	Ocaña et al. 2017 (IFAPA)	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Contig9100	SNP	Ocaña et al. 2017 (IFAPA)	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Contig9100	SNP	Ocaña et al. 2017 (IFAPA)	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Contig17534	SNP	Ocaña et al. 2017 (IFAPA)	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Locus_10591	SNP	Ocaña et al. 2017 (IFAPA)	
Vicia faba	RIL 29H x Vf136	Ascochyta resistance	Contig19144	SNP	Ocaña et al. 2017 (IFAPA)	

Growing legumes in Europe

Which species and which variety?

In the WP6 of the LEGATO project, a network of variety trials of many legume species was sown during two years (2015 and 2016) across a wide range of environments by the different partners involved in the project and in different European countries. Locations are indicated on the map. Contributing plant breeders are listed in Annex 1. Report at: [*http://intranet.iamz.ciheam.org/forms/Legato/WP6/index.php*](http://intranet.iamz.ciheam.org/forms/Legato/WP6/index.php)). Three main areas were considered: the Mediterranean area, the Maritime Area and the Continental Area (see Maps below).



In the deliverable 6.3, results allowed to estimate better the adaptations of the legume species in the different climatic zones:

- **Mediterranean area:** in this sector, pea (winter and spring) reached higher yields than winter faba bean, lupin, chickpea or lathyrus (grasspea) (see figure below).
- **Maritime area:** Spring pea gave high yields in UK, Germany and France. Yield potential of spring and winter faba bean reached high levels in UK and Germany but lower yields

in France. Winter lupin reached quite high yields in the west of France (JD GIE Prolupins). (See figure below).

- **Continental area:** Except in Austria in 2015 (due to hail damage), spring pea gave higher yields in all locations than spring faba bean and other species (spring lupin and Lathyrus). Winter pea gave interesting yields in the two locations where it was tested with only one cultivar.(See figure below)

This table, elaborated in LEGATO Deliverable 6.3, summarizes the species adaptation to European climatic zones:

	Mediterranean	Continental	Maritime
Spring Peas	***	***	***
Winter Peas	***		**
Spring Beans		***	***
Winter Beans	**		***
Chickpeas	**		
Grass Pea	**	*	
Spring white Lupin	*	**	*
Winter Yellow Lupin	*		**

Legend : *Can be grown but not ideally adapted for the climatic zone. ** Varieties are adapted for this zone but some problems may arise in certain years.*** Varieties are adapted for this zone and will perform well in most years.

Our results have shown that some cultivars are quite adapted to all trial sites tested and especially to high yielding environments. In contrast, other cultivars are more adapted to poor yielding environments. The table below shows the varieties best adapted to the different climatic zones among those that were tested in the trials network of WP6 of LEGATO project:

	Mediterranean	Continental	Maritime
Spring Peas	Kayanne Bluemoon	Gambit	Astronaute Prophet
Winter Peas	Galactic Isard	NS Mraz	Balltrap
Spring Faba Beans	-	Alexia Julia Espresso Fury	Fuego Tiffany
Winter Faba Beans	Favel Polikarpi Irena	-	Wizzard Diva
Chickpeas	-	-	-
Grass Pea	Rhodos	Sitnica	-

	Grao da comenda	Studenica	
Spring white Lupin	Estoril	Dieta	Estoril
Winter Yellow Lupin	Orus/Magnus	-	Orus/Magnus

Results coming from the trials network of WP6 of the LEGATO project have furnished information on the sowing dates in the different countries. This is summarized in the tables below (intervals spanning the observed sowing dates are given):

Climatic zone	Country	Spring species	Sowing date
Mediterranean	Greece	Pea, lupin, chickpea, Latyrus	20 Dec-20 Jan
Mediterranean	Italy	Pea,, chickpea	31 Oct – 10 Nov
Mediterranean	Spain	Pea, chickpea, Latyrus	15-30 Dec
Mediterranean	Portugal	Pea, lupin, Latyrus	30 Nov – 20 Jan
Maritime	France	Pea, lupin, faba bean	10 – 20 Mar
Maritime	UK	Pea, faba bean	20 Mar – 10 Apr
Maritime	Germany	Pea, faba bean	5 – 15 Apr
Continental	Czech Republik	Pea, lupin, faba bean	10 20 Apr
Continental	Serbia	Pea, lupin, faba bean, Latyrus	15 – 25 Mar
Continental	Austria	Pea, faba bean, Latyrus	20 – 25 Mar
Continental	Estonia	Pea, faba bean	1 – 5 May

Climatic zone	Country	Winter species	Sowing date
Mediterranean	Greece	Pea, faba bean	20 Dec – 20 Jan
Mediterranean	Italy	Pea, faba bean	31 Oct – 15 Dec
Mediterranean	Spain	Pea, faba bean	25 – 31 Dec
Mediterranean	Portugal	Pea, lupin, faba bean	25 Nov – 15 Dec
Maritime	France	Pea, lupin, faba bean	25 Oct – 10 Nov
Maritime	UK	Pea, faba bean	20 Oct -5 Nov
Maritime	Germany	Pea, faba bean	1 – 5 Oct
Continental	Czech Republik	-	-
Continental	Serbia	Pea	25 – 31 Oct
Continental	Austria	Pea	1 – 5 Nov
Continental	Estonia	-	

Further sources of information

Project Web sites

The LEGATO project ends officially on the 31st of December 2017. The LEGATO website (www.legato-fp7.eu/), however, will continue to be active for several years. Data resources created during the project will be accessible via the site. The annual reports and deliverables will be accessible, as will the newsletters. The site will also include updates on publications arising out of LEGATO research.

Current EU H2020 projects involving grain legumes :

- EUCLEG http://www.eucleg.eu/
- REMIX https://www6.inra.fr/remix-intercrops/The-Project
- LEGVALUE http://www.legvalue.eu/
- DIVERIMPACTS http://www.diverimpacts.net/about.html
- TRUE https://www.true-project.eu/

Practical guides to legume cultivation

Sustainable cropping system design.

MASC website: http://wiki.inra.fr/wiki/deximasc/package+MASC/

Legume cultivation guides:

- Pea and Faba bean growing guide: http://www.pgro.org/agronomy-guides-publications/
- PGRO Pea and Bean Agronomy App: http://www.pgro.org/pgro-agronomy-app/
- Pea (in French): http://www.terresinovia.fr/publications/guides-de-culture/guide-de-culture-pois-2017/

- Faba bean (in French): http://www.terresinovia.fr/publications/guides-de-culture/guide-de-culture-feverole-2016/
- Pea (in Spanish): http://www.juntadeandalucia.es/agriculturaypesca/ifapa/servifapa/contenidoAlf?id=06fca9f2-7199-4743-b275-efe54fc4086b&sector=efbcf1a0-9a2c-11df-accb-f3b48ff48fa1&sectorf=efbcf1a0-9a2c-11df-accb-f3b48ff48fa1&l=guias

Growers associations

Terres Inovia(France) http://www.terresinovia.fr/

PGRO(UK) http://www.pgro.org/

Demonet (Germany) http://www.demoneterbo.agrarpraxisforschung.de/

Demonstration sites

INRA UE Agroécologie, Domaine d'Epoisses, http://www6.inra.fr/plateforme-casys

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