Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed

Report

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Final Report Summary - EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed)

Executive Summary:
In the final Report of the EUROLEGUME project (2014-2017), we describe the foregrounds of the project, which clearly show how it has contributed to the scientific progress of the legume value-chain, focused on three plant species (faba beans, peas, and cowpeas), involving the selection and characterization of genetic plant resources, using tools such as molecular biology, NIR, and root phenotyping, as well as field crop management using diverse techniques and technologies, and considering the development of novel food products and feeds. The outcomes achieved in this project have a great impact on the legume production across Europe. In the
northern European countries, due to their very short growing season it is extremely difficult to grow legume crops given the lack of varieties with the proper capacity to mature under these agro-climatic conditions. To overcome this situation, the EUROLEGUME project has recovered traditional cultivars of faba beans and peas, which were compared with an array of other cultivars from other regions in Europe, to ascertain the possibility of expanding the growth of these crops in the northern regions. In this regard, it was performed a comprehensive characterisation of this plant material within the 4 consecutive years of the project time-life, which, by chance, indeed represented a large climate variation when compared to the average climate data. Apart from have identified the best cultivars, featured by the best phenotypical adaptation (external morphological parameters) and yield and quality parameters (i.e. protein and amino acid content) the experiments conducted during the project revealed the economic feasibility of growing faba beans in high latitudes with specific cultivars—as evidenced in the Baltic countries and in Norway. Thus, in turns, these findings will have a great socio-economic impact on the agricultural production systems in these regions.

On the other hand, in the southern Europe regions, apart from having identified the best faba beans and peas, the knowledge regarding the possibility of growing cowpea as a multipurpose crop, and its insertion in the Mediterranean cropping system, contributing to their sustainability, particularly when the region is facing severe abiotic and biotic challenges was significantly expanded. This novel outcome will also have a high socio-economic impact.

Addressing the best agricultural management practices for improving sustainability, it is stressed the relevance of including any of these three legume species in crop rotations with other horticultural crops, providing multiple benefits for the legume crop, for other crops, for the income for farmers, for the environmental sustainability, and for the overall competitiveness of the European farming system. However, specific attention should be given to the cultivars traditionally used and thus adapted to each specific growing environment. From the foregrounds on this topic it has been revealed the best management practices, such as growing legume crops with inoculation with specific strains of Rhizobium and arbuscular mycorrhizal fungi (AMF). In this issue, new formulations with combinations of rhizobium and AMF have been developed as inoculants.

Another set of foregrounds that is described is the development of an array of novel foods products, which are close to new market trends and preference of consumers to improve diets and health, under the concept of eating protein produced in a more sustainable growing system with a reduced carbon footprint. To support the creation of these novel food products have been developed new packaging materials and the respective storage conditions, representing a great advance on TRL to support the food industry in bringing these products to the market. In the same way, a novel approach of using the plant co-products from legume production for feed has been developed, creating an added value product, with the potential to be traded as functional, prebiotic feed to reduce the use of antibiotics. A novel feed processing is also presented.

For all these foregrounds was tried to present the respective impact and how they could be further explored in the future. There is an overall need to continue to invest in the legume crop scientific and technological development, trying to focus in the concept of multipurpose crops of short season in a close connection with farmers and industry to expand the use of these crops, beyond the present concept of pulses- dry seeds.

Project Context and Objectives:
The demand of food in 2030 is foreseen to be 50% higher than today. Given the constraints associated
with farming production, this situation would entail severe undernutrition in several developing regions, which are the focus of the attention of the international community on the increase of food productions in a sustainable way. In this context, Fabacea (legumes) is the second most important plant family of crop plants after the grass family Poaceae. This family is the third largest within flowering plants, and includes 650 genera and around 18000 species. Grain legumes represent almost 27% of the world crop production and provide about 33% of the dietary protein consumed by humans.

In many countries, the consumption of dry legume seeds is residual and many of the legume species are not even known by consumers. This situation raises three major questions related to human consumption: 1) how to increase the plant protein consumption and how to make them aware of the relevance of legumes in their diet, and dissipate many of the concerns that they have related to ii) the long food-preparation time and iii) the existence of anti-nutritional proteins (lectins, protease inhibitors) and non-antinutritional compound (angiotensin I-converting enzyme (ACE) inhibitor, raffinose-series oligosaccharides, tannins, phytic acid). Indeed, given the presence of such antinutritional compounds, various deleterious effects may occur as a consequence of the continued dietary ingestion of legumes’ seeds or flours, such as hemagglutination, bloating, vomiting, and pancreatic enlargement. However, on the other hand, the “antinutritional” compounds of legumes hold also many beneficial properties in the treatment and/or prevention of disease when properly processed. A second thematic field is related to economic and management aspects hampering increased legume production, making the respective productions systems more profitable and attractive to farmers by showing the multiple advantages of inserting these crops in the agricultural growing systems and the new techniques and technologies likely to be used to improve their efficiency and yield.

In Europe, the importance of legume crop species grown differs widely from one country to another—driven by cultural and environmental influences. This situation underlies the occurrence of a broad genetic diversity of resources of pea, common beans and faba beans across Europe, which in the southern countries is also extended to cowpea and lentil. They have been kept in numerous collections in gene banks, research institutions, and also in farms and peasant farming systems. On the other hand, to date it is recognized world-wide that there is a great potential to use local plant material and accessions from germplasm banks to contribute to the sustainability and competitiveness of European farming systems. Actually, locally grown legumes are adding value not only to agricultural and environment resources (improved soil properties through Rhizobium, intensified cropping systems in sustainable way, new feed possibilities, etc.), but also gain interest from agro-industries to develop innovative food products. This possibility is gaining more and more relevance under the increasing and often climate change-driven biotic and abiotic challenges that agricultural systems are facing. Indeed, climate change-enhanced modifications of water availability and temperature (extremes), and the intensified occurrence of pests and diseases are certainly the major challenges that need to be addressed now. However, it is necessary to gather further knowledge on the characterization of this genetic material and the respective plant x environment interaction. In addition, using legume plants as “buffer” for different unpredictable impacts in agriculture ecosystems (soil erosion, greenhouse gas emission, and C footprint) can contribute to the sustainable development of Europe ensuring both safe environment and food supply for its citizens.

Climate changes, featured by the occurrence of more frequent extreme events, particularly high temperature in the southern region of Europe (northern regions also tend to have similar effects), require the development of new crop management skills in which inoculation with specific Rhizobium strains and arbuscular mycorrhizal fungi (AMF) can play a major role, particularly by increasing water (WUE) and nutrient use efficiency (NUE), major challenges under Life Cycle Assessment. In this context legumes and
the development of inoculation procedures and new formulations including Rhizobium, AMF and other plant growth promoting rhizobacteria (PGPR) is a major challenge to sustain the agriculture and rural development and counteract the foreseen desertification process (already observed in some areas of the southern European countries). The negative impact of this occurrence represents a drawback for the common agriculture policy, lagging behind several countries with a negative socio-economic impact, requiring a quick intervention.

Legume crops are usually linked to pulses, a more generic concept, which includes all the dry seeds produced from legume crops. If we accept this concept, and extrapolate to the world-wide legume production, then it will be included a wide array of crops, one more common (i.e. soybeans, dry beans, peas, and faba beans) than others (i.e. cowpeas and lentils), which globally represent a group of superfoods, and which can be grown without reliance upon inorganic nitrogenous fertilizers or with only a fraction amount of this expensive and high environmental impact fertilizer. Actually the effective legume agronomy, linked to optimized crop rotation, can deliver ecosystem services and minimize the use of inorganic fertilizers, protecting environments from further aggressions and lowering costs to producers, whilst also delivering highly nutritious commodities.

As mentioned above, the sustainability of the agri-food systems will be compromised in the near future because of the higher consumer demand of the growing world-population expected for the coming years. In this regard, the present consumption of meat as a dietary source of animal protein is a major concern, since this system is not sustainable in a long run. Hence, it should not be envisaged a completely and utopic substitution of animal protein by vegetable protein but as overall trend the human consumption of vegetable protein (also of high biological value) needs to increase. Indeed, an increased consumption of legumes in the EU is highly desirable taking into account the great nutritional value and the beneficial health effects of legumes. Legumes contain high level of protein and adequate proportions of slow absorption carbohydrates and polyunsaturated fatty acids making them valuable as health-promoting foods. This is especially relevant taking into consideration that, on average, 51 g of protein needs to be consumed per person per day (46 and 56 g per adult female and adult male, respectively). A ½-cup serving of legumes contains on average 7 g of protein, or about 15 percent of the recommended daily allowance. Moreover, besides the protein supply provided by legumes, one serving yields more than half of the recommended daily amount of folate, a quarter of the amount of zinc, and 1/5 of calcium. Legumes are also valuable dietary sources of B vitamins, antioxidants, and dietary fibre. The nutritional facts of legumes have been associated with a reduction in the incidence of cancers, cardiovascular diseases and metabolic disorders. The combination of proteins and amino acids makes legumes interesting also for vegetarians and vegans to ensure a balanced diet.

The increase of the number of vegetarians, meat avoiders, and meat reducers over the last decades has encouraged the use of plant-based foods and ingredients with functional and high-protein composition to replace meat products. The interest on increasing the presence of legumes towards a more balanced diet and to a better health status of the population, expanding the traditional concept of the use of legume crops not just for dry seeds (pulses) but also for many other plant parts (i.e. leaves, flowers) and the transient stages to dry seeds (i.e. pods and immature fresh seeds), closer then to vegetable production, consumers, and farmers are faced with a innumerous possibilities, which will make this plant family much more attractive for consumers and markets. Taking this approach, it is required to introduce the concept of multipurpose use crops. Apart from natural products, EUROLEGUME aimed also at considering the processed legume foods, which can be further expanded from the conventional use as frozen or canned foods. In fact, nowadays, new opportunities to use legume seeds for the production of novel foods or as
ingredients to other food products are still far from being fully exploited. This will require the development of new preparation procedures and food processing methods beyond the conventional heat processing. Along with the development of new food products, packaging including approaches to reduce the amount of plastic in packaging needs further research. Thus, the most appropriate processing, packaging and storage techniques should be selected and optimized for application in new products in order to provide high quality and safety of products.

In addition, a further use of legumes as feed (beside soybean, which is already used at large scale) must also be considered; for this purpose, other parts of the plant and the co-products can also be taken into consideration. To support this view are the large imports of protein crops, which bring a large dependency and instability to the agricultural production in Europe, and affect agricultural producers and cause price hikes. The dependence of EU food producers on feed market price fluctuations considerably affects the production cost of food. In the period 2011–2020, according to FAO forecasts, the grain (maize) price could rise by 20%, consequently resulting in an increase of the price on meat (poultry) by 30% (AVEC, 2014). A lower production cost could be achieved by using domestically grown products – grain as a nutritional source of energy and faba beans and peas as a source of protein – in poultry diets. It has to be emphasised that the provision of domestically grown feed for livestock production contributes to the independence of the agricultural industry from feed price fluctuations in the world market and the sustainable exploitation of agricultural lands.

It is in this context that the EUROLEGUME project was designed, a partnership of 18 members, including scientific/academic actors and stakeholders, from 10 European countries, across northern to southern regions, representing a large diversity of environmental conditions and legume production countries. For the research studies more common legume species (pea and faba bean) and one minor used crop (cowpea) were selected.

The overall objective of the project was to improve the agricultural management of these crops towards sustainable production of legumes, as well as to enhance and diversify their multipurpose use, which is especially relevant in a context of changing climate. The successful achievement of this general objective would contribute to broad the production area and thus, to identify new valuable varieties for each agro-climatic condition, and to design new food and feed products fitting properly the nutritional requirements and the hedonic expectation of the diverse European populations. To achieve these goals would contribute to turn EU more competitive and sustainable. This general objective was completed by the following specific objectives:

1. Source and characterize the genetic diversity of local genetic resources of pea (Pisum sativum L.), faba bean (Vicia faba L.), and cowpea/black-eye-bean (Vigna unguiculata (L.) Walp.) for the development of new varieties by breeding programs to be grown under northern and southern Europe agro-climatic conditions, especially under the perspective of increasing effects of climate change;

2. Develop new legume-based food and feed products featured by attractive physical and organoleptic features, with a comprehensive characterization of the nutritive value from available European varieties of pea, faba bean, and cowpea;

3. Collect from different locations across Europe and select from germplasm banks native strains of most appropriate rhizobium and arbuscular mycorrhizal fungi to support nitrogen fixation. Develop new commercial inoculants for the different agro-climatic conditions in Europe;

4. Introduce leguminous (pea, faba bean, and cowpea) crops in the European agricultural systems to enhance the sustainability (i.e. a reduction on greenhouse gas emissions), by designing the most proper agricultural practices (specific crop rotation and inoculation practices for legume species and agro-climatic conditions in Europe).
conditions) to improve yield and economic returns to farmers and to reduce the environmental impact of legumes productions;
5. Develop new high efficient alternatives for legume by-products processing (silage and fungi treatments) to add value as feeds to these currently under exploited materials.

The main long-term progress of EUROLEGUME will be the increase of legume production in the European Union (EU), thereby reducing the import and turning local agriculture into more competitive, by identifying more sustainable growing conditions and environment conservation. Moreover, increasing the proportion of legumes in the diet will contribute to provide more balanced and safe foods featured by high quality protein sources to European citizens. The most remarkable novel achievements beyond the state-of-the-art include:

- Breeding – European genetic diversity of legume crops will be accessed and new elite genotypes for production and/or breeding will be selected. Near Infrared Reflectance (NIR) calibrations will be created for use in the fast and efficient evaluation of breeding material;
- Food and feed production – novel food and feed products will be developed with increased nutritional value and easy consumption. New processing & packaging technologies and materials will be developed for particular foods;
- Biological nitrogen fixation – Rhizobium leguminosarum and Bradyrrhizobium spp. strains of diverse geographical origin will be described and evaluated. New commercial Rhizobia and AMF products will be developed;
- Cultivation practice – sustainable cropping systems will be developed for particular agro-ecological conditions and to overcome changing climate conditions;
- Environmental sustainability – agronomical and biotechnological strategies will be used for efficient soil management, valorization of residual biomass and nitrogen fixation.

Project Results:
EUROLEGUME is a multidisciplinary project focused on the development of scientific and technological outputs, which are intended to create added-value on the pea, faba bean, and cowpea value chains. Thus, the project addresses several challenges across the whole value chain, starting on the selection of the best cultivars up to the processing technology of novel food products, as well as new feed products and their effects on animal production.

This section was designed to easily understand how the project outputs match the initial set of objectives for each WP, mentioned in a previous section.

During the four years of the project (2014-2017), the work was conducted according to the initial plan, involving in a very dynamic and interactive mode the different partners, on the key activities of coordination (WP1), research (WPs 2, 3, 4, 5, and 6), and dissemination (WP 7), which represented the person-month effort referred in Annex 2 for each WP and partner.

EVALUATION OF THE PERFORMANCE OF LOCAL GENETIC RESOURCES OF PEA, COWPEA, AND FABA BEAN ACCESSIONS FOR POTENTIAL BENEFITS IN REGARD TO SELECTIVE BREEDING FOR SITE-SPECIFIC ABIOTIC AND BIOTIC CONDITIONS

Increasing growing area of legumes has given rise to a demand of new varieties; while during the last decades, investments in breeding programmes towards improved legume varieties were unattended,
relegated by the priority set up on cereals. However, socio-economic changes have entailed the disappearance of a number of local genetic resources, whilst only small-scale farmers continue growing diverse local landraces, selected according traditional knowledge on their adaptation capacity to local environmental conditions. Thus, local genetic resources represent important sources for genetic variability, as they contain valuable co-adapted genes for future cultivation practices, higher yield, and better compositional quality.

Despite the significant available number of accessions of local faba bean, pea, and cowpea germplasm across Europe, there are still a lot of local genotypes, not collected, evaluated and included in breeding programmes. Thus, one of the project objectives was focused on collecting and characterizing local genetic resources. According to this strategy, the inclusion of genotypes in preliminary screenings was determined by two main criteria: 1) Local genetic resources (landraces) without sufficient previous characterization; and 2) Genetic resources featured by valuable traits, although presenting a gap of information on interesting descriptors, especially grain nutritional quality, required to make decisions on their suitability to be included in breeding programmes as donors of particular traits. Within the EUROLEGUME partnership it was gather a total of 263 faba bean, 211 pea, and 139 cowpea accessions from diverse European countries (Estonia, Norway, Latvia, Sweden, Albania, Portugal, Spain, and Greece), which were exchanged between partners and included in multilocal trials, carried out under Northern, Central, and Southern Europe agro-climatic conditions, for primary description regarding morphological, phenological, and quality traits, as well as for evaluation of genetic diversity. These activities involved the interaction of all WP’s. Different sources for collecting genetic resources were used, including Gene Banks, local breeders’ collections, farmers, Non-Governmental Organizations (NGOs), and hobby gardeners. Carried out scientific activities revealed new sources of local genetic resources that can potentially be used as donors in breeding programmes or directly used in agricultural production.

Genetic diversity of faba bean (Vicia faba L.), pea (Pisum sativum L.), and cowpea (Vigna unguiculata L.) Following the sourcing and characterization of genetic and phenological diversity of local genetic resources of cowpea and faba bean, the major foregrounds are:

i. Evaluation of 69 new local faba bean landraces in Latvia and 3 local faba bean landraces in Greece, which were collected in expeditions during project implementation.

ii. Evaluation of a total of 263 faba bean, 211 pea, and 139 cowpea local accessions from diverse European countries (Estonia, Norway, Latvia, Sweden, Albania, Portugal, Spain, and Greece), which showed a broad variation between locations (agro-climatic conditions) and accessions. New sources of local genetic resources were identified regarding their value as potential donors of appreciated traits in breeding programmes (flowering initiation and full ripening phase, resistance to diseases and pests, productivity and morphology traits, and nutritional quality (protein content) of grains). As a result of this activity, it was concluded that the spectrum of the most promising accessions for each region shows a different phenotypic response, due to differences in agro-climatic conditions (site specific adaptation). According to the results from this work, the information gathered supports the selection of the most promising genotypes to be included in breeding programmes under Southern and Northern Europe conditions.

EUROLEGUME project also brought new advanced knowledge on the root traits present in currently available genotypes and their adaptable plasticity under environmental constraints and developed novel statistical methods to facilitate future, root system-targeted, breeding approaches. This is of high relevance, because especially the modification of root system architecture (RSA) is envisioned
contributing to future major improvements of desirable agronomic traits, such as yield, drought tolerance, and resistance to nutrient deficiencies. Furthermore, RSA was described of underlying a second green revolution-improving resource use efficiency of crops. The major foregrounds are:

i. Development of a new method to classify pea genotypes based on 5 identified, important root traits, by using a machine learning (ML) approach. The most frequent trait of mature pea cultivars used for differentiation was total surface area of lateral roots originating from taproot segments at 0-5cm depth - a trait highly correlated to resource uptake from the top soil (water in irrigated systems, immobile nutrients) or (by “absence” indicating a deeper root placement) in deeper soil horizons (water in rain fed systems, mobile nutrients). This novel statistical methodology can provide reliable information on the differentiation of mature root systems in breeding programmes and can, thus, be utilized, by breeders and researchers, alike to identify promising germplasm in respect of root architectural traits for targeted breeding efforts. The high classification rate determined is implying that culturing did not lead to a major loss of variability in root system architecture of European pea cultivars, evidencing that selection from the available germplasm collections for root-targeted breeding strategies is possible without, e.g. backcrossing.

ii. A second major technological step forward is the development of a RSA model-based upscaling from seedling to mature root systems. In breeding, generally large numbers of candidate genotypes have to be screened to determine phenotypic variability in a given target trait. Therefore, breeders require phenotyping approaches with sufficient throughput to cope with large screening populations (mostly seedlings growing in artificial media). A key question in phenotyping was, thus, whether observations are platform specific or can be transferred to other environments, particularly soil grown, mature plants. Using a set of European pea genotypes, it was demonstrated that RSA models can overcome the apparent lack of correlation, taking into account the biological rules of root system formation within the model algorithms. The results prove that easily accessible root elongation and branching traits from an agar-based phenotyping platform are sufficient to predict the ranking of fully developed root systems. Root system architecture models are thus an integral part of a phenotyping pipeline, translating high-throughput early stage traits into mature root system predictions, and can now be used by breeders to efficiently integrate root traits in their screening and breeding efforts on legumes and, potentially, similar crop species.

iii. The assessment of the morphology and architecture of the root has shown large differences between accessions from the same country. For faba bean, a supervised, i.e. hypothesis-driven, ML approach revealed that cultivars from Southern Europe are featured by a greater and coarser but less frequent lateral roots at the top of the taproot, potentially enhancing water uptake from deeper soil horizons, than accessions from Northern Europe. Unsupervised clustering analysis revealed that trait differences between Northern and Southern cultivars are not predominant, but that two cultivar groups, independently from major and minor types, differ largely in overall root system size. However, a similar difference could not be found for pea, indicating different selection criteria in both species during past breeding and adaptation to target environments. The larger rooting depth for southern faba bean accessions may be one factor governing the larger drought tolerance of this species, compared to pea accessions utilized in Southern agro-ecosystems (compared to respective cultivars in Northern environments). It was evidenced that differences between accessions are more pronounced than differences between species, whilst no general influence of Northern or Southern European origin has been found on root morphology.

iv. New advanced knowledge was gained in terms of pea breeding for abiotic stresses tolerance, particularly on the adaptation capacity to suboptimal temperatures of pea genotypes, following the evaluation of roots growth and morphology. The large diversity between pea genotypes in traits such as root length, root volume, number of tips and forks, and root specific area was found to influence the
adaptation capacity to suboptimal temperatures. This knowledge will allow more targeted screening approaches for identifying cultivars with an increased tolerance to low temperatures during seedling establishment, as relevant in Northern European agro-ecosystems.

Genetic resources exploration for efficient breeding programmes
The diverse identified genepool was explored for further pre-breeding and breeding activities, with the objective of developing new cultivars of high plasticity and multipurpose use. The major foregrounds on this topic are:

i. In EUROLEGUME, new underexplored cultivars have been identified between evaluated accessions. According to this newly generated knowledge, it was concluded that local landraces and old varieties of faba beans could be directly used in commercial production due to their high yield and protein potential that is similar or higher than in currently used commercial varieties. The most promising genotypes with good adaptation capacity to high yielding environments, candidates to be introduced in commercial growing under Northern Europe, are the Latvian landraces ‘Bauska’, ‘Priekulu 32’, and ‘Priekulu vietejas’, with the highest mean protein yield under tested environments (1.27 1.18 and 1.17 t ha⁻¹ respectively), as well as the field bean old variety ‘Lielplatones’ (Latvia) (1.21 t ha⁻¹). Local landraces of the faba bean varieties ‘VF_002’ and ‘VF_005’ (Latvia), with mean protein yields of 0.96 and 1.03 t ha⁻¹, respectively, were found as the most promising for low yielding environments.

ii. Among the pea accessions evaluated, the most promising for commercial growing are the Estonian commercial variety ‘Kirke’, the Latvian grey pea variety ‘Bruno’, and the old Estonian variety ‘Leili’, which showed the highest mean protein yield (1.03 0.93 and 0.92 t ha⁻¹, respectively) and general adaptation to all tested environments (b=1). Between the tested genotypes, none featured a very good performance under low yielding environments.

iii. The Portuguese cowpea accessions ‘Cp 5553’, ‘Vg 73’, ‘Cp 5051’, and ‘Vg 60’, with the highest mean protein yield (0.54 0.51 0.42 and 0.43 t ha⁻¹ respectively) and regression coefficient values higher than one (b>1), were found to be more adapted for favourable environments, whilst the accession ‘BGE044375’ (Spain) with high protein yield (0.43 t ha⁻¹) and regression coefficient lower than one (b<1) is better adapted to unfavourable conditions.

iv. New promising pea, faba bean, and cowpea accessions were found to have high content of protein, amino acids, starch, dietary fiber, tannins, and phenolic compounds. Genotypes featured by combined good quality and high yield potential were suggested for direct introduction in production, as valuable varieties for food and feed (pea variety ‘Bruno’ (Latvia) and faba bean variety ‘Lielplatones’ (Latvia)). Accessions with some good quality parameters, but with lower yield potential were suggested as sources for increasing quality parameters in breeding programmes, for example pea landraces ‘k 4171’, ‘k 4831’ (both from Latvia), ‘Eesti kollane söödahernes’ and ‘Loomig’ (both from Estonia), as possible sources of high protein content and amino acid content, and landrace ‘k 4172’ (from Latvia) as a source of high dietary fibre content, tannin content, and phenolic compounds. In faba bean, in respect to high protein donors, the results from several experiments recommend the use of the varieties ‘Aqua dulce’ (from France) and the landrace ‘VF_001’ (from Latvia). When evaluating the genetic diversity regarding their quality, the variety ‘VF_066’ (from Latvia) and the local landrace ‘VF_066’ (from Latvia) were identified as good sources of dietary fibre, while the commercial cultivar ‘Fuego’ (Germany) was revealed as a rich source of tannins and phenolic compounds. In cowpea, the local accessions ‘Vg58’ and ‘Vg51’ (Spain) are suggested as the most promising accessions for breeding.
New methods for selecting legume plant material for food and feed

A significant foreground of the project was the acceleration of selection methods by using Near Infrared Reflectance (NIR) in genotype assessment. Hence, NIR calibrations were developed for cheap and fast evaluation of quality traits in breeding programmes, as well as for detecting of dry matter (%) and crude protein content (%) in pea, faba bean, and cowpea. Beyond the initial plan, it was defined additional calibration equations for the ash, total phenolic compounds, and total dietary fibre evaluation in pea samples, and crude fat, crude ash, and total dietary fibre evaluation in faba beans seeds. Qualitative calibration equations were also obtained with the purpose to fast screen accessions and cultivars for amino acids arginine, aspartic acid, glycine, serine, leucine, and tyrosine in faba bean, and alanine, arginine, glutamic acid, isoleucine, leucine, proline, tryptophan, and valine in pea.

Deeping insights in the genetic selection methods and single Nucleotic Polymorphism Markers

The foregrounds on the area of molecular genetics, focused on the enhancement of selection methods to identify new, genetically diverse accessions to be included in further breeding programmes, are:

i. A novel and economic protocol using Next-Generation Sequencing for the genotyping of P. sativum and V. faba, with a barcoded amplicon library and selected Nucleotic Polymorphism Markers (SNP), was developed to accomplish the genetic characterizations foreseen in EUROLEAGUE.

ii. Analysis of the genetic variation and relationships between Iberian Peninsula and worldwide cowpeas shows their structure in three different subpopulations and sustains possible dispersion routes of cultivated cowpea:
- One subpopulation clustered the accessions from Europe, including Iberian Peninsula’s, North of Africa, and Cuba, suggesting that Spaniards introduced this crop to Cuba;
- Accessions from South and Southeast Africa, South of America, and Asia were grouped in another subpopulation, possibly as a result of the Portuguese sailing routes that established direct contact between Europe, Southern Africa, and India.
- West Africa accessions were included in a different subpopulation emphasizing the specificity of the native cowpea gene pool.

Additionally, the in-depth analysis of genetic diversity in Iberian Peninsula cowpeas revealed three accessions featured by high genetic variability, appearing as be very interesting to be used in breeding programmes to increase genetic diversity in new cultivars;

iii. Mostly clades of P. sativum and V. faba were exclusively formed by accessions from a certain country. So, it is concluded that selection in breeding programmes as parents for hybridisation accessions from different geographic origin will help to increase genetic diversity in new breads. On the other hand, other accessions were interspersed, indicating that genetic and geographic distances do not always match.

iv. New candidate SNP markers were found associated to phenotypic traits: days to flowering (SNP_100000350), pod yield (SNP_100000736), and protein content (SNP_100000543, SNP_10000255) for pea and, as well as with the traits: days to flowering (SNP_50001040), pod yield (SNP_500000764, SNP_500000965), protein content (SNP_50001040), pod length (SNP_50001872, SNP_50001365) and plant height (SNP_5000911, SNP_5000089, SNP_5001679) for V. faba. These associations are now available to foster the breeding process.

ENHANCE BIOLOGICAL NITROGEN FIXATION AND P UPTAKE BY LEGUMES THROUGH SYNERGIC INFLUENCE OF RHIZOBIA AND ARBUSCULAR MYCORRHIZAL FUNGI IN ORDER TO OBTAIN HIGH NUTRITIONAL VALUE FOOD AND FEED PRODUCTS IN SUSTAINABLE
AGRICULTURE

Selection of effective and competitive rhizobial strains for enhanced Biological Nitrogen Fixation and well adapted to a wide range of climatic and edaphic conditions including acid, arid, and semi-arid regions. Improvement of legume production in many world regions requires isolation and selection of effective abiotic stress tolerant microbial symbionts. In this context, one of the aims of this study was to select the best strains of geographically different rhizobia, other plant growth promoting bacteria and arbuscular mycorrhizal fungi (AMF), which were collected across Europe on different legume genotypes and in different climatic zones.

Our research is in line with worldwide studies on indigenous rhizobia, in fields without rhizobial inoculation history, which were revealed of chief importance for the selection of novel strains well adapted to the local environmental conditions. Such strains often exhibit a better performance in similar habitats being more suitable and preferable when designing new inoculant formulations.

From experiments under greenhouse conditions and open field, was gained relevant information for the selection of the best inoculant strains for particular legume species and the adequate carrier media, envisaging the development of commercial products. These strains are currently being tested in several geographic regions aiming to define their compatibility under diverse edaphic-climatic conditions.

Identification molecular markers for field studies in order to monitor the inoculated strains through pot and field experiments

The results of the present study were far beyond the state-of-the-art, providing the first analysis on the phylogenetic diversity of indigenous cowpea-nodulating rhizobia in Greece and Portugal, and further confirm the promiscuity of cowpea, also suggested from the genetic studies and biodiversity conducted in other WPs of EUROLEGUME, extending our knowledge on the diversity, distribution, and evolution of cowpea-nodulating rhizobia in European soils. Increased knowledge was gained about the diversity of nodulating bacteria associated to faba bean and pea in several European countries. The rhizobia isolated and characterized in the present study showed a high genetic diversity including putative new species and symbiovars.

It was shown that the isolates can be discriminated by Multilocus Sequence Analysis (MLSA) or Enterobacterial Reetitive Integenic Consensus (ERIC)-fingerprinting analysis; however ERIC evidences a higher discriminatory power than BOX-PCR and REP-PCR in identifying rhizobial strains, with the following advantages: can be performed without previous cultivation of the strains before analysis, reveals the genetic heterogeneity of the strains at a genome scale, and does not need to sequence specific genes for identification, which permits a rapid and cost-effective technique for identification of large number of isolates from pot and field experiments. However, when no previous genetic information is available, MLSA is required to obtain the information at species and symbiovar level. The rhizobia taxonomy can be based on housekeeping or core genes, usually present in the cromossome; however, host specificity is determined by nodulation genes (nod A, nod C), often carried in plasmids. Therefore, bacteria of the same species can have different host specificity. Due the importance of symbiotic compatibility when developing inoculants, it is required that symbiovars should be determined and supported by symbiotic gene sequence information.

Develop improved microbial inoculants addressed to obtaining marketable products

From the intensive research carried on in EUROLEGUME, it was shown that co-inoculation of rhizobia and
AMF significantly increased plant growth and contributed to plants resistance against drought stress; however, the results depend on the type of rhizobia strain. A finding of great impact for the optimization and high efficiency of Biological Nitrogen Fixation (BNF) under field conditions is that this depends on the symbiosis with bacteria, which must be well adapted to particular edaphic-climatic conditions, as well as on the plant genotype.

Due the variable features of the microbial symbionts, the inoculation of legumes with a mixture of beneficial microorganisms could improve the efficiency of the inoculants. Therefore, the new advanced formulations are based in a mixture of rhizobia and AMF, being dependent on the legume species:

i. For cowpea plants, mainly for regions of southern Europe, selected rhizobia strains are Bradyrhizobium sp. and Bradyrhizobium elkanii. Both are adapted to drought and present tolerance to acidic soils.

ii. For faba bean was selected the strains R. laguerreae and Paraburkholderia xenovorans, adapted to acidic soils and drought, isolated in Portugal, and two Rhizobium leguminosarum bv. viciae strains isolated in Latvia.

iii. A mixture of AMF strains is proposed by for the new commercial inoculants: Rhizophagus irregular, Funneliformis mosseae, and Clarodeoglomus claroideum.

iv. For a multipurpose inoculant, other plant growth promoting rhizobacteria (PGPR), isolated from the EUROLEGUME studies in legume plants, could be added to the mixture, such as the phosphate solubilizers from genera Bacillus and Klebsiella.

Carrier material used in the inoculants and the storage temperature influences the success of immobilization and the survival of rhizobia inoculants. Our studies showed that sterile peat with a moisture content of about 60% is a good carrier, being recommended storage at a temperature of -18 °C or 4 °C. However, even under proper storage conditions, the bacteria population declines over time. Therefore, further improvements in the formulation and in the packaging are still required for a longer shelf-life. A new packaging bag is now being developed in collaboration with a Belgian company.

It must be emphasized that bacterial inoculants, selected in Southern Europe, are featured by a resistance to moderate water stress. Although not yet fully proved, it is likely that, under field conditions, these inoculants might persist over drought periods, though maintaining the mutualistic capacity with the host. Thus, still further work is required involving inoculum companies, directed to test the selected inoculants in different field conditions in order to define also their degree of adaptation to the farmers’ agricultural practices.

**IMPROVE AN EFFICIENT ASSESSMENT OF HIGH PROTEIN LEGUMES OF LOCAL ORIGIN (PEA, BROAD BEAN, AND COWPEA) IN FOOD AND FEED, AS AN ALTERNATIVE TO IMPORTED HIGH-PROTEIN FEED MATERIALS (SOYA BEANS, CORN, ETC.), AND THEIR IMPACT ON THE QUALITY OF FINAL INNOVATIVE PRODUCT, COST-EFFECTIVENESS, AND ENVIRONMENTAL SAFETY**

There is an actual trend to increase the consumption of natural and local produced foods, with low carbon footprint, and healthier foods based on a gradual literacy and awareness of consumers about the influence of diet on health and well-being. According to this new trend, there is an increased market demand for innovative natural foods and food industry to produce healthy, ready-to-eat, high quality, and safety products. Previous studies have shown that faba bean is an interesting product to develop innovative foods, due to its beneficial health implications. The project aimed to develop new food products, particularly from local available European varieties of pea, faba bean, and cowpea, to satisfy consumer needs for minimally processed, ready-to-eat, and value added products, ensuring sustainable protein
sources and contributing to the nutritional and sensory quality of final innovative products, cost-effectiveness, and environment safety.

The local genotypes and varieties with high nutritional value selected for food/feed processing

One of the main objectives of the project was related to the development of new legume-based food products and feeds, based on a refined second-stage selection process of elite genotypes with the best quality parameters, from the large genetic material gathered and available within the project. The material selection was done for each main region covered by the partnership (Baltic countries, Nordic countries, which were represented by Sweden and Norway, and the Southern European region including Portugal, Spain, Greece, and Albania) since a significant cultivar × environment interaction occurred. The following attributes were used in the selection process: high productivity, yield stability, high protein content for food or low protein content for feed, as well as high content of selected amino acids, and low anti-nutrient content.

Following a Principal Component Analysis (PCA), the weight of each variable for the distinct PC’s, in the form of ‘Loadings’, Partial Least Squares Regression (PLS-R), the Wold’s iteration, and the Cross-Validation process, it was found that the best faba bean cultivars used for further development of novel food and feeds were ‘Aqua dulce’, ‘VF_001’, ‘Gloria’, ‘Lielplatones’, and ‘Bauska’ for Latvia, as well as ‘Gloria’ for Norway. The cultivars ‘Favel’ and ‘Fuego’, irrespective of growing location, do not seem to present nutritional characteristics to be selected, due to their low protein and low essential amino acid content.

Regarding peas, the most suitable cultivars were ‘Bruno’, as well as ‘Kirke’, and ‘Capella’, since they have a relatively high productivity (3.45–3.94 and 3.72 t ha⁻¹, on average, respectively) and excellent nutritional characteristics represented by high protein content (26.4–24.9 and 25.0 g / 100 g DM, respectively); high content of dietary fiber (23.7; 23.5 and 22.1 g / 100 g DM); and high content of phenolics (3.6–4.9 and 2.3 g/kg DM, respectively). The lowest integrated assessment values were established for the variety ‘Bruno’, indicating the smallest deviation from the target values among the analysed samples.

From several experiments performed in the Southern European region, it was revealed that cowpea is a very promising crop for the future, which deserves special attention. Indeed, most of the tested cowpea accessions/varieties produced fresh pods of high or superior quality – being rich in proteins, chlorophylls, carotenoids, and phenolics, and showing high antioxidant activity and low concentrations of nitrates and raffinose-family oligosaccharides. These features prompt their use to produce new varieties with high yields, and superior quality and dietary properties upon breeding programmes. Pod fresh weight ranged between 3 and 6 g in the accessions of unguiculata ssp., whereas it was significantly heavier (8–17 g) in sesquipedalis ssp., which produced pods of comparable weight to the unguiculata ssp. Accordingly, pod length in the unguiculata ssp. accessions ranged between 11 and 20 cm and in the sesquipedalis ssp. between 30 and 60 cm. In addition, growing cowpea for green pods requests a much shorter growing season with fewer inputs than other vegetables, making the crop more sustainable and much more adapted to several abiotic stresses imposed by climate change.

In addition, the results from the characterization of promising legume varieties – three varieties of faba beans (‘Columbo’, ‘Gloria’, and ‘Julia’) and two varieties of peas (‘Clara’ and ‘Capella’) as feed ingredients for ruminants in Norway – suggested that nutritional value can be improved upon proper heat processing (ordinary steam pelleting, expander pelleting, and extruder processing, as well as roasting). Dry seeds of faba bean, pea, and cowpea were evaluated for the production of typical livestock feeds for dairy cows, broiler chickens, and milking goats. When evaluating legumes for inclusion in dairy cow and broiler diets, it was found that the biochemical composition of faba beans and peas, when compared to
soybeans, was as a promising ingredient to be included in dairy cows and broiler chickens rations, being a valuable source of locally grown dietary protein.

Innovative food and feed products from locally grown legumes
New legume-based foods
EUROLEGUME has expanded and given evidence of new opportunities of using legumes for food products of improved nutritional value. Within the project, a range of innovative products was developed using selected legume genotypes: immature seeds of peas and beans with extended shelf-life, pesto sauce made from fresh faba bean seeds, cowpea fresh pods as a novel legume vegetable, faba bean ‘cheese, ready-to-eat-pulse spreads, extruded snacks made from dry pea and bean seeds, and protein and fibre rich legume bars with various flavours.

Cowpea fresh pods are, on average, featured by higher crude protein and dietary fibre 31.0 and 40.0% DM, respectively, when compared with fresh green beans (20.0 and 22.0% DM, respectively). Thus, cowpea accessions grown in Southern Europe could be introduced in the market as a novel legume vegetable and be considered as valuable genetic material for the production of cowpea fresh pods of even higher quality and dietary value. Pods are rich in proteins, chlorophylls, carotenoids and phenolics, especially when compared to fresh pods of other vegetable species (e.g. snap beans, okra). Pods also exhibited high antioxidant activity and low concentration in nitrates, when compared to green vegetables (in particular leafy ones), as well as low content in raffinose-family oligosaccharides (RFOs), especially when compared to dry legume seeds.

Another step towards innovation was to extend the shelf-life of the developed minimally processed products of high nutritional value: immature faba bean, pea, and cowpea seeds for fresh consumption, and the frozen cowpea grains and pods. Fresh faba bean and peas are an important source of proteins, carbohydrates, vitamins, and minerals, green pea being a legume product rich in proteins (ca. 24%) and complex carbohydrates, important for humans. For these reasons, pea and faba seeds are recommended to be consumed as a part of a healthy diet. Aiming to extend the shelf life of these new products beyond the usual 3-4 days of green beans, our results revealed that a cold temperature and modified atmosphere packaging (MAP) can extend the shelf life of immature pea and bean seeds. Thus, the development of minimally processed immature pea and faba seeds meets the objective of promoting the consumption of fresh legumes. Indeed, following storage and commercialization, if packaged in a suitable container, might be consumed as fresh or directly microwaved, which converts these products in a quick available healthy cooked food with high organoleptic and nutritional quality.

In addition, an innovative pesto sauce was made of fresh faba beans seeds. Its physicochemical properties, especially colour, were greatly influenced by heat treatments; microwave (MW) treatment was able to preserve greenness, improve the consistency, texture, and taste (see further section on processing and packaging technologies).

Additionally, two innovative products of “humus-like” have been developed, namely, green pea puree from fresh seeds and ready-to-eat legume spreads from dry seeds of pea and cowpea. Both products underwent short storage time, thus, efforts have been made to extend their shelf life. These puree present different soft textures and represent an excellent and convenient alternative to promote the daily consumption of the five servings a day of fruits and vegetables recommended. Pea purees could satisfy this trend by increasing the intake of legumes, which are an important source of nutrients. Green pea puree is rich in proteins (6.54 g 100 g-1 of product), fiber (4.66 g 100 g-1 FW), and vitamin C (22.13 mg 100 g-1 FW, on average).
The developed technology and selected raw materials ensure the production of pulse spreads with high protein and dietary fibre content, which are safe for consumption. Seasonings had a significant influence on the degree of preference among consumers, dietary fibre and total phenolic content, colour and firmness of pulse spreads. Nutritional value analysis showed that protein content of all four developed pulse spreads meet the requirements of EU Regulation (EC) 1924/2006 for a nutrition claim of ‘high protein’ since 21.7–22.9% of energy value of pulse spreads is provided by protein. The same regulation allows labelling of all developed pulse spreads as ‘high in fibre’ considering they contain ≥6.0 g of fibre 100 g-1. Energy value of pulse spreads ranged from 537 to 550 kJ 100 g-1. Protein and dietary fibre coverage (%) by one serving (50 g) of pulse spreads was dependent on the selected consumer age and gender group.

Another novel product, the faba bean based blue mould ‘cheese’ (“Bean Blue”) was developed. It is based on a 75–25% mixture of faba beans and soya beans. The production steps include dehulling of beans (faba beans only, not the soya beans), soaking during night, and mixing together with vegetable oil into a milk that is cooked and coagulated into a yoghurt-like substance. Blue mould culture and lactobacillus culture is added. The coagulate was placed in forms to drain excess water, dry salted, and matured for 2–3 weeks.

Other novel developed products were sweat and salty extruded snacks based on peas, faba beans, and cowpea. Assessment of the chemical and physical properties of extruded products revealed that no additional raw materials for pea and bean extrusion are needed to ensure acceptable product size, volume density, and hardness. It is possible to obtain extruded legume products having considerably higher protein content, comparing to cereal extrudates: in the product from grey peas the protein content was 26.2 g 100 g-1, from broad beans – 28.5 g 100 g-1 and from field beans – 27.7 g 100 g-1. Sensory panels have chosen products with added flavour, the preference being for barbecue and almond taste. Used extrusion parameters, packaging materials, and technology proved to ensure stable microbiological quality during whole storage time of 24 months for non-flavoured products, but for flavoured products, it should not exceed 12 months in any of materials tested in experiments.

Extruded pea product was further used in the development of crunchy legume bars, with increased protein and reduced carbohydrate and fat content, providing an alternative to cereal bars for health-conscious consumers. Totally, six formulations of extruded pea bars of various flavours were developed – light caramel, goji berries, hemp seeds, sun-dried-tomatoes & garlic, sour cream & onions, and ginger & wasabi. The protein content of the bars ranges from 13 to 16 g 100 g-1 of product, and it is about 17.0% of the energy value of the bars. The content of sugars ranges from 26 to 33 g 100 g-1 of product. The fat content is about 2 g 100 g-1 of product (very low). The energy value per 100 g of developed bars is between 320 and 366 kcal.

New legume-based feeds
Soybean can be replaced in the diet with local sources of vegetable protein, peas and pea straw in the Murciano-Granadina dairy goat diet, since they did not affect yield and gross chemical composition of the milk; actually, they contribute to improve the fatty acid profile. However, the replacement is still weak because pea prices are not competitive. Moreover, the local production of peas is not steady from one year to the other. Therefore, further effort is necessary to rise the farmers’ and political awareness about this issue.

Grain legumes can be an alternative as a vegetable protein source in dairy goat diets, since their inclusion did not affect milk production and presented advantages in fatty acids profile that may be beneficial to
consumer health. This was shown in a dairy goat feeding trials, barley straw was replaced by pea straw during the first month; and during the second month peas were included and replaced part of the cereals and 33.0% of soybean were developed to evaluate effect of legumes on milk production. In the fatty acid (FA) profile, polyunsaturated FA increased in the milk from goats fed pea straw, which may be due to the tendency (p<0.12) of oleic acid C18:1n9c to increase. In addition, saturated FA tended (p<0.08) decrease, and unsaturated and Cis FA to increase. When peas were also added to the diet, milk showed a higher Omega 3 FA percentage. Moreover, short and medium chain FA (p<0.06) tended to decrease and monounsaturated (p<0.09) and Cis (0.07) FA to increase.

Based on the experimental data from dairy cow and broiler feeding trials, it can be concluded that feed rations may comprise ‘Bruno’, ‘Vitra,’ ‘Pinochio’ peas and ‘Lielplatone’ faba beans grown in Latvia, thereby reducing the use of imported soybeans. The cost of crude protein from peas and faba beans is by 0.18-0.38 EUR/kg lower than from soybean meal.

Feeding dairy cows with feedstuffs rich in protein (peas and beans) is economically beneficial, as it reduces the cost of feed and increases the productivity. Summarising of the results from different trials revealed that the diets comprising only one kind of legumes (peas or faba beans) were the most economically efficient, while the highest production efficiency was achieved if incorporating 22-24% ‘Lielplatone’ faba beans into the diet for dairy cows.

The inclusion of rations of peas and faba beans in broilers diet, may replace soybean oil meal, meeting the physiological requirements. It well provides the required nutrients for an adequate animal performance and live weight increase, with a reduction of feed costs and a consequent increase on economic returns. It is further recommended a diet with 200 g of peas kg-1, which ensures an increase in live weight gain and a decrease in the feed conversion ratio and accordingly, lower feed consumption and a lower production cost per unit produced. These alternatives give a chance to decrease the amount of imported soy and thus, to reduce feed costs and raise the competitiveness and productivity.

It was shown that locally grown peas and beans can be included in the feed cow ration by incorporation in animal feed by 4.1%, or 20.0–24.0% from concentrated feed. The results of the study confirmed that the use of legumes in dairy cow feeding as a self-produced feed for improving nutritional value and protein balance in feed rations is significant and promising, keeping better milk yields in lactating cows and raising milk yields while maintaining normal animal health.

Similarly, locally grown faba beans and peas can be included in broiler chicken diets with the aim to reduce the amount of imported protein source - soya meal. Cross Ross-308 broiler chicken were used in the experiment. Various amounts of peas ‘Bruno’, ‘Vitra’, and ‘Pinoccio’ (Pisum sativum) and faba beans ‘Lielplatone’ (Vicia faba var. minor) were tested in chicken diet, as well as their effect on chicken health status, meat quality and chemical composition of faeces were evaluated. The optimum additive of 200 g kg-1 in broiler chicken feed was established, which provides live weight gain and good feed conversion rate, resulting in reduced feed consumption. The study revealed that bigger amounts of peas (300 g kg-1) are not acceptable, because broiler chicken productivity was decreased and it had negative effect on chicken health.

The cowpea stover can be part of a rabbit diet up to 100 g kg-1, following the treatment with Pleurotus citrinopileatus, creating an added value to this plant biomass residue as explain in the following section.

New processing and packaging technologies and materials

Very fast loss of freshly harvested legume quality is typically observed due to their very high respiration rate. Within EUROLEGUME, it was studied the quality parameters of fresh peas and faba beans stored in
MAP to provide valuable alternatives that give response to the scarce information available regarding the optimum storage conditions in this regard for ready-to-eat legume-based products. The best quality preservation was observed at 1 °C, when compared to 4 °C. Under the former condition, it was achieved a high quality peas, even after 12 days of storage, independently of the sanitizer applied (sodium hypochlorite (NaOCl) or acidified sodium chlorite (ASC)). Similar results were obtained for seeds from faba beans. However, shelf life was shorter than for peas, reaching 9 and 7 days at 1 °C and 4 °C, respectively. Moreover, acidified sodium chlorite might be a good alternative to sodium hypochlorite for pea and faba seed’s disinfection, even though more research is needed to study its effects on other quality parameters. Although there was a browning effect of faba seeds, which reduced their shelf life, the quality was still acceptable. Overall, immature green pea seeds can be stored for 14 days under MAP at temperatures between 1 and 4 °C without any noticeable quality loss, while 7 days was the limit for faba seeds. The use of ASC as sanitizer during processing is a good alternative to SH since it led to seeds with a better sensory quality and a higher content of vitamin C.

The use of UV-C light (UV) or NaOCl plus edible coating, Naturcover P (NAT), as sanitizers during processing of fresh faba seeds could be an alternative to NaOCl alone, since they improve the sensory, microbial and nutritional quality of the final product. Both fresh and microwaved NAT samples showed the highest values for vitamin C and total phenolic content (TPC), while UV samples presented the highest sugars concentration. Therefore, these samples (NAT and UV) achieved the highest sensory quality. These results suggest that typical quality loss due to processing can be reduced by using those coadjuvants. As proposed here, fresh seeds can be microwaved into the same package obtaining a very tasteful product with reduced tannins content. The package developed for the fresh product can be used for microwaving—decreasing the risk of contamination and, at the same time, requires less time of preparation.

While the nutritional analyses showed a significant decrease in cowpea fresh pods crude protein content during the freezing process, the content of the individual amino acids stayed rather constant. Furthermore, blanched cowpea pod samples were shown to have significant lower amounts of crude protein after all different freezing periods, which were correlated to the loss of water-soluble proteins, as well as to the denaturation of proteins at high temperature. While the amounts of total dietary fibre increased after freezing, none of the storage options had an effect on the total fat, ash or organic matter content. The evaluation of the phenolic composition showed a significant decrease in ortho-diphenol and tannin concentration, while the amount of total phenols and flavonoids did not change significantly during freezing. According to these results, freezing was revealed as a good option to maintain fresh products non-perishable, while additional blanching treatments have a positive impact on phenolics, but reduces the protein content.

The alternative MW processing instead of conventional heat treatment was applied for faba bean pesto sauce evaluating its microbial, sensory, and nutritional quality, as well as its bioactive compounds’ content during the storage for 20 days at 5 °C. Physicochemical properties are greatly influenced by heat treatments, especially colour, with an increase in a* values (loss of greenness), whilst MW treatment is able to preserve greenness and improved the consistency, texture, and taste. In addition, the observed degradation of condensed tannins by MW is an interesting result to replace conventional pasteurization for astringent cooked food. That degradation has been previously observed for seeds but, up to our knowledge, this is the first report of that effect in a pesto sauce. Therefore, MW is an efficient technology to apply in faba beans pesto sauce to improve its physicochemical, nutritional, and sensory quality. This sauce seems to be a novel food product that will stimulate the legume’s consumption.
In the study, high hydrostatic pressure (HHP) treatments allowed to decrease the viscosity of green pea puree at higher shear rate, which is favourable for the industrial processing of the puree, since it is more fluid. Furthermore, HHP treatments improve the rheological properties in pea based puree, where the consistency properties are very important for consumer’s acceptance, without changes in sensorial properties like colour or taste until the end of storage (36 days at 5 °C). The initial shelf-life testing of newly developed pulse spreads showed that total plate count in pulse spreads reached the critical 3.69 log CFU/g after three days of storage at refrigerator temperature. Therefore, two advanced processing methods – sous vide treatment and high pressure processing – were evaluated to provide microbiological safety, sensory quality, and shelf-life improvement of pulse spreads. Both processing technologies maintain the microbiological quality of pulse spreads during 62-day storage, without significant differences between spreads packaged in transparent polyamide / polyethylene (PA/PE) and lightproof polyethylene-terephthalate / aluminium / polyamide / polypropylene (PET/ALU/PA/PP) film pouches. Sensory quality of sous vide treated spreads was classified as good at the end of storage (57 days for spreads with seasoning, 62 days for spreads without seasoning), whereas high pressure processed spreads maintained very good sensory quality (62 days). Both sous vide treatment and high pressure processing are suitable to ensure the production of high quality pulse spreads with significantly longer shelf-life.

For extruded snack production, the optimum ingredient proportions and extrusion parameters were determined selecting the most promising products according to physical and sensory evaluation of the developed samples. To maintain a high quality product during storage and establish the product shelf life, 24-month storage experiments have been completed. On average, during whole storage time yeasts and mould count increase, but aerobic colony plate count decreased. The biggest increase of yeasts and moulds were observed between month 12th and 18th, but aerobic plate counts decrease more linearly. However, none of tested microbial parameters exceeded the acceptable limit. Overall, the best or the worst packaging material and technology for extruded legume products among tested ones could not be clearly established. Oxygen (O2) absorbent reduced the changes in peroxide value and the speed of changes in colour components, but in unglazed samples both of these parameters did not change significantly throughout the storage time. Peroxide value in PET / ALU / PP / PA packaging was larger than in OPP / CPP packaging. Moisture content in storage time changed significantly in all packaging materials. From the economical point of view OPP/CPP packaging without O2 sorbent, is recommended, when storage does not exceed 12 months. Storage time of non-flavoured products can be set at 24 months, but for the tested flavoured products, it should not exceed 12 months.

Biodegradable polymer VC999 BioPack lidding film PLA with good barrier properties was used for extruded snack bar packaging. For shelf life extension, the use of oxygen scavenger in the pouch was investigated. In packages containing oxygen absorbent, the moisture content increase was slower. During storage, there was a tendency to an increase in water activity after 12 months, – water activity increased by approximately 17%. The most significant changes in hardness were observed after 9 months of storage, where the hardness of the bars, packaged without oxygen absorbents, had risen by an average of 20 units, whilst in packages with oxygen absorbent the rise was of only 10 units. After 12 months of storage, hardness increased by 40 units in samples without oxygen absorbents and 20 units in samples with oxygen absorbents. The data obtained are not sufficient to draw conclusions on product shelf-life, therefore further studies would be required prior to commercialization.

Improving feed value of field peas and field beans for ruminants through processing has been the main aim in heat processing activities. The heat processes examined have been ordinary steam pelleting, expander
pelleting, and extruder processing and roasting. The effect of the various processing methods has been examined through analyses of main nutrients upon rumen nylon bag incubations (in sacco). The preliminary conclusion is that nutritive value of field peas and field beans for ruminants can be improved by processing, but the effect depends on processing conditions. When expander processing, the results indicate that rumen escape of protein can be increased with ca. 30 g kg\(^{-1}\) DM in field beans. When roasting, care should be taken to avoid overtreatment. At 160 °C for 10 minutes, reactions between carbohydrates and proteins reduced protein and energy value of field beans. Roasting at temperatures of 160 °C and treatment time longer than 5 minutes are suitable to decrease amino acids concentration in faba beans and field peas. The cooperation established with a commercial slaughter pig producer provided the opportunity to try the inclusion of toasted faba beans in feeds for pigs, which improved taste and partly reduced tannin content. From these results, it was concluded that toasting is a simple and easy method for use of faba bean cultivars high in tannins to pigs (and tentatively monogastrics in general); there is thus less need to use white-flowering low tannin cultivars for these animals.

CONTRIBUTE TO THE IMPROVEMENT OF SUSTAINABILITY OF FABA BEAN, PEA, AND COWPEA CROPS IN TERMS OF YIELD PARAMETERS, NUTRITIONAL VALUE, TOLERANCE TO BIOTIC AND ABIOTIC STRESS, EFFICIENCY OF BIOLOGICAL NITROGEN FIXATION, AND NITROGEN UTILIZATION EFFICIENCY, IN EUROPE, THROUGH EFFICIENT CULTIVATION SYSTEMS

Biological nitrogen fixation

Biological nitrogen fixation (BNF) reduces the dependence on inorganic N fertilizers, and may contribute to products of higher nutritional quality, for instance relatively to the level of protein, which is of interest for the development of valuable foods and feeds. Within the project, new scientific knowledge was obtained to better understand the effects of inoculation in three legume species- faba bean, pea, and cowpea, under different growing systems. From the field trials developed in EUROLEGUME, it was shown that cowpea can establish efficient symbiotic relationships with diverse bacteria, mainly slow-growing rhizobial species belonging to the genus Bradyrhizobium. A genetic and symbiotic diversity of indigenous rhizobia were isolated from field-grown cowpea nodules, from five geographically different regions of Greece and evaluated for their efficiency to improve yield, protein content and BNF under normal and stress conditions. This work revealed that, in alkaline soils, cowpea is nodulated by fast-growing rhizobia belonging to the genus Ensifer, which constitutes a novel symbiovar. However, in acid-neutral soils, nodulations involve more frequently Bradyrhizobium lineages. It was expanded the knowledge regarding cowpea nodulation diversified ability, distribution, and evolution of cowpea-nodulating rhizobia in European soils that ensure the formation of root nodules, thereby stimulating BNF.

To evaluate the efficiency of different cowpea landraces to fix atmospheric N\(_2\), it was set a field experiment, which involved inoculation with different rhizobia strains (Bradyrhizobium sp. and Sinorhizobium sp.) and combinations between them. Regarding the %Nd\(_{\text{fa}}\) values (proportion of nitrogen derived from the atmosphere), significant differences were observed between the non-inoculated control (19.8%) and the rhizobium treatments (22.1-60.8%). Inoculation with Bradyrhizobium sp. strain 1 resulted in a higher total BNF than all the other treatments (26 kg ha\(^{-1}\)). With the exception of the combining inoculation with Sinorhizobium sp. and Bradyrhizobium sp. strain 2, all the other rhizobial inoculations were able of fix significantly more atmospheric N\(_2\) than the control treatment (9.5 kg ha\(^{-1}\)).
In addition, it was found that cowpea crops, featured by relatively short-season, increased the N content of soils, independently of the management practices, and stimulated the growth of microbial populations, which can release nutrients to soils by degradation of organic matter. The dual inoculation with Burkholderia sp. and AMF did not increase the nodulation efficiency in the crop. From a hydroponic experiment with cowpea, it was concluded that, applying different rates of inorganic N before and after the formation of N2-fixing nodules could be an effective strategy to promote root nodulation, thereby reducing the supply of inorganic N fertilizers via fertigation, without compromising yield. The nodulation with Ensifer spp. either alone or in a mixture with Bradyrhizobium spp., enhanced cowpea nodulation and BNF (30.0 and 35.0%, respectively). However, the use of Bradyrhizobium spp. alone was less efficient in nodulating cowpea plants.

The use of N2-fixing Burkholderia species as inoculum in a faba bean crop increases the amount of inorganic N as ammonium in the rhizosphere soil (293.0 and 484.0% in ‘Muchamiel’ and ‘Palenca’, respectively). ‘Palenca’ cultivar influences soil fertility, increasing some parameters such as N content, while ‘Muchamiel’ shows better quality parameters (higher pod length). Plant available mineral N in soil has a negative effect on rates of N2 fixation in nodules. The amount of N added via N2 fixation in shoot and root has a positive influence on crop quality parameters (pod length and weight of 100 seeds).

In general, inoculation of faba bean with Nitrogen-fixing bacteria increases the amount of biologically fixed N (up to 30 kg ha-1 in shoots- inoculation with Burkholderia vietnamiensis) and protein content (up to between 74.0-127.0%, with respect to the control), but is dependent on environmental conditions. However, crop yield did not seem to be affected by inoculation treatment, presumably because indigenous rhizobia can efficiently nodulate roots and fix sufficient amounts of atmospheric N2. Nevertheless, to maintain the microbial diversity of the soil, a sustainable management must be developed, such as avoiding the use of herbicides or introducing crops in rotation.

From rotation experiments (2015-2016 and 2016-2017- Greece) in organic and conventional farming system, with different rotation treatments between pea, faba bean, and cabbage, it appeared that pea and faba bean (especially pea) have the ability to leave considerable amounts of soil N to the following crop, resulting in increased yield of head cabbage under organic farming practices.

In an experiment conducted from November 2014 to June 2015 in Greece with four different pea landraces, which were cultivated following either organic or conventional farming practices, the percentage of N derived from the atmosphere (%Ndfa) fluctuated between 74.5 and 89.0%. These values, which were obtained without rhizobial inoculation, showed that pea is a legume with a high N2-fixation efficiency. The total amounts of biologically-fixed N2 by pea, which were estimated by taking into consideration the %Ndfa, the total-N concentrations in the plant biomass and the total dry biomass production, ranged from 45.2 to 125.3 kg ha-1 in the different treatments.

In a similar experiment with faba bean, which was conducted from November 2014 to June 2015 in Greece, the nitrogen fixation ability of faba bean landraces originating from different regions of Greece under both organic and conventional farming systems was investigated. Although faba bean was not inoculated with N2-fixing rhizobia, the plants exhibited a high efficiency in fixing atmospheric N2, as indicated by the percentage of N2 derived from the atmosphere (%Ndfa), which exceeded 75.0% in all tested cultivars, and the total amount of biologically fixed N up to full anthesis, which fluctuated from 118.5 to 193.9 kg ha-1 in the various cropping systems and cultivars.

In a second experiment conducted in the next season (2015-2016) with the aim to test different rotation schedules in organic and conventional farming systems, the pea plants exhibited a reduced ability than in 2014-2015 to fix atmospheric nitrogen, as indicated by the estimated %Ndfa values, which on average
amounted to 63.5% over all rotation schedules and cropping systems. However, when this experiment was repeated in 2016-2017, the %Ndfa values were comparable to those measured in 2014-2015 (89.4 on average over all treatments).

Also in faba bean the %Ndfa values in a similar rotation experiment conducted in 2015-2016 were slightly lower (71.3% over all rotation schedules and cropping systems) than in 2014-2015. However, when the same rotation experiment with faba bean was repeated in 2016-2017, the %Ndfa was as high as in the first experiment conducted in 2014-2015 (87.4% on average over all treatments).

These seasonal differences in the percentage of N derived from the atmosphere and concomitantly in the total amount of fixed N2, are ascribed to differences from year to year in climatic conditions at critical stages for the establishment of the rhizobia and the root nodulation.

With respect to the impact of different rotation schedules on BNF, it was found that the highest %Ndfa and BNF were obtained for both faba bean and pea in the organic plots, when the same legume was the preceding crop (i.e. pea after pea and faba bean after faba bean) in the experiment conducted in 2016-2017. However, in 2015-2016, no significant differences in %Ndfa and BNF were found between the rotation treatments or the cropping systems. The increased N2 fixation by both pea and faba bean when pea or faba bean, respectively, was the preceding crop is ascribed to a higher availability of indigenous rhizobia in the soil, when the same legume species was the preceding crop. This finding indicates that rhizobia inoculation of both pea and faba bean at sowing might increase the amounts of fixed atmospheric N2, at least in organic crops.

Sowing densities
Lower sowing densities for faba bean and higher for pea (30-40 seeds m-2 in faba beans and 120-144 seeds m-2 peas) can be used in the Northern countries. The field trials performed in ECRI and PHRC to test different sowing densities in faba bean crops, showed that there are no significant (p>0.05) interactions between cultivar and plant density, either in yield or on protein content. From these results, it can be concluded that sowing rates of 30 seeds m-2 can be used for faba bean in Estonia and 40 seeds m-2 in Latvia to obtain a good yield and protein level. Regarding pea sowing density, the results obtained in ECRI indicated that yield increased for varieties ‘Capella’, ‘Clara’, and ‘Kirke’ by 28.0 41.0 and 23.0%, respectively, when increased sowing rates were applied (from 120 to 144 seeds m-2). In 2016 there was only a tendency for sowing rate to increase pea yield on most of the tested varieties.

Effect of the three legumes species in Intercropping and crop rotations
Concerning the total biologically-fixed N in trials aimed to test the impact of legume intercropping on strawberries, higher amount of BNF was obtained when peas were used as intercropping. In particular, the total amount of fixed atmospheric N2 ranged between 27 and 53 kg ha-1. In another experiment in which faba beans were used as intercropping, the total amount of fixed atmospheric N2 ranged from 11 to 22 kg ha-1. After analysing the strawberry plants, the highest amount of N derived from atmosphere that had been acquired by strawberries was found in treatments with pea as intercropping (6.2 kg ha-1), while in treatments with bean it was 1.4 only kg ha-1.

Faba bean and pea are good preceding crops for vegetables crops such as white cabbages. According to our results, the improvement of soil properties depends on the specific rotation scheme applied, the accessions/cultivars considered, and the management practices (conventional/organic). However, the pea and faba bean cultivars that have been used in the field trials had no immediate influence on the levels of nitrogen and organic matter in soils that were available to following crops.
Onion and faba bean, when grown in intercrop, compete for moisture and nutrients. The detrimental effect of this competition impairs mainly onion, especially when grown under unfavourable conditions (high temperature and low precipitation). For example, in 2014, the onion yield, when grown with faba bean as intercrop, was almost half of the yield obtained in fertilized and unfertilized controls. In particular, the onion yield amounted to 0.89 kg m\(^{-2}\), on average, in onion crop grown with faba bean as intercrop, 1.79 kg m\(^{-2}\) in fertilized control treatment, and 2.08 kg m\(^{-2}\) in unfertilized control treatment. Therefore, onion/faba bean intercrop is not recommended in onion crops aimed at high yields.

Carrot and faba bean plants are equal competitors and share available soil resources (carrot yield in intercropping variant was 2.28 kg m\(^{-2}\) and did not differ significantly from the control variant developed without N fertilization (2.68 kg m\(^{-2}\)). In addition, faba bean has a positive influence on soil properties and could contribute to lower the application of nitrogen fertilizers needed to fulfil the requirements of the following crops.

Cabbage, can be grown in intercropping with faba bean without yield losses. However, there is no benefit for the cabbage crop during the same cropping period. This was the conclusion from an experiment conducted in 2014 under unfavourable weather conditions for cabbage development, in which the cabbage yield was 1.69 kg m\(^{-2}\) in the control treatment (no intercropping, no N fertilization), and 1.63 kg m\(^{-2}\) in the intercropping plots. Thus, any benefits from BNF of faba bean is anticipated in the following crop.

Soil biological activity fluctuates during the vegetation period and is closely related with environmental factors, rather than with plants grown in the soil. Nonetheless, faba bean crop has a positive influence on the biological activity of soils and can be used towards sustainable improvement of soil resources. Hence, in 2014, the assessment of DHA in vegetable/faba bean intercropping trials revealed significantly higher DHA values in the plots with faba bean intercropping (121 µL L\(^{-1}\) h\(^{-1}\), on average) relative to N fertilized and unfertilized variants (77 and 107 µL L\(^{-1}\) h\(^{-1}\) on average, respectively, at p<0.05).

Organic versus conventional growing systems
As overall conclusion on the field trials established aimed at gaining a further insight in the advantage of organic vs conventional growing systems, it was noticed that, on average, organic cropping systems are better adapted to stand a stress conditions (water scarcity).

Legumes, especially pea and faba bean enhanced yield of head cabbage under organic farming systems due to legume ability to leave considerable amounts of soil N (12.0% increase, on average) to the following crop. The conclusion for the growers and, in particular, organic farmers from these results is that higher yields at a lower cost can be achieved if legume crops, such as peas and faba bean, are incorporated in rotation schedules with vegetables. Pea and faba bean as preceding crops, could increase the yield of head cabbage by 21% and 14%, respectively (on average), in organic farming systems compared to two year of cabbage monoculture.

Selection of cultivars for an improved adaptation to different and stress conditions
Although benefiting from irrigation, cowpea is recognized by a good adaptation to water stress conditions, which depends also on the genotype of each cultivar. For cowpea, the most productive accessions were BGE038474 and IT97K-499-35 in Cartagena, Spain, but IT97K-499-35 and ‘Vg60’ in Greece.

After several trials developed in the frame of EUROLEGUME under different environmental conditions, the most suitable pea and faba bean cultivars suggested for different regions across Europe, particularly in the Northern regions where the knowledge to date was very scarce, are:
Selected pea cultivars:
- Greece: ‘AUAANDRO001’, ‘AUASHIN001’, and ‘Onward’.
- Sweden: ‘Bruno’ and ‘Clara’.
- Latvia: ‘Bruno’ and ‘Kirke’.

Selected faba bean cultivars:
- Albania: ‘AUT005’ and ‘Aguadulce’.
- Norway: ‘Julia’ and ‘Lielplatones’.
- Sweden: ‘Julia’ and ‘Lielplatones’.
- Latvia: ‘Julia’ and ‘Lielplatones’.

The gene VuEF1A should be used for normalization when comparing different tissues under stress in cowpea. The gene CPRD65 is the most reliable one to study drought stress in cowpea. This gene should be a candidate to analyze germplasm for drought resistance and assess response to drought in direct transcriptomic experiments.

The circadian expression of protein synthesis genes indicates that sampling time may be crucial in order to have comparable results between experiments, places varieties etc. Sampling time has to be referred to the subjective time from dawn.

GENERATE ADDED VALUE PRODUCTS FROM LEGUME GRAIN PRODUCTION RESIDUES-
ADDITIONALLY, CROP MANAGEMENT TOOLS ARE EXPECTED TO BE DEVELOPED IN ORDER TO MITIGATE GREEN HOUSE GASES EMISSIONS

Characterization of residual biomass. Evaluate silage with mix of legume residues and apples. Use of solid state fermentation with 3 fungal strains. Test of diets on rabbits

Following the harvest of pods or dry beans from cowpea, a significant plant biomass (cowpea stovers) remains, which was evaluated for feed. According to our experience it is not feasible to separate leaves from stalks and other plant parts in the field. However, leaves have higher protein values (21% vs. 12%, in DM basis), lower fibre contents (40% vs. 68%, in DM basis), and higher digestibility (60% vs. 35%) compared to stalks. The cowpea stovers were collected at the end of the dry seed harvest and evaluated under two different approaches:

i. Ensiling with discarded apple.

ii. Enhance its nutritive value by solid state fermentation (SSF), using white-rot fungi.

Ensiling with a mixture of 15% and 85% of cowpea stover and discarded apples in a fresh weight basis, respectively, was found as a very promising mixture. According to the aerobic stability data, the ensiling period was successful at only 45 days of ensiling. Trying to improve the ensiling process, it was used the addition of microbial inoculants, to spare the fermentation of soluble carbohydrates; however, there was no positive effect of the selected inoculant, and soluble carbohydrate fraction still decreased by 90%. In addition, although not initially planned, it was decided to evaluate if treated cowpea stover by SSF, could also be used in the same way. This could be important, as the treated stover could be conserved by a
certain period of time and given to animals during feed shortage periods. Thus, this processing alternative was found capable to reduce soluble carbohydrates by only 80%. Nevertheless, dry matter losses were low (3%) and the aerobic stability was high. Overall, according to the project results, it could be possible to demonstrate that cowpea stovers and discarded apples can be used and conserved through ensilage.

In addition, in the frame of EUROLEGUME, it was tested to increase the nutritive value of cowpea stover through its incubation with white-rot fungi, aiming to decrease the concentration of antinutritional factors and thus, increasing the biological availability of cell wall structural carbohydrates. Following a screening trial, performed with 5 (initially only 3 strains were foreseen) fungal strains (Ceriporiopsis subvermispora, Pleurotus citrinopileatus, Pleurotus eryngii, Lentinula edodes, and Ganoderma lucidum), and 6 incubation times (7, 14, 21, 28, 35, and 42 days) in a 5x6 factorial experimental design, it was shown that the strain P. citrinopileatus and the incubation period of 22 days is the treatment that promoted higher delignification (46%), higher increase in the digestibility values of the treated cowpea stover (30%), and a net crude protein increase by 13%. This material was tested as feed in a commercial rabbit production upon which a 5% incorporation in the diet bur indicated that this level of inclusion could be increased. Thus, it was tested 5 and 10% of incorporation in the feed in commercial rabbits’ diets prepared by NANTA (Animal Feeding, SA), using untreated and treated dried legume cowpea stover mowed at 2.5 mm particles. These diets were given to weaning rabbits and its effects on growth performances (live weight, weight gain, feed intake, and feed efficiency) were evaluated. To achieve this objective, five experimental diets were prepared containing 0 g/kg (US0) and 50 g/kg or 100 g/kg of cowpea stover untreated (US5 and US10) or treated with P. citrinopileatus (TS5 and TS10).

It was shown that live weight at 63 days was affected by the level of inclusion of untreated cowpea stover (p=0.0408). Animals fed with US10 diet had 10% lower final live weight (2196 g) compared with the control (US0; 2365 g). However, the final live weight of animals fed the control diet (2365 g) and those with treated cowpea stover (2356 g) did not differ, suggesting that the treatment of cowpea stover with P. citrinopileatus diminished the negative effects of the inclusion of untreated cowpea stover on rabbit performance. The analysis of the digestive tract histology indicated that there were no changes in the height (µm), tip width (µm), junction width (µm), and crypt depth (µm) of the jejune and ileum for the different diets. Following the same trend already reported for the gastrointestinal histology, no differences of the haematology of the animals were detected between diets. Further analysis of animals’ serum biochemistry and gut microbiology was done. It was shown that animals fed diets containing up to 10% inclusion of treated cowpea stover had lower cholesterol levels, indicating that P. citrinopileatus may induce a hypercholesteremic effect. Furthermore, microbiota data indicate some changes within the microbial population of animals fed diets containing up to 10% inclusion of treated cowpea stover. Overall, a 10% level of inclusion is perfectly attainable, indicating that higher levels might be possible. In addition, the inclusion of treated cowpea stover implicated the increase in the proportion of wheat bran and a decreasing in lucerne and beet pulp proportions in the diets, which generated a reduction of the cost of the diets (13.6€/ton feed) up to 6.2% for the 10% level of inclusion (TS10).

Summarizing, the main results retrieved indicate that cowpea stover can successfully be conserved through an ensilage process using mixtures (at a 15% concentration in fresh weight basis) containing discarded apples. This is an important achievement as both raw matters are produced at the same period, thus increasing its feasibility and decreasing potential environmental drawbacks arising from the removal of these residues. Cowpea stover treated with P. citrinopileatus can be included in commercial rabbit’s diets up to a level of 10%. Animal performances and the reduction cost at this level of inclusion indicate that higher levels of inclusion (up to 20/25%) would also allow a higher decrease in the economic costs of
feed production with not detrimental effects on growth performances.

Enhancement of nitrogen availability to following crops. Effect of cultivar on the following crops
The findings from EUROLEGUME, supported by experiments across Europe (Spain, Portugal, Greece, Albania, and Norway) support the previous evidences that legume inclusion benefits crop rotations. Peas and faba beans can increase the nitrate concentrations in the soil as benefit to the next crop (on average by 18%) particularly in the early stages of the next crop, when compared to cabbage as the preceeding crop. This effect is mainly due to the contribution of the root biomass rather than the N content in the roots, being more evident in soils with low fertilization levels. The above ground biomass incorporation into soil did not significantly increase the yield of following crops.

The legume species and the cultivars within each species also influence the nitrogen available to the next crop in the rotation. The detailed data revealed that, on average, faba bean and pea crops can increase the soil total N by 2.33 and 1.94 g kg-1, respectively, when compared to wheat (1.05 g kg-1); the subsequent brassica crops (broccoli and cauliflower) had a significant higher total biomass and curd yield compared to those grown after pre-crop of wheat. The best results were achieved in a rotation with faba bean, where the biomass production of broccoli and cauliflower was increased by 37 and 59% respectively, whilst there was a significant increase of curd yield versus wheat rotated plot by respectively 52% in broccoli and 64% in cauliflower.

When pre-crop of faba bean was compared with pea, a 50% increase in yield of broccoli (2nd crop) and 2-fold increase in yield of mini Romano lettuce (3rd crop in rotation) occurred. This can be partly explained by faba beans having higher root biomass compared to peas (5.08 vs. 2.41 kg-1 m-2). Nevertheless, for nutrient demanding crops like broccoli, legumes, as pre crop, can only partly provide the requirement of fertilizers, as additional mineral fertilizers (80 kg N ha-1) provided a 2 and 3-fold increase in lettuce and broccoli yield, respectively, when compared to non-fertilized plots.

Winter cover crop efficiently conserved nitrogen during wintertime as showed by a 4-5-fold reduction of N concentration in soil without cover crops on a heavy soil due to nitrogen leaching, whilst the nitrogen stored in the cover crop will be available to the following crops. Thus, these results are very relevant for yield performance in soil types with high drainage properties exposed to leaching conditions.

According to our findings, the relevance of the above ground biomass on soil N enhancement, after harvest, or its incorporation in the soil to reduce the greenhouse gas emissions, are negligible. Thus, the best use of this biomass would be for feed (i.e. rabbits), eventually as functional feed creating an added-value in the legume crop value chain.

Advanced knowledge on the C sequestration and GHG of different cropping systems with legumes
The extra input of N and C to agricultural systems provided by legume crops may contribute to soil C sequestration and, in addition, to increase soil microbial activity that may stimulate carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) and greenhouse gas (GHG) emissions. Within EUROLEGUME, following the experiments in three locations across Europe (Portugal- tested species and crop residues, Spain- tested cultivars on organic and conventional farming, and Greece- tested species and organic and conventional farming), in three consecutive years (2014-2017), overall, it was found that legume crop residues management and, in some of the rotations evaluated, also the legume species contributed to a significant reduction of GHG emissions. The cultivars and organic/conventional farming practices did not have any effect on GHG emissions. Regarding the C sequestration no clear effects were detected for all the factors under study, certainly due to the limited time frame of the experiments to obtain consistent
changes in this type of soil characteristics.

Inserting legume crops in crop rotations with whole-crop cereals for forage did not produce an increase in GHG emissions. Nitrous oxide (N2O) emissions observed during legume crops were negligible (below 1.5 kg N2O-N ha-1). Whole-crop triticale forage in rotation with cowpea, faba bean or pea, with legume residue incorporation into the soil showed lower or similar cumulative emissions of N2O (between 0.19 and 0.41 kg N2O-N ha-1) than a monoculture of whole-crop triticale for forage moderately fertilized with mineral N (values between 0.67 and 0.88 kg N2O-N ha-1). Moreover, when emissions of GHG were yield-scaled (i.e. emissions expressed by yield unit), cereals forage monocultures fertilized with mineral-N showed a 2-fold increase when compared to similar crops in rotations with legumes, which reveals the environmental advantage of changing from a mineral N fertilized-based system to a legume-based system, particularly when the legume residues are incorporated into the soil.

In specific experiments carried out with faba bean and peas in rotation with cabbage, under both organic and conventional farming systems, it was observed that neither the legume species nor the farming systems caused significant differences in the cumulative GHG emission values. However, it was found that cabbage as a previous crop can affect the emissions of N2O and CO2 from the soil of the next crop, since higher emission values were observed at the earliest stages of the cultivation of peas and faba bean, when the previous crop was cabbage, which may be due to the incorporation in the soil of the post-harvest biomass of the cabbage crop. Results also showed that, although no differences were observed on cumulative emissions for the entire crop season, at the early stages of crop growth N2O emissions were significantly higher in the conventional than in the organic farming system. Moreover, it was found that, at the beginning of pea crop, there were significantly higher emissions in the conventional farming system compared to organic farming system, 5.04 and 0.92 g N2O ha-1 d-1, respectively. This may be explained by the fact that the mineral fertilizers applied to the soil started to release N2O gases more quickly than the application of manure (slower incorporation and decomposition of organic matter into the soil).

Experiments with the crop rotation system cowpea/broccoli developed also under two different management practices (conventional and organic) with two cowpea cultivars (‘Feijão frade de fio preto’ and ‘Feijão frade de fio claro’) showed that cumulative N2O emission in cowpea fields did not follow a clear pattern, while in broccoli fields, there were no significant differences caused by previous grown cowpea cultivar or management practice.

The total Soil Organic Carbon (SOC) stocks, after the three experimental years, did not vary significantly with cropping practices such as the inclusion of legumes in the crop rotation or the two different management practices (conventional and organic) with two cowpea cultivars (average of 12 mg C kg-1 soil). Although, when total SOC was fractionated in labile and recalcitrant fractions, it was observed that in the experiment with the crop rotation system cowpea/broccoli, soil labile carbon at cowpea harvest showed significant higher values in legume crops than in fallow treatments under both conventional and organic management practices, but recalcitrant carbon and total soil carbon values after the broccoli crop and the end of the rotation did not exhibit significant differences between treatments.

Potential Impact:
In this section the impacts and the specific associated dissemination activities, which intend to bring research to society and advocate the need to keep the investment on legume research, will be addressed for each of the major foregrounds of EUROLEGUME.
The outcomes of EUROLEGUME represent a fully integrated, very intensive, and collective initiative focused on the development of legume research, across all the very different steps of the legume value-chain, which have some fairly wide socio-economic implications, mostly embedded into rural development, and sustainability, and to foster the competitiveness of legume-based food industries.

The scientific activities of EUROLEGUME, and the respective outcomes, have covered quite a wide range of topics within the legume value-chain, which can be grouped in three major areas:

i. Collection, characterization, and selection of new pea, faba bean, and cowpea cultivars, featured by relevant agronomic and quality traits;

ii. Development of novel food products and the respective suitable packaging materials and storage conditions, which were intended to meet the specific interest of consumers and industry; simultaneously were developed and evaluated new feed products, processing methods and rations for some of the most common animal species;

iii. Identification of agricultural practices, which are intended to increase the yield, quality, and income of the farmers and reduce the impact of changing climatic conditions on legume crops.

These results will obviously have an impact in the different steps of the legume value-chain, which have already been disseminated by several tools and actions and, which will be further expanded in the near future.

As foreseen in the initial plan, there will be a gradual enhancement of the cultivated area of the 3 legume species, in Northern and Southern European areas, with a clear benefit for the environmental and economical sustainability of the agricultural systems, based on the specific knowledge, techniques, and technology that has been disseminated by the stakeholders within the value-chain, particularly farmers and their respective associations. At the same time, an increased consumption of these legumes species is also expected, based on an intensive programme of dissemination at various levels of consumers (young population in schools and universities, as well as other consumers), and using both classical media and social networks to achieve a wide distribution range. A great emphasis has been given to the added nutritional value of these legumes, to the high quality protein and the respective benefit for human health (i.e. fighting obesity and some types of cancer), which was well received by the different classes of population and distribution chains, not fully aware of the relevance of including these legumes regularly in the diet. Thus, as planned, and foreseen, there will be an increase of the consumption of these and eventual other legumes species with a positive impact on human health, with a consequent reduction of the Health Care costs, and in the quality of leaving of our society. The impact will be even higher when it is widely perceived that these legumes can be used at other transient stages of the plant development and in novel products, which have received, in some public demonstrations, an excellent acceptance by a diverse class of consumers. Industries can then bring these products into the market with a clear impact on the bio-economy.

If we accept that some of the plant residues can be used for feeds with functional properties, creating added-value for these products and reducing the inputs of antibiotics in animal production, it will create a major impact in this sector, resulting in an improvement on animal welfare and meat and milk quality. Thus, the research and innovation outcomes from EUROLEGUME, some of them quite up on the Technology readiness level, since it was already demonstrated the feasibility of their use by the industry, have also contributed to the implementation of a bio-based economy (legumes are indeed a true sustainable bio-based value chain), in geographical areas with low bio-based activities, pursuing the critical path towards 2020 in the acceleration of the development of sustainable value chains. This was achieved by: i) using the whole plant biomass in a more efficient way and with increased income for the
farmers and feeding industry, which is likely to increase job security (green jobs) in rural areas; ii) the development of novel foods and feed products, using the concept of multipurpose crop, which respond to the market needs; iii) answering the consumers concern on eating products with a less carbon footprint. EUROLEGUME had also a clear impact on the scientific development of the partner institutions and the respective scientists involved, fostered by an intensive cooperation, which ended with a share of cutting-edge knowledge published in peer review Journals of high impact. The project has already resulted in additional collaborative projects of different groups within the EUROLEGUME partners—both in similar and adjacent fields of R&D activities. This action has contributed to increase the role of Europe in innovation pathways to impact in legume research and to reveal the leadership of partners in addressing some complex research topics.

EVALUATE THE PERFORMANCE OF LOCAL GENETIC RESOURCES OF PEA, COWPEA, AND FABA BEAN ACCESSIONS FOR POTENTIAL BENEFITS IN REGARD TO SELECTIVE BREEDING, FOR SITE-SPECIFIC ABIOTIC AND BIOTIC CONDITIONS

The new fully characterized local landraces, in which the specific traits have been emphasized, will be delivered to Gene Banks for long-term storage and available for breeding programmes for further exploitation. This action is supported by a Handbook (Deliverable 2.5) which summarizes the results after screening the broad gene pool of 100 faba beans, 104 field pea, and 68 cowpea landraces, old varieties, and unexplored varieties from different origin. Upon these characterizations, it was reflected the phenotypic variability of evaluated genetic resources under Northern and Southern Europe agro-climatic conditions. This information is of relevant use by Gene Banks, breeders, and researchers, particularly under the abiotic and biotic challenges that agriculture systems are facing, and the consequent fast erosion of natural resources. The availability of this information will save time and resources to face the urgent needs to tackle these challenges. The Handbook, available for downloading in the website of project partners, will be electronically distributed to gene banks, researchers and breeders, and seed companies, to ensure a wide audience.

Project partners AREI (Latvia), ECRI (Estonia), INIAV (Portugal), and AUT (Greece) will continue their breeding programmes, including in the hybridisation the EUROLEGUME accessions that revealed desirable agronomical and quality traits. In the near future, improved cultivars with higher yield, quality, and improved adaptation to the local environmental conditions will be available for distribution, which will benefit the farmers and respective associations, consumers, and the whole economy, in general. The attraction of these crops to farmers, and increased cultivation area, certainly will reduce the imports of legume protein.

In the meantime, a legume-plant variety database will be registered in the European Commission, the local Latvian faba bean variety ‘Lielplatones’ and landrace ‘Džūkstes’, which will go to seed production. Similarly, it will be initialized for identified elite cowpeas landraces in Portugal, Greece, and Spain. To promote the increase of the cultivation area of the best accessions identified in the project, AREI (Latvia) has already established a cooperation with local farmers, to launch at least 4 new licencing contracts for the cultivation of the grey pea cultivar ‘Bruno’. AREI (Latvia) also plans to sign a Contract of Agreement with 2 companies in Sweden, to grow Latvian local varieties, evaluated during project (grey pea ‘Retrija’ and faba bean variety ‘Lielplatones’). In cooperation with LTD ‘ALOJA STARKELEN’, AREI (Latvia) is planning to increase, until 2019, the seed production and commercial growing area of grey pea ‘Bruno’ in organic farming, up to 1500 ha, using the growing techniques and technologies designed during the project (Deliverable 5.2).
RISE (Sweden) plans the multiplication of the cultivars ‘Lielplatones’, ‘Retrija’, ‘Bruno’, ‘Looming’, and other selected beans and peas, to support the new developed foods under the project, in cooperation with Örebro University.

Near-infrared reflectance (NIR) calibration for the assessment of pea (Pisum sativum L.), faba bean (Vicia faba L.), and cowpea (Vigna unguiculata L.) on protein and amino acids content is the most practicable, quick, and cheap analytical technique for fast and efficient evaluation of genetic resources in breeding programs. Project partners AREI and ECRI already used evaluated calibrations in faba bean and pea breeding programmes, saving time and resources. Calibrated NIR equipment and the respective support have been also available for other partners and stakeholders, which contribute to the impact of the project and for the dissemination of the foregrounds on this topic.

The impact, dissemination and exploitation of the new cultivars, intercropping, rotations with legumes, inoculations, organic versus conventional farming, have also been achieved with great success by the organization of several field days, directed to farmers, across the different regions covered by the project. These demonstration actions showed how farmers are motivated to adopt the new concepts of farming systems and push forward the use of legumes in their agricultural systems.

**ENHANCE BIOLOGICAL NITROGEN FIXATION AND P UPTAKE B LEGUMES THROUGH SYNERGIC INFLUENCE OF RHIZOBIA AND ARBUSCULAR MYCORRHIZAL FUNGI IN ORDER TO OBTAIN HIGH NUTRITIONAL VALUE FOOD AND FEED PRODUCTS IN SUSTAINABLE AGRICULTURE**

Essential natural resources for agriculture, such as, mineral nutrients, soil and water, will become limiting factor in the near future. It is broadly recognised that the current rates of application of chemical fertilisers and water usage are neither economically viable nor environmental desirable. Hence, the selected rhizobium strains represent a substantial contribution to the improvement of the efficiency of microbial inoculants for legumes available in the market, as a solution to intensify the BNF and phosphorous uptake using sustainable procedures. Legumes and their symbiotic microorganisms are very relevant to tackle the challenges faced by the agriculture systems, particularly environmental degradation and water scarcity. The development of novel microbial inoculants is, indeed, one of the major research activities with impact in the future of the agriculture productivity and sustainability with a strong influence of farmers income and the respective attractiveness for legume production. A four-year research for such a complex issue and to reach a high TRL has been short.

Selection of elite microbial inoculants, including rhizobia and AMF, are in line with the emerging driving force for enhancement of more biologically based cultivation of agricultural crops and the attempts within EU regulations to reduce inputs of agrochemicals and water.

One of the great impacts of the molecular analysis, rely on providing the tools to select appropriate molecular markers, which will enable to monitor the survival, persistence and efficiency of the selected strains used as inoculants, in pots and in field experiments, under natural conditions in competition with native rhizobial populations.

Due the short period of the project for selection and field experiments, the microbial strains selected in EUROLEGUME are being tested in other ongoing projects in Europe, and a field experiment is also planned under a collaborative study with researchers in the state of Goiânia, Brazil, in cowpea and chickpea plants, where warm temperatures are almost constant whole year round. These experiments will certainly improve our knowledge about their range of adaptation to edaphic-climatic conditions.

Apart from the partner’s SMEs in EUROLEGUME, it was created a spin-off for the production of rhizobia inoculants for relevant crops in Southern Europe, such as cowpea, chickpea and soybean. A new
packaging bag is in development with the aim to improve the shelf life of the inoculant, since the bacteria viability is one of the main requirements for the efficiency of the inoculation. The developed cowpea inoculants have been submitted to a registration process as biofertilizers and biostimulants and will be available in the market in the next few months. The selected inoculants have great potential for the improvement of legume productivity under drought conditions and reduction of the use of agrochemicals. Moreover, other techniques of inoculation with the selected strains are currently being tested, particularly the seed coating, in order to evaluate if the coating of the grain legume seeds with AMF and rhizobia inoculants, with the help of sticky coating materials, could reduce the amount of the inoculant applied without reduction in the efficiency.

IMPROVE AN EFFICIENT ASSESSMENT OF HIGH PROTEIN LEGUMES OF LOCAL ORIGIN (PEA, BROAD BEAN, AND COWPEA) IN FOOD AND FEED AS AN ALTERNATIVE TO IMPORTED HIGH-PROTEIN FEED MATERIALS (SOYA BEANS, CORN, ETC.) AND THEIR IMPACT ON THE QUALITY OF FINAL INNOVATIVE PRODUCT, COST-EFFECTIVENESS AND ENVIRONMENTAL SAFETY

Food
The developed technological solutions, for the production of novel products from legumes (pulse spreads, extruded legume snacks and bars) open the possibility for a more extensive use of legumes, creating the opportunity for farmers (leguminous crop cultivation) and producers (diversified processing), promoting local raw material sales and production of value added products. There are contacts with companies for licence agreements, for instance in Latvia, and patents in Sweden. The innovative pulse products developed within the project can reduce or exclude time-consuming preparation and are an excellent option for consumers today, as modern lifestyle has substantially changed eating habits, leading to a significantly higher demand for ready-to-eat foods. The development of novel products from legumes clearly has a positive impact on the advancement of the European economy, with a great potential export. Given the great impulse of the project in the legume production in Baltic countries, it is envisaged a specific positive impact on the advancement of the Latvian economy, with a potential to export the novel foods. At the moment LLU has also received a verbal request from a foreign investor who is interested in buying the developed technology. Technology Transfer Office at LLU is managing the required documentation (announcement of auction, licensing agreement etc.) to prepare the technology for sale.

From our partner in Sweden (JTI) it was found that the production of fresh faba beans for processing (freezing) can be done in Sweden south of the latitude 59°N and that ordinary cultivars already used for feed purposes, can be used for fresh harvest; the small seeded cultivars are important due to the fact that the market price is significantly higher for the size 8–10 mm (‘super baby’) compared to sizes above 16 mm. The results of the EUROLEGUME project have played a significant role in the decision to start cropping of faba beans for fresh harvest in Sweden.

Fresh pods of cowpea (of both ssp. unguiculata and sesquipedalis) are traditionally consumed in the Mediterranean countries, mostly used boiled in salads. However, they are scarcely found in folk markets as fresh food, which in addition must be purchased and consumed soon after harvest, as pods are highly perishable. The seasonal production of cowpea fresh pods during summer (from field crops) and their limited postharvest life are the main constraints for marketing and consumption of this product. Apart from the possibility of frozen storage it can be explored an out-of-season production of greenhouse cowpea in Southern Europe; packaging in plastic bags of proper permeability to water and gases can prolong storage
of pods at 5-10 °C for 10 days. However, in order to manage the introduction of this novel food to the market further research is still needed. The impact of the greenhouse cowpea production, not yet explored, will create an added-value for the farmer and another opportunity to expand the diversity of greenhouse crop production.

The proposed minimally processed immature pea and faba seeds, also represent a new opportunity to promote the consumption of fresh legumes. In addition, after storage and commercialization, that product, if packaged in a suitable container, might be consumed fresh or directly microwaved. In that way, a healthy cooked food with high organoleptic quality can be quickly obtained. As a result, consumers’ health will benefit from increased proportion of legumes in their diet. There is a company ready to sell minimally processed immature seeds from dehulled faba-beans packaged in modified atmosphere but further market studies need to be done to support the launch of the product.

For the new legumes-based products, like faba beans based sauces, pulse spreads and puree, the processing methods still need to be optimized (i.e. low energy consumption, low CO2 emission) however, some SME’s already showed interest on producing and marketing these products.

In the case of extruded legumes, which have high protein content, could be the base material for the development of high protein snack bars, since taste is sufficiently neutral, and so they can be used for both salt and sweet snacks preparation. An added-value can be given by adding functional supplements - such as hemp seeds, Goji berries, ginger, and others. These products are creating great opportunities to SME’s, which need a dedicated commercial exploitation. In this sense, for instance, these products are in line with the consumer’s trends demand, on rich protein and fibre, being suitable to use on the go.

Regarding the production of faba bean based yoghurt there are promising contacts in Estonia and to a company in Sweden, to produce vegetable ice-cream. We need to look into shelf-life and suitable technology to reduce the faba bean taste and vicin-convicine. There were also contacts to develop "ready-to-heat" hamburgers following a first prototype developed by Örebro University. Further technological developments are required for the production system, shelf life, frozen or chilled products.

The promoted advanced processing and packaging technologies provide a long shelf-life, which represent a great advantage both for the industries and consumers, and eventually for exported products, with a clear gain for the EU economy.

Feeds

As already showed, faba beans and peas are very productive crops, and may be used as a high-quality feed concentrate for poultry and agricultural animals, as they contain 22–35% protein, in some cases even 26–38% of cheaper locally grown protein. This means that faba beans and their mixtures with peas that contain 20–23% protein can meet the protein requirement of poultry. Taking into account that overall, feed makes up about 70% of the total cost of poultry products, the increase of domestic protein-rich feedstuffs proportion in feed consumption would decrease the production cost of poultry, contributing to an increased efficiency and significant socio-economic impact in poultry farming, improving broiler productivity.

The inclusion of local protein in feeds also decreases the amount of imported soya and the carbon footprint of the feed in the diet. Thus, the use of traditional (regional) European protein crops (faba bean and pea) for domestic feed contributes to greater independence of the agricultural industry from feed price fluctuations in the global market and to sustainable use of the agricultural area.

The results from the use of legumes as domestic feedstuffs for the purpose of raising the nutritional value of the feed and balancing protein in the feed ration for dairy cows is very promising, as legumes help to better maintain the milk yield level during lactation, and enhance the milk quality indicators, providing
consumers with healthier products.
The inclusion of pea straw as a by-product, which can contribute to an added value of the Murciano-
Granadina goat diet, is an alternative to soybean in the diet of goat milk, increasing the level of
unsaturated fatty acids (6%) in milk and decreased saturated fatty acids (6%). This information has been
promoted by the distribution of leaflets between farmers and breeders. The process of changing diets is
still weak due to the fact that pea prices are not competitive. Moreover, the local production of peas is not
steady from one year to the other. However, there is a net benefit and impact on health of consumers,
particularly in the protection of cardiovascular diseases due to the high quality milk. Further dissemination
actions are needed to ensure a higher consumer’s impact.
The new feed products, from expander or extruder pelleted peas or field beans, and feeds produced from
roasted peas or field beans, improve animal performance with economic impact. The peas and field beans
can be processed and fed alone, or processed and included as an ingredient in compound feeds.
Expander and extruder pelleting requires rather large investments and is usually restricted to specialized
feed processing plants producing compound feed. Roasting is an alternative heat processing method
using heat from an external heater (like propane burner), allowing for heat processing at lower cost and at
farm level. Thus, roasting of peas and field beans can be exploited by the agricultural sector for improving
feed quality. The cooperation established with a commercial slaughter pig producer provided the
opportunity to try the inclusion of roasted faba beans in feeds for pigs, which improved taste and partly
reduced tannin content.

CONTRIBUTE TO THE IMPROVEMENT OF SUSTAINABILITY OF FABA BEAN, PEA, AND COWPEA
CROPS IN TERMS OF YIELD PARAMETERS, NUTRITIONAL VALUE, TOLERANCE TO BIOTIC AND
ABIOTIC STRESS, EFFICIENCY OF BIOLOGICAL NITROGEN FIXATION, AND NITROGEN
UTILIZATION EFFICIENCY, IN EUROPE, THROUGH EFFICIENT CULTIVATION SYSTEMS
The role of legumes (pea, faba bean, cowpea) in sustainable agriculture is multidimensional and the
benefits are many. The contribution of leguminous crops, in addition to feeding people and animals (at
lower production costs), improving soil fertility, protecting the environment due to low inputs and can also
be seen as a tool to manage the risk of upcoming climate change.
From the results/foreground, it can be said that the socio-economic impact is remarkable. More
specifically, new data, suggest new cultivation practices and provide new information on different
productive sectors benefiting agriculture, industry, and universities - research institutes.
Farmers (both, organic and conventional farming) are the end users of these results, which have been
already disseminated in practical and scientific conferences and seminars of national and international.
Farmers are introducing already in their cropping systems new cultivars (e.g. ‘Lielplatones’ is under
process of registration in the variety list of old cultivars in Latvia) and its production acreage is increasing
rapidly. Findings about inoculation efficiency for faba bean and pea support the idea of inoculation
necessity only in particular cases of unfavourable growing conditions and new soils previously not used for
production of these crops. This knowledge gives economical profit by resources using efficiency and cost
savings on inoculum where it is not necessary. These results are disseminated to farmers already by
different dissemination tools - publications in farmers’ magazines, seminars, and field days. The list of the
most suitable and productive cultivars also is directly and instantly used by farmers to increase the
productivity of their crops. All these findings lead to more sustainable farming and diminish environmental
lead by economical using of resources (diminished seeding rate, high productive cultivars, cultivars of high
nutritional value, cultivar mixtures exhibit plant potential of particular cultivars). Therefore, all these
findings support idea of diminishing load on environment, therefore benefits all the society. New rotation schemes with legume species and vegetables are recommended for farmers, so they can increase their yield at lower cost, especially under organic farming practices. The importance of cultivation of legume traditional varieties (landraces) lies in the fact that they are suitable for low-input farming and they have wide genetic resistance to stress, so they can be used in breeding programmes from companies to provide new commercial varieties with better adaptation and higher yield. Also, identification of a set of genes that can be used as transcriptional markers for drought stress in cowpea. Some of the results obtained from WP5 indicate that the tested landraces are more suitable for organic cropping systems than the respective commercial varieties. Furthermore, the obtained results revealed the potential of utilizing some of the tested landraces in breeding programmes, due to their high weed competition ability, and their high BNF efficiency, in combination with their ability to provide similar green seed yields with the commercial varieties. Nevertheless, in most cases, the commercial varieties contained more or similar amounts of protein with the landraces.

From legumes, industries can produce high quality and nutritional products (food) that can be linked to tradition and branded processing, to broaden the consumer’s choice, as well as to offer a greater variety of organoleptic and food choices. Feed industries have new information about new varieties with high nutritional value for animal feed.

**GENERATE ADDED VALUE PRODUCTS FROM LEGUME GRAIN PRODUCTION RESIDUES** - **ADDITIONALLY, CROP MANAGEMENT TOOLS ARE EXPECTED TO BE DEVELOPED IN ORDER TO MITIGATE GREENHOUSE GASES EMISSIONS**

Characterization of residual biomass. Evaluate silage with mix of legume residues and apples. Use of solid state fermentation with 3 fungi strains. Test of diets on rabbits

The above ground plant residual biomass from the three legume species (faba bean, pea, and cowpea) can be used to be incorporated in the soil however, its use for feed can represent an added value improving the crop competitiveness. EUROLEGUME studies were focused only in cowpea, which is the crop with lowest above ground plant biomass.

The ensiling of cowpea stover with discarded apples has the potential to be used by local farmers in order to produce feedstuffs that can be used for ruminant feeding during periods of feed shortage. Furthermore, as these by-products are seasonally produced at the same time, the ensiling may decrease the environmental impact.

Apart from silage, new feeds have been obtained through the incubation with white-rot fungi, leading to a cost reduction of commercial compound feeds for rabbits around 13.6€/ton feed. This new feed could potentially be used in higher proportion in rabbits’ diets up to 20-25%, thus leading to even higher cost reduction, with great impact on animal feed industry. New trials are already being prepared in order to evaluate this possibility. Data from the growth performances of rabbits have shown that animals fed diets containing up to 10% inclusion of treated cowpea stovers had lower cholesterol levels, indicating that P. citrinopileatus may induce a hypercholesteremic effect. This fact could be of great importance, and future trials are being prepared in order to evaluate the potential impact on animals’ health status as microbiota analysis have also pointed out possible effects on the gastro-intestinal tract microbial population.

Proposals for new research projects are now being envisaged in order to evaluate the prebiotic effects of this feed, so that it can be used as a functional feed as well. This constitutes a very valuable information for the European animal production industry due to the prohibition of using antibiotics as growth promotors since 2006.
In order to cope with the potential utilization of this new feed the production methodology must be upgraded and adapted to an industrial scale. Contacts have been promoted with a Portuguese mushroom production company in order to evaluate the possibility to produce this feed using the same inoculation and incubation procedures they are using to produce mushrooms.

Enhancement of nitrogen availability to following crops. Effect of cultivar on the following crops
As a rule of thumb, farmers should base their fertilisation planning on analyses of nitrogen in the soil to determine the levels of biomass incorporation, which will reduce the imported fertilizers accordingly. Our findings are, together with soil analyses, useful for farmers and consultants when planning fertilization to a given crop.
These results are a contribution to be used as a starting point for further studies on other legume species and cultivars, with different physiological and yield performances and allometric relations, to provide a full set of data for planning the fertilisation in crop rotations. The end users of this information will be farmers and their consultants with a clear impact on the efficient use of plant biomass to reduce fertilizers input. Apart from a reduced production cost there will be a reduced environmental impact since the production of mineral nitrogen fertilization represents one of the main agricultural practices, with a high emission of pollutants in the atmosphere, soil, and water.
Moreover, reducing the environmental load of nitrogen mineral fertilizers, which is due to the activities of the technological subsystem (production, transport, and application), to the alteration of some soil microbial processes and to the excess supply not absorbed by the cultivated crops, will have an overall positive environmental impact. Supplying N in the form of plant legume biomass is then an overall friendly process.

Advanced knowledge on the C sequestration and GHG of different cropping systems with legumes
Although no evidences have been found concerning the contribution to carbon sequestration in the soil, introduction of legumes in rotations with other crops and incorporation of their residues into the soil showed advantages regarding the emission of GHG and so must be recommended to farmers as a sustainable practice for organic or conventional agriculture. Cropping of legumes species in addition to being an important source of quality protein for humans and animals have massive implications for the sustainability of agricultural. The ability of legumes to fix atmospheric nitrogen symbiotically increases the availability of N to the following crops when legumes are included in rotations. Moreover, legumes may contribute to improve soil organic matter (SOM) content, soil structure, and availability of soil phosphorus. Maximum advantages of cropping legume grains arise when residual stubble is incorporated into soil after harvest. When legumes are used as a break crop in rotations dominated by grasses (cereals) or plants from other botanical families, they play an important role in the pest and diseases suppression by interruption of life cycles of plant enemies and allow decreasing pesticides application with important economic and food security benefits.

Further series of longer studies are necessary to draw clear conclusions. This aspect is more relevant to carbon sequestration data, where it is fundamental to allow the stabilization of the soil-plant system to changes in agricultural practices when different techniques or crop management are applied. It is also relevant to know the mineralization time-pattern of the legume crop residues incorporated into the soil. In future work, a deeper understanding of those processes and consequences for the soil-plant system is required and it will be of great help to apply methodologies of SOC fractionation or using methodologies of fractionation of organic matter based on labelled isotopes.
Another key issue, especially for dryland agriculture in the Mediterranean areas, is the use of no-tillage or minimum-tillage methods with legume crops. Under these conditions and practices, the mineralization kinetics of the legume residues left at the soil surface or removed at the harvest need to be clarified; in addition, the impact of this soil management technique on GHG emissions should be fully investigated.

Other general dissemination and exploitation activities
The specific results are available to everyone, which shows interest on knowing more about the project and the respective outcomes. To ensure that this can happen all non-confidential documents are available at the project website and free to access.

The EUROLEGUME project has been followed in social networks and by several scientists and stakeholders around the world. It is our intention to give sequence to these contacts and disseminate extra findings from the large amount of data that are still being handed by some partners. A majority of results will certainly reach the scientific community, whilst some topics require further studies to support robust conclusions and future exploitation. For instance, the contacts already established with SMEs will proceed to foster the production and marketing of some of the novel products, feeds and inoculants. Other SMEs will be contacted by the partners that have developed the products to proceed with this aim. The SMEs involved in the project are very motivated to pioneer these initiatives.

Several dissemination actions have been scheduled for 2018 and 2019, addressed to inform farmers about the major project results and their potential applications thus, rising the interest of key stakeholders to cultivate those varieties selected for the diverse European agro-climatic conditions. In the sequence of this dissemination programme, the first seminar is planned in March 2018 at AREI (Latvia), while at least 4 presentations are planned to be given in different events to farmers and farmers organisations in Latvia, Estonia, and Greece. Additional relevant dissemination activities planned to be developed in the near future are as follows: Publications in JCR scientific journals, Dissemination of project results in farmers’ magazines; At least 2 publications in Latvia, 1 in Estonia, 1 in Norway, 1 in Sweden is planned for reflecting of results about best performing varieties; Dissemination of EUROLEGUME results in Field days: Activities scheduled to summer 2018, organised by AREI (Latvia), ECRI (Estonia), and INIAV (Portugal).

In field days farmers and other stakeholders can visit the experimental fields and obtain information. Using the popular research gate https://www.researchgate.net/ repository for sharing the most relevant information on EUROLEGUME results and achievements, also linking the website to download databases. Building on knowledge developed in EUROLEGUME, a new national research project FoodProFuture NIBIO, Norway is underway. The new project will implement some knowledge developed about accessions and legume production in the EUROLEGUME project. Some field trial data may be used to aid in future Life Cycle Assessment (LCA) analyses.

Apart from these, some general exploitations activities are foreseen, e.g. to ensure that leaflets will be sent to an increased number of stakeholders according to their potential interest for the application of the knowledge and technology. Skype and face-to-face meetings will be conducted to get a feedback on the potential use of the available information and to address potential questions how these results can be applied. The website of the project will be kept online for the next couple of years and fed with activities of the partners on this topic to ensure further visibility.

List of Websites:
www.eurolegume.eu